European Union Risk Assessment Report

2-BUTOXYETHANOL

CAS No: 111-76-2
EINECS No: 203-905-0

RISK ASSESSMENT

GENERAL NOTE

This document contains two different reports:

- **Volume 68 Part I Environment** (Publication: EUR 22501 EN) – pages 2-97
- **Part II Human Health** (Final approved version awaiting for publication) – pages 98-419

European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

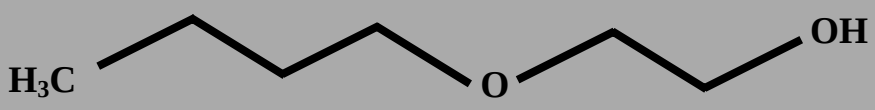
Existing Substances

European Union Risk Assessment Report

CAS No: 111-76-2

EINECS No: 203-905-0

2-butoxyethanol (EGBE) Part I - environment



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The mission of the IHCP is to provide scientific support to the development and implementation of EU polices related to health and consumer protection. The IHCP carries out research to improve the understanding of potential health risks posed by chemical, physical and biological agents from various sources to which consumers are exposed.

The Toxicology and Chemical Substances Unit (TCS), commonly known as the European Chemicals Bureau (ECB), provides scientific and technical input and know-how to the conception, development, implementation and monitoring of EU policies on dangerous chemicals including the co-ordination of EU Risk Assessments. The aim of the legislative activity of the ECB is to ensure a high level of protection for workers, consumers and the environment against dangerous chemicals and to ensure the efficient functioning of the internal market on chemicals under the current Community legislation. It plays a major role in the implementation of REACH through development of technical guidance for industry and new chemicals agency and tools for chemical dossier registration (IUCLID5). The TCS Unit ensures the development of methodologies and software tools to support a systematic and harmonised assessment of chemicals addressed in a number of European directives and regulation on chemicals. The research and support activities of the TCS are executed in close co-operation with the relevant authorities of the EU MS, Commission services (such as DG Environment and DG Enterprise), the chemical industry, the OECD and other international organisations.

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RISK ASSESSMENT

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Part I – Environment

CAS No: 111-76-2

EINECS No: 203-905-0

RISK ASSESSMENT

Final Report, 2006

France

The environmental part of the risk assessment of 2-butoxyethanol (EGBE) has been prepared by Ministry of the Environment (MEDD) on behalf of the European Union.

The scientific work on this report has been prepared by:

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Final report:	**2006**

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.
The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.
The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.
If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.
The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.
This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

Roland Schenkel
Director General
DG Joint Research Centre

Mogens Peter Carl
Director General
DG Environment

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[1] O.J. No L 084, 05/04/199 p.0001 – 0075

[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011

[3] Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS No:	111-76-2
EINECS No:	203-905-0
IUPAC Name:	2-butoxytehanol
Synonyms:	EGBE (this synonym will be used in the present study to refer to the chemical Ethylene Glycol Butyl Ether). Other synonyms: 2-BE; butoxyethanol; 2-butoxy-1-ethanol; n-butoxyethanol; butyl ethoxol; 3-oxa-1-heptanol; o-butyl ethylene glycol; butyl glycol; butyl monoether glycol; ethylene glycol butyl ether; EGBE; ethylene glycol n-butyl ether; ethylene glycol monobutyl ether; glycol butyl ether Commercial trade names: Dowanol EB; Butyl Cellosolve; Butyl Icinol; Butyl Oxitol; Eastman EB Solvent

Environment

Conclusions to the risk assessment for the aquatic compartment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Conclusion (ii) is applied to all levels of the life cycle of EGBE: production, formulation, processing and private use.

Conclusions to the risk assessment for the terrestrial compartment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Conclusion (ii) is applied to all levels of the life cycle of EGBE: production, formulation, processing and private use.

Conclusions to the risk assessment for the atmospheric compartment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Conclusion (ii) is applied to all levels of the life cycle of EGBE: production, formulation, processing and private use.

Conclusions to the risk assessment for secondary poisoning

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Conclusion (ii) is applied to all levels of the life cycle of EGBE: production, formulation, processing and private use.

Human Health

(to be added later).

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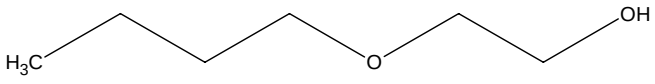
1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 111-76-2
EINECS Number: 203-905-0
IUPAC Name: 2-butoxyethanol
Molecular formula: $C_6H_{14}O_2$
Structural formula: CH_3-CH_2-CH_2-CH_2-O-CH_2-CH_2-OH



Molecular weight: 118.17 $g.mol^{-1}$
Synonyms: EGBE (this synonym will be used in the present study to refer to the chemical Ethylene Glycol Butyl Ether). Other synonyms: 2-BE; butoxyethanol; 2-butoxy-1-ethanol; n-butoxyethanol; butyl ethoxol; 3-oxa-1-heptanol; o-butyl ethylene glycol; butyl glycol; butyl monoether glycol; ethylene glycol butyl ether; EGBE; ethylene glycol n-butyl ether; ethylene glycol monobutyl ether; glycol butyl ether
Commercial trade names: Dowanol EB; Butyl Cellosolve; Butyl Icinol; Butyl Oxitol; Eastman EB Solvent
Annex I entry: 603-014-00-0

1.2 PURITY/IMPURITIES, ADDITIVES

Purity: the purities quoted in IUCLID were all ≥ 99% w/w

Impurity: as the synthesis reaction produces other glycol ethers, small concentrations of them can be found.
2-butoxyethoxyethanol (CAS 112-34-5) ≤ 0.3% w/w
1,2-ethanediol (CAS 107-21-1) ≤ 0.3-0.5% w/w
1-butanol (CAS 71-36-3) ≤ 0.1-0.2% w/w
water < 0.1-0.2% w/w

Additives: 0.008-0.012% w/w 2,6-bis(1,1-dimethylethyl)-4-methylphenol (CAS 128-37-0) added to prevent the formation of peroxides.

1.3 PHYSICO-CHEMICAL PROPERTIES

Note: When the reliability of values do not enable a clear choice between one and another, a median value is chosen or calculated taking into consideration all figures supplied by the industry and only once the values found in handbooks or reports and which differ.

The physico-chemical properties are discussed below and summarised in **Table 1.1**.

Table 1.1 Physico-chemical properties

Property	Value
Physical state	Liquid
Melting point	-74.8°C
Boiling point	171°C
Relative density	0.9 at 20°C
Vapour pressure	1.41 hPa, calculated at 25°C (initial value: 1 hPa at 20°C)
Surface tension	26.6 mN/m at 20°C
Water solubility	Highly miscible
Partition coefficient n-octanol/water (log value)	0.8
Granulometry	Not applicable
Flash point	67°C
Autoflammability	244.5°C
Flammability	Upper limit: 12.7% (volume) Lower limit: 1.1% (volume)
Explosive properties	Not explosive
Oxidising properties	No oxidising properties
Viscosity	3.28 mPa.s at 20°C
Henry's constant	0.08 Pa.m^3/mol at 25°C
Conversion factors (101 kPa, 20°C)	1 ppm = 4.9 mg/m^3 1 mg/m^3 = 0.204 ppm

1.3.1 Physical state

EGBE is a neutral, colourless liquid with a very weak pleasant odour (Ullmann, 2000). An absolute perceptible limit in air of 0.1 ppm (50% recognition = 0.35 ppm and 100% recognition = 0.48 ppm) was referred to EGBE (Verschueren, 2001).

1.3.2 Melting point

Several values are reported in literature. The value of -77°C appears in several reports and studies (ECETOC, 1994; OECD, 1996; Dow Chemical Co, 2001) and handbooks show values

ranging between -75 and -70°C (-74.8°C Lewis, 1999; < -70°C Ullmann, 2000; -70°C Howard, 1989; -75°C Eastman, 2001; -70.4°C BASF, 2002; -70°C Verschueren, 2001).

The median of all different values found is retained for this study: -74.8°C.

1.3.3 Boiling point

Most of the values found in literature are in the following range: 168-172°C (BASF, 2002; Ullmann, 2000). A boiling point of 170.8°C for EGBE can be found (ECETOC, 1994) which is close to those appearing in handbooks (170°C in Kirk-Ohtmer, 1983 and Verschueren, 2001). Two other handbooks show values between 171 and 172°C (Lewis, 1999 ; Howard, 1989). Boiling point of 171°C was found in technical data sheets or product information reports (BP, 2002; Dow Chemical Co, 2001).

The median of all the different values above is 171°C. This value will be used for the risk evaluation.

1.3.4 Relative density

Several relative density values are available too. Most of them are about 0.9 (Verschueren, 2001 - value measured at 20°C; Merck, 1996). Technical data sheets or product information reports give values of 0.9011 and 0.902, at 20°C (BP, 2002; Dow Chemical Co, 2001).

A rounded value of 0.9 will be chosen for this study.

1.3.5 Vapour pressure

Vapour pressures ranging from 0.79 to 1.3 hPa, at 20°C have been reported. Data come from technical data sheets: 0.8 hPa (Shell Chemicals, 2001), 0.89 hPa (Dow Chemical Co, 2001), 1.3 hPa (BP, 2002). The same range appears in Sax, 1986 and other chemical handbooks give almost the same values: 0.89 hPa (Verschueren, 2001), 1 hPa (Ullmann, 2000).

The rounded value of the median gives a vapour pressure of 1 hPa, at 20°C. It will be used in the risk assessment (based on this value, a vapour pressure of 1.41 hPa has been recalculated at 25°C, by EUSES, EC, 2004).

1.3.6 Surface tension

Several values of surface tension appear in technical data sheets for commercial EGBE: Dow Chemical Co, 2001 reports a value of 27.4 mN/m at 25°C, Eastman, 2001 gives a value of 26.6 mN/m at 20°C and Shell Chemicals, 2001 another of 28.9 mN/m. A value of 27.4 mN/m at 25°C is also reported in a handbook (Kirk-Ohtmer, 1983).

The value reported in Eastman, 2001 will be retained for the study: 26.6 mN/m, at 20°C, the temperature recommended in the OECD guideline No 115.

Surface active properties can be assumed for glycol ethers, and especially for ethylene glycol butyl ether, because of its quite long carbon chain. The values reported in the literature for EGBE tend to indicate that this substance is a surface-active reagent even if no indication has been found about the concentration of the substance during the tests listed above. Indeed, OECD

guideline n°115 suggests that surface tension measurements should be performed using a concentration of 1 g/L for soluble substances.

The fact that EGBE shows surface-active properties could thus lead to the disturbance of analytical method employed to measure some physico-chemical characteristics.

However, there is a difference between the surface activity of traditional surfactants and substances that can reduce the surface activity of solutions like EGBE. What is observed with the glycol ethers during the surface tension measurements is the typical non-ideal behaviour of a mixture of a water miscible solvent such as methanol and ethanol. The reason for the observed relationship between surface tension and concentration is the disruption of the hydrogen bonding of the water causing non-linear behaviour of the surface tension against the concentration. In this case the substance is not migrating to the surface; it is not acting in the traditional surface-active manner. Therefore it would not affect the measurements of the physical chemical properties. One should also notice that glycol ethers do not form micelles. They are fully miscible with water and form clear solutions.

Furthermore, considering the other properties of this substance (EGBE is highly miscible in water, hydrosphere is the preferential target of EGBE in the environment: > 90%, see Section 3.1.1.2), surface active properties of EGBE will not be considered in this assessment.

1.3.7 Water solubility

EGBE is often reported as miscible with water in several data sheets and product information reports (Hoechst, 1992; Hoechst, 1993; BP, 2002). A solubility of about 50,000 mg/L is reported by Eckel et al., 1996, 63,500 mg/L by ASTER, 1996. A value of 1.10^6 mg/L has been measured (Riddick et al., 1985 cited in HSDB, 2000).

EGBE can be considered as highly miscible in water and the upper value of the range advised by EUSES (EC, 2004), 1.10^5 mg/L, is retained for modelling purposes.

1.3.8 Partition coefficient n-octanol/water

Several QSAR calculations of partition coefficient n-octanol/water for EGBE have been performed. They are presented hereafter using an ascending order of logarithmic values: 0.57 (calculated with KOWWIN v.1.66. - US EPA and Syracuse Research Corporation, 2001); 0.81 (Verschueren, 2001), 0.84 (ASTER, 1996).

Partition coefficient measurements have been performed (Tanii and Hashimoto, 1982): 0.77 is the mean value of three determinations. Measurements have been carried out with gas chromatography.

Other measured log K_{ow} can be found in literature: 0.74 (Verschueren, 2001), 0.81 (Staples et al., 1998), 0.83 (Korenman and Dobromyslova, 1975; Hansch and Leo, 1985, cited in Prager, 1995; Staples et al., 1998).

The rounded value of the median obtained with all measured data gives a log K_{ow} of 0.8 that is retained for this study.

1.3.9 Granulometry

Not applicable: the substance is a liquid.

1.3.10 Flash point

Values reported in literature range from 60 (INRS, 1996 and Merck, 1996) to 68°C (BP, 2002). Both values are measured with closed cup method. Two other values were obtained using this method: 67°C (Shell Chemicals, 2001; BASF, 2002) and 61°C (Sax, 1986), while methods leading to values of 65°Dow Chemical Co, 2001) and 67°C (Ullmann, 2000) are not mentioned.

The median of the values measured using a closed cup is retained: 67°C.

1.3.11 Autoflammability

Two temperatures of autoflammability have been found in technical data sheets: 244°Dow Chemical Co, 2001) and 245°C (BP, 2002). The median value of 244.5°C is retained.

1.3.12 Flammability

Limits of flammability are reported by BP, 2002 for EGBE. The upper limit is 12.7% (volume) and the lower one is 1.1% (volume).

1.3.13 Explosive properties

Not explosive.

1.3.14 Oxidising properties

There are some references which suggest that glycol ethers can be prone to the formation of peroxides on storage. However data from one of the producers, shown below, indicates that peroxide production levels for EGBE remain low, even during prolonged storage under adverse conditions, as shown in **Table 1.2**.

Table 1.2 Peroxide levels in EGBE during storage under adverse (daylight) and recommended (dark) conditions. No antioxidants used. Results in mmol active oxygen/litre

	In daylight	In the dark
Time 0	0.018	0.018
3 months	0.040	-
6 months	0.052	0.019
18 months	0.95	-

The National Fire Protection Association's code for the reactivity of EGBE is 0 indicating minimal hazard. In consequence, there is no requirement for classification R19.

1.3.15 Viscosity

The range of viscosity values found in technical data sheets for EGBE goes from 2.9 mPa.s (at 25°C, Dow Chemical Co, 2001) to 3.3 mPa.s (Shell Chemicals, 2001). A value of 3.28 mPa.s is also reported by BP, 2002, at 20°C. BP Chemicals, 1998 also reports a range of viscosity values for several concentrations of EGBE in water (between 20 and 100% volume): 2-6 mPa.s at 20°C. Several values have been found in handbooks: 3.26 mPa.s, at 20°C (Ullmann, 2000), 2.83 mPa.s at 25°C (Prager, 1995) and a catalogue of commercial substances gives a viscosity of 5.83 mPa.s, at 20°C (Merck, 1996).

The most common values are rounded and a viscosity of 3.3 mPa.s (at 20°C) is retained for this study.

1.3.16 Henry's constant

Different QSAR estimation methods (calculated with HENRYWIN v.3.10. - US EPA and Syracuse Research Corporation, 2001) give values of Henry's law constant presented in **Table** 1.3**.**

Table 1.3 Henry's law constant values (at 25°C) in EPI suite (US EPA, 2001)

Method	Value ($Pa.m^3/mole$)
Bond method	0.009 9
Group method	0.002 1
Experimental value quoted in EPI suite database	0.162 1
Calculated with VP.M / WSol ratio using EPI suite estimated values (QSAR)	0.116 1

Henry's law constant values have also been calculated using another program (MPBPVP), at 25°C. Depending on the method used, values of 0.01 and 0.002 $Pa.m^3/mol$ are reported (SRC, 1988, cited in Envirofate).

Another study shows a calculated Henry's law constant of 0.551 $Pa.m^3/mol$ at 25°C ASTER, 1996).

Henry's law constant can also be estimated from the ratio of the vapour pressure to the water solubility using selected values from this study: 141 Pa for vapour pressure and 1.10^6 mg/L for water solubility. Calculation gives a Henry's law constant of 0.017 $Pa.m^3/mol$.

Experimental measurements of Henry's law constant have also been Kim et al., 2000). Two different methods were used to determine Henry's law constant for the equilibrium partitioning of EGBE contained in an aqueous solution and the air. Results of measurements are presented in **Table 1.4**.

Table 1.4 Henry's law constant values as measured in Kim et al., 2000

Bag method for equilibrium partitioning	Batch stripping method
T = 20°C ; H = 0.041 Pa.m³/mol	T = 22°C ; H = 0.063 Pa.m³/mol
T = 25°C ; H = 0.081 Pa.m³/mol	T = 23°C ; H = 0.068 Pa.m³/mol
T = 30°C ; H = 0.101 Pa.m³/mol	T = 25°C ; H = 0.082 Pa.m³/mol
	T = 30°C ; H = 0.135 Pa.m³/mol

In the previous study, there is a good correlation between both method used, bag method for equilibrium partitioning and batch stripping method, and particularly at 25°C. Moreover, direct measurement of the Henry's law constant is recommended for water miscible compounds (TGD - EC, 2003). So a Henry's law constant of 0.08 $Pa.m^3/mol$ will be used.

1.4 CLASSIFICATION

1.4.1 Current classification

There is no classification for the environment.

1.4.2 Proposed classification (environmental part only)

According to the data presented and the criteria of Directive 67/548/EEC EGBE is not classified as dangerous for the environment.

2 GENERAL INFORMATION ON EXPOSURE

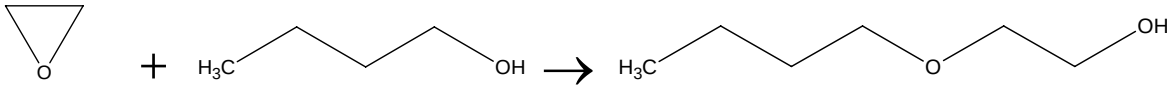
2.1 PRODUCTION

The chemical 2-butoxyethanol (here referred to as EGBE) belongs to the group of glycol ethers, which are mainly used as solvents. During 2003, the production of EGBE in the European Union was approximately 161,000 tonnes (personal communication from CEFIC). According to information provided by industry and compiled by SICOS, 1999, the total EU production of all glycol ethers was 350,000 tonnes in 1998.

2.1.1 Production processes

EGBE is manufactured by adding n-butyl alcohol and ethylene oxide. The standard ethoxylation reaction for glycol ethers is as follows:

Equation 2.1 EGBE synthesis reaction



ethylene oxide *butanol* *EGBE*

Ethylene oxide and butanol react together with a typical industrial catalyst. This catalysed reaction produces a range of butyl glycol ethers (monoglycol, diglycol and triglycol ethers) which are separated by fractionation using, for example, vacuum distillation (personal communication from OSPA).

2.1.2 Detailed production capacity

The production and sales data for years 2001 to 2003 are given in **Table 2.1**.

Table 2.1 Overview of EGBE production and sales in Europe for years 2001 to 2003 (data provided by CEFIC)

(In kilo tonnes)	2001	2002	2003	Figures retained
Production	135	158.5	161	155.1**
Imports	9	6	6.5	6***
Exports	49	59	68	64***
Net into stock	*	7.5	3	-
Captive use (EGBEA production)	9	8	10	9

Table 2.1 continued overleaf

Table 2.1 continued Overview of EGBE production and sales in Europe for years 2001 to 2003 (data provided by CEFIC)

(In kilo tonnes)	2001	2002	2003	Figures retained
Sales in EU	86	90	86.5	88.1
Total use in EU	95	97.5	96.5	97.1¤

* Included in exports figure
** The production volume has been obtained using the following calculation: (Exports + Captive use + Sales) – (imports)
*** Mean of 2002 and 2003 figures. This figure has been retained in order to take into account the evolution of import and export volumes the last two years
¤ See Table 2.3 for a breakdown of total use in EU

The figures presented above show that there is a trend for an increase in production year by year: 135, 158.5 and 161 kt for years 2001, 2002 and 2003 respectively. However this is almost entirely due to increased demand for exports: 49, 59 and 68 kt each year between 2001 and 2003. The overall demand within the EU remains flat.

A production volume of 155,000 tonnes/year will be retained for the risk assessment.

The production in the European Union is located at five different sites (see **Table 2.2**).

Table 2.2 EGBE production sites in EU (larger than 1,000 tonnes/year)

Company	Location
BASF AG	Ludwigshaven, Germany
BP*	Lavera, S. France
Dow Chemical Company**	UCL, Wilton International, Middlesbrough, United Kingdom
Sasol Gmbh***	Marl, Germany
Shell Nederland BV	Hoogvliet Rt, the Netherlands

* EGBE business of Hoescht renamed as Clariant in 1998 and then sold to BP (2001)
** EGBE business of ICI sold to Union Carbide in 1995 and then to Dow in 2001 / EGBE business of Enichem sold to Dow in 2001
*** Huels sold to Condea in 1998 and then EGBE business sold to Sasol in 2001

From the **Table 2.2**, it appears that some production sites are located in the same area. Consequently the locations of both German sites and the Dutch one have been checked so as to establish whether they could pertain to the same region (TGD definition EC, 2003). Distances between Marl and Ludwigshaven on one hand and Marl and Hoogvliet on the other hand are 259 and 188 km, respectively. So, in the regional assessment, none of these sites will be considered in a same region.

Ten EGBE importers are also reported. Most of them are paint or detergent manufacturers and some are distributors. They import less than 1,000 tonnes/year except one which has reported a higher tonnage (but detailed figures are not available).

2.2 USES

EGBE has a wide range of uses as a solvent in paints and surface coatings, detergents and surface cleaners, inks or dyes. A breakdown of the uses of EGBE in Western Europe has been established based on the data collected for years 2001 to 2003 by CEFIC (see **Table 2.3**).

Values presented in this table are based both on volumes shown in **Table 2.1** and on the EGBE volumes declared by uses reported in 2001, 2002 and 2003. The analysis of this set of data has led to a choice which is meant to represent a reasonable worst case. The final data choice is based mainly on averages but some expert judgement has also been applied to adjust for market knowledge and the fact that supply via distributors adds some uncertainty to the numbers. This uncertainty explains the significant fluctuation in the annual tonnage figures for the smaller uses. Typically, 25-40% of volume goes via distributors. To reflect these uncertainties, the figures are quoted as rounded numbers. 2002 and 2003 data should be given more weight as some errors have possibly been made during assessment of the 2001 data in allocating users to the appropriate end use categories.

Table 2.3 Breakdown of EGBE uses in Europe

End use	Stage of the life cycle	Industry category	Use category	2001[a]	2002	2003	Retained proposal	
							Quantity used (Tonnes)	Percentage of total use
Paints and coatings (including estimation for indirect sales via distributors)	Formulation Processing Private use*	14: paints, lacquers and varnishes industry	48: solvents	51,275	63,000	56,700	57,000	58.70
Detergents, cleaners	Formulation Private / public use	5: personal/ domestic 6: public domain	9: cleaning, washing agents	1,4921	7,000	10,000	11,000	11.33
Chemicals used in synthesis	Processing	3: chemicals used in synthesis	33: organic intermediates	6,916	13,600	11,000	10,000	10.30
Captive use (EGBEA production)	Processing	3: chemicals used in synthesis	33: organic intermediates	9,000	8,000	10,000	9,000	9.27
Printing inks	Formulation Processing	12: pulp, paper and board industry	48: solvents	5,340	1,100	2,500	2,500	2.57
Oilfield chemicals	Formulation Processing	6: public domain	48: solvents	0	3,000	2,000	2,500	2.57
Metal cleaning	Formulation Processing	8 : metal extraction, refining and processing industry	9: cleaning, washing agents	0	50	2,000	2,000	2.06
Electronic industry	Processing	4: electrical / electronic industry	48: solvents	1,590	0	30	600	0.62

Table 2.3 continued overleaf

Table 2.3 continued Breakdown of EGBE uses in Europe

End use	Stage of the life cycle	Industry category	Use category	2001¤	2002	2003	Retained proposal	
							Quantity used (Tonnes)	Percentage of total use
Leather treatment operations	Processing	7: leather processing industry	48: solvents	1,349	0	160	500	0.51
Pharmaceuticals	Processing	3: chemicals used in synthesis	48: solvents (extraction agents, reaction media)	2,073	400	400	500	0.51
Adhesives	Formulation Processing	6: public domain	48: solvents	223	500	500	500	0.51
Cosmetics / Personal care	Formulation Private use	5: personal / domestic 6: public domain	15: cosmetics	102	0	700	500	0.51
Fire foams	Formulation Processing	6: public domain	25: foaming agents	65	200	330	300	0.31
Agricultural products	Formulation Processing	1: agricultural industry	48: solvent	207	0	140	200	0.21
Paper industry				0	0	20	0	0.00
Textile manufacture				0	50	20	0	0.00
Miscellaneous (unknown)				917	0	0	0	0.00
Rubber, oil industry				674	0	0	0	0.00
Oil spill dispersants				191	0	0	0	0.00
Construction chemicals				153	0	0	0	0.00
Total				94,996	96,900	96,500	97,100	~ 100

* For personal decorative paints only

¤ Original 2001 data provided by CEFIC have been modified so as to integrate sales via distributors and volume of EGBE used for EGBEA production on site (captive use)

Four major uses can be identified from sale figures. The use of EGBE in paint and lacquer industry represents ~ 58% of the total volume used in EU. The two other main uses, intermediate for EGBEA synthesis (including captive use) and cleaning agent, represent respectively ~ 20% and ~ 11% of the total quantity of EGBE used.

Information for other minor uses for EGBE is also available (light dimmed cells in **Table 2.3**). The sum of the other uses represents about 10% of the total use of EGBE. Due to the low toxicity of the substance, these uses might be evaluated in a second time, if they show any concern. Moreover, some uses have been reported in the past that seem to no longer exist or errors could have occurred when allocating volumes to end-uses (dimmed lines in **Table 2.3**). For some of these uses, the percentage of total use has been set at 0 since no information has confirmed that EGBE was still used in this sector. For some other uses (e. g. oil spill dispersant),

figures reported does not seem to indicate a real annual use of the substance since stockpiles could be made during several years without using the product (see Section 3.1.2.2.5).

Another information source (OECD, 1996), presented in **Table 2.4**, shows supplies of EGBE to EU market. It gives a more detailed view on the major uses for EGBE.

Table 2.4 EGBE end use information (OECD, 1996)

	End use	kt/year (% of total use)
Industrial use	Anticorrosion coatings	2.6 (2.9%)
	Can coatings	9 (10.0%)
	Coil coatings	6.5 (7.2%)
	Decorative trade coatings*	15.5 (17.2%)
	General industrial coatings*	16.8 (18.7%)
	Automotive OEM coatings	1.3 (1.4%)
	Automotive OEM coatings*	6.5 (7.2%)
	Wood coatings*	1.3 (1.4%)
	Detergents / cleaners	3 (3.3%)
	Inks (screen)	5 (5.6%)
	Feedstock for EGBEA production	11 (12.2%)
	Subtotal	78.5 (87.3%)
Consumers uses	Decorative retail coatings*	10.4 (11.6%)
	Detergents / cleaners	1 (1.1%)
	Subtotal	11.4 (12.7%)
	Total	89.9 (100%)

* Water based

In this study, the detailed percentages for paint and detergent uses presented in **Table 2.4** will be applied to the volumes retained in **Table 2.3**. These figures will be presented with further explanations given in the following paragraphs concerning each reported use.

2.2.1 Paints and coatings

Glycol ethers and their acetates are effective in many surface coating technologies including, for example, high and low solid coatings, waterborne emulsions, water-reducible coatings. As ‘true solvents’ glycol ethers are able to dissolve many resins in all proportions, and are particularly useful in promoting flow properties. They also encourage good gloss and film properties by maintaining the resin(s) in solution throughout the film formation process (Shell Chemicals).

Detailed percentages for different paint types were calculated from the figures in **Table 2.3** and end use information for EGBE (OECD, 1996). They are presented in **Table 2.5**.

Table 2.5 Detailed view of paint uses

End use	% of total use	Typical maximum % of EGBE in formulated products
General industrial coatings*	14.11	3
Decorative trade coatings*	13.02	1.5 (3**)
Decorative retail coatings (consumers uses)*	8.73	1.5 (3**)
Can coatings	7.56	7
Coil coatings	5.46	7
Automotive OEM coatings*	5.46	8
Anticorrosion coatings	2.18	1
Automotive OEM coatings	1.09	2
Wood coatings*	1.09	2
Total	58.70	

* Water based
** Personal communication, OSPA (value used for the assessment)

2.2.2 Detergents, cleaners

Glycol ethers can be used in all types of cleaners. They reduce surface tension and are excellent coupling agents. Working in conjunction with a surfactant, they facilitate wetting of the surface, penetration of the soil, and help to suspend the soil in water until it is wiped or rinsed away.

The analysis of uses of 434 cleaning products identified during risk assessment of EGBE in cleaning products (NICNAS, 1996) revealed the range of EGBE concentrations below.

Table 2.6 EGBE concentrations in cleaning products

EGBE conc.	% of total products	Examples of cleaning products
< 10%	68	Surface cleaner (multi-purpose cleaners)
10-30%	14	Floor stripper
30-60%	1	Glass / window cleaner ; Oven cleaner
10-60%	2	Carpet cleaner
> 60%	1	Laundry detergent
unknown	14	Rust remover

In this study, most of the cleaning products have an EGBE concentration < 10%. However, extreme values go from < 1% to 94%. The fraction of chemical in formulated detergent for both industrial and consumer uses (0.1) quoted in the OECD, 1996 will be retained for this study. In this reference EGBE end use volumes show that ¾ (~ 8.50% of total use) of EGBE used as a detergent takes place in the industrial domain and that only ¼ (~ 2.83% of total use) remains for consumers uses.

2.2.3 Chemicals used in synthesis: intermediate for EGBEA production

EGBE is used in the synthesis of acetate esters as well as phthalate and stearate plasticisers. Although the major use of EGBE as an intermediate is in 2-butoxyethanol acetate synthesis, it is also employed for the synthesis of di(-2butoxyethyl) phthalate (ATSDR, 1998).

2.2.4 Printing inks

Combination of glycol ethers and other solvents can be used to achieve the right evaporation rates, levelling properties, water miscibility and solubility for ink formulations. They can also be used to adjust surface tension and viscosity, promoting deep and even penetration of inks in soft substrates (Shell Chemicals). The typical maximum percentage of EGBE in formulated printing inks is 20% (OECD, 1996). Specific information shows a decline in this particular use due to the switch to radiation cured screen printing inks.

Rastogi, 1991 analysed a series of printer's inks in order to characterise their levels of organic solvents. EGBE was found in 2/29 samples and identified, for example, in printer's inks for screen printing on paper and paper boards. The EGBE concentrations range in such formulated products was 0.1-0.4%.

The value quoted in OECD, 1996, 20%, will be retained as the typical percentage of EGBE in printing inks.

2.2.5 Other uses

Oilfield chemicals (2.57%): oilfield chemicals include brines and brine components used as completion, workover[4] and drill-in base fluids. Other end-use and intermediate applications include antifoams, drilling lubricants, emulsifiers, corrosion inhibitors, surfactants, oilfield biocides and scale inhibitors. They can be used in offshore platforms. Based on a search of patents on such products (personal communication, OSPA), typical concentrations are in the range 5-40%. However, one product used for the cleaning of oilfield equipment has been reported with concentrations of EGBE between 60 and 100%. This latter product is a concentrated one intended for dilution before use.

Metal cleaning (2.06%): metal cleaning/degreasing is used in industries where the production process includes fabricating and/or assembling metal parts: mainly the automotive, aviation, appliance and railroad industries. During the various steps of the production process, metal parts must be cleaned of oils, fluxes and grease.

Electronic industry (0.62%): among the uses of glycol ethers in electronic industry, those in copper clad laminating, laminates or photoresistors are reported (Shell Chemicals).

Pharmaceuticals (0.51%): glycol ethers are reported to be process chemicals in the synthesis of pharmaceuticals. Usually they provide the medium where reaction takes place but they are also used in separation steps in order to obtain the desired drug or to maximise its purity.

Leather treatment operations (0.51%): use as a solvent in leather protection system confirmed by appropriate EU trade association (OSPA enquiries). Nevertheless, EGBE is being substituted by other solvents (personal communication from the European Leather Industry).

[4] Major maintenance or remedial treatments.

Glycol ethers are used in aqueous degreasing systems for sheep and goatskin leather at concentrations of 1-5% (worst case). Only EGBE is reportedly used in this part of the process. Usage rates can therefore be calculated as up to 3 g/kg of dry finished leather (worst case based on 5% EGBE in formulation). Based on the total production information in the TGD of 314 Mm^2 leather per year, 25% of which is sheep/goatskin, and assuming a leather weight of 2 kg/m^2 would suggest total GE use in this application of around 500 tonnes/year.

Glycol ethers are also used in leather finishing. EGBE is quoted as being used in this application, which is effectively a "coating" operation where a preparation is applied by air atomised spraying in a spray booth. Actual usage rates are quoted as up to 0.4 g/kg of dry finished leather, which, following the above assumptions, would lead to total GE consumption of up to 250 tonnes/year. There will in addition be wastage from over spray and emissions to water from the over spray control systems. However, since this is a coating operation, it is already covered by the existing painting scenarios. Consequently, there will not be a separate risk characterisation for this use.

Adhesives (0.51%): in the industry of adhesives and sealants, glycol ethers are employed as solvents and enter formulated products. They are employed both in domestic and industrial adhesives. EGBE is most likely used in water-based adhesives. A percentage of 10% of EGBE in formulation will be adopted for the assessment. This figure is similar to the highest figures used for water-based coatings (personal communication, OSPA).

Cosmetics/Personal care (0.51%): the solvent and coupling properties of glycol ethers are utilised in cosmetic formulations. They can be used to stabilise emulsions used in lotions, preventing the active ingredient particles from dropping out of suspension. This helps to maintain the aesthetic appearance of the product and can increase shelf-life. That usage is now believed to be superseded by DEGBE (OSPA enquiries). Actually, EGBE is used in few cosmetic formulations and is only permitted in hair dyes up to a concentration of 10%.

Fire foams (0.31%): EGBE can be used in synthetic foam formulations. No firm information was available but an estimation of the market size would be 1-3 kt/year. Some of this would be exported however (OSPA enquiries). Most synthetic foams use glycol ether solvents as a foam booster and freezing point depressant. Butyl glycol ether use is now more and more superseded by other glycol ether. The solvent composition of fire foams is typically 16% as the concentrate but 0.5% in use (personal communication, OSPA).

Agricultural products (0.21%): the use of EGBE as a solvent or a co-solvent in pesticide formulations represents a minor application for this chemical. EGBE is used as a coupling solvent to help disperse the active component in water when the concentrate is diluted for use. It may be used at a significant level in the concentrate but these are usually diluted manyfold before use. For a conservative worst case "in use" figure, 1% will be retained (this would correspond to 1 L diluted in 100 L which represents a low dilution factor compared to typical use of pesticides for field application).

Construction chemicals: EGBE is an additive for construction materials. It is used as a water-reducing agent for example.

Cutting oils: declining use in soluble oils. Glycol ethers used 'very occasionally' at use levels of 5-7%. Total EU sales in 1996: 110 kt/year for such oils. As a worst-case, if 5% of oils use glycol ethers and all assumed to be EGBE, total market would be a maximum of 400 tonnes/year (~ 0.41% of total use) (OSPA enquiries).

Oil spill dispersants: this use was reported to be declining. It is now only found in three of the 44 dispersants approved for use in UK waters. Total stocks of dispersants in EU are estimated at 5 kt and the estimated use in EU would be < 300 tonnes/year (OSPA enquiries).

Photographic solutions: EGBE seems to be no longer used in photographic solutions.

Rubber, oil industry: EGBE is reported as used in polymer manufacturing.

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

The level of exposure of the environment to a chemical depends on the quantities and compartments of release and subsequent degradation, distribution and accumulation in the environment. This section discusses the behaviour of EGBE and its releases into the environment.

3.1.1 Environmental fate

3.1.1.1 Degradation in the environment

Hydrolysis

No experimental data are available on hydrolysis. However, alcohols and ethers are generally resistant to hydrolysis (Lyman et al., 1990).

Photodegradation

If EGBE is present in ambient air it is expected to exist almost entirely in the vapour phase, based on a vapour pressure of 1 hPa at 20°C, where vapour phase reactions with photochemically produced hydroxyl radicals may be important. A QSAR method is applied for a first estimation of primary transformation rates (SRC, 1988, cited in Environment Canada and Health Canada, 2000): the overall OH rate constant for EGBE has been calculated by the AOP program v1.90 and is estimated to be $2.3.10^{-11}$ cm^3/molecule.second at 25°C. The estimated value corresponds to an atmospheric half-life of about 16.7 hours at an atmospheric concentration of 5.10^5 hydroxyl radicals/cm^3. Atkinson, 1987 has calculated an OH rate constant for EGBE of $1.96.10^{-11}$ cm^3/molecule.second.

Estimated half-lives values for EGBE, based on photooxydation, are also mentioned in Howard et al., 1991, cited in Environment Canada and Health Canada, 2000. They range between 3.3 and 32.8 hours.

Values retained in this study to represent the photodegradation ability of EGBE were taken from a study performed by Aschmann and Atkinson, 1998. EGBE rate constants were measured using a relative method, i.e. measures were made indirectly using a well-known compound, for three different sensitizers: OH, O_3 and NO_3. Test conditions were as follow: light spectrum > 300 nm, EGBE concentration was $4.71.10^{-3}$ mg/L. Measurements revealed that reactions between EGBE and O_3 or NO_3 are of minor or negligible importance as tropospheric loss processes. Indeed, rate constants with NO_3 and O_3 have been measured at 3.10^{-15} cm^3/molecule.second and $< 1.1.10^{-19}$ cm^3/molecule.second respectively. The main loss process for EGBE in ambient air is the reaction with OH radicals. The corresponding measured rate constant was $2.94.10^{-11}$ cm^3/molecule.second.

Using this last data, a pseudo first order rate constant for degradation in air is calculated using TGD (EC, 2003): $K_{deg\text{-}air} = 1.27\ d^{-1}$. This rate constant gives a half-life value of ~ 13 hours for EGBE in air.

Degradation products of EGBE after its reaction with OH radicals have also been measured by Tuazon et al., 1998. An EGBE concentration of $4.7.10^{-3}$ mg/L was used for the experiment performed at 25°C. Several products have been identified: propionaldehyde (CAS 123-38-6), butyraldehyde (CAS 123-72-8), butyl formate (CAS 592-84-7) and 2-hydroxyethyl formate (CAS 628-35-3), 3-hydroxybutyl formate, 2-hydroxybutyl formate and an organic nitrate.

Since EGBE does not absorb ultraviolet radiation within the solar spectrum (> 290 nm), direct photolysis in the atmosphere is not expected to occur (Silverstein and Bassler, 1963, cited in HSDB, 2000).

Biodegradation

The available aerobic biodegradation test results for EGBE are summarised in **Table 3.1**. A number of tests were carried out according to (international) standard test guidelines. Although the current information on several technical aspects is incomplete for some biodegradation tests the total data set available is regarded as being sufficient to draw conclusions on the biodegradation potential of EGBE.

Table 3.1 Biodegradation test results for EGBE

Test #	Type of test	Detection	Result	Day	Method	Conc. Of TS	Conc. Of inoculum	Reference	Validity
1	BOD_5-test	O_2 uptake	73% (BOD/ThOD)	5	APHA n°219, 1971	Unknown	10 mL[2,c]	Bridié et al., 1979a	Valid with restrictions
2	BOD_5-test	O_2 uptake	31% (BOD/ThOD)	5	APHA n°219, 1971	Unknown	10 mL[1,c]	Bridié et al., 1979a	Valid with restrictions
3	BOD_5-test	O_2 uptake	8% (BOD/ThOD)	5	DEV H5 A2	Unknown	River water taken below a factory effluent	Knappe and Popp, 1974	Valid with restrictions
4	BOD_{20}-test	O_2 uptake	75% 70% 64% 29% (BOD/ThOD)	20 15 10 5	APHA n°219, 1971	3, 7 and 10 mg/L**	3 mL[4] (filtered seawater seed)	Price et al., 1974	Valid with restrictions
5	BOD_{20}-test	O_2 uptake	88% 82% 74% 26% (BOD/ThOD)	20 15 10 5	APHA n°219, 1971	3, 7 and 10 mg/L**	3 mL[1,a]	Price et al., 1974	Valid with restrictions
6	In-herent biodeg Test	DOC	95% 92% 8%	8 7 4	Zahn-Wellens test (OECD 302B)	750 mg/L	33 mg/L[1,b]	BASF AG, 1980	Valid with restrictions

Table 3.1 continued overleaf

Table 3.1 continued Biodegradation test results for EGBE

Test #	Type of test	Detection	Result	Day	Method	Conc. Of TS	Conc. Of inoculum	Reference	Validity
7	In-herent biodeg Test	DOC	100%	28	OECD 302B	500 mg/L	Unknown[1,a]	Huels, 1982*	Lack of data
8	In-herent biodeg Test	DOC	100% 63% 22%	5 3 1	OECD 302B	450 mg/L	Unknown[1,a]	Hoechst, 1976	Valid with restrictions
9	Ready biodeg Test	O_2 uptake	88%	28	OECD 301C	100 mg/L	30 mg/L[3,b]	CEFIC, 1993*	Lack of data
10	Ready biodeg Test	DOC	95%	28	OECD 301E	10 mg/L DOC	Unknown[1,a]	Huels, 1982*	Lack of data
11	Ready biodeg Test	O_2 uptake	72% 57% 5% (BOD/ThOD)	20 10 5	Unknown	Unknown	Inoculum from a WWTP	Dow cited in Gonsior, 1990	Valid with restrictions
12	Ready biodeg Test	O_2 uptake	96%	14	OECD 301C	100 mg/L	30 mg/L[1,a]	CITI, 1992	Valid with restrictions
13	Ready biodeg Test	O_2 uptake	75% 70% 47%	28 15 5	OECD 301D	Unknown	Unknown[1]	Waggy et al., 1994	Valid with restrictions

a) Activated (domestic) sewage
b) Activated sludge from (industrial) STP
c) Filtered effluent from STP
1) Non-adapted inoculum
2) Adapted inoculum
3) No information on adaptation or non-adaptation of inoculum available
4) Seed source maintained by adding small amounts of settled raw wastewater
* Original report not available
** Results of the biodegradation test were expressed taking into account all concentrations of EGBE used ("weighted average")

Several tests have been conducted according to APHA (American Public Health Association) method n°219. In tests 1 and 2, the Biological Oxygen Demand was measured after five days whereas in tests 4 and 5, the BOD was measured after 20 days. The four tests were performed with an inoculum constituted by filtered effluent from a STP excepted for test 4 in which the inoculum came from a filtered seawater seed. The domestic source of the inoculum was only specified for test 5. In tests 2, 4 and 5, the inoculum was reported as non-adapted contrarily to that used in test 1. In test number 4, the inoculum source was maintained by adding small amounts of settled raw wastewater. In the four tests, the biodegradation was quantified comparing the measured BOD to the Theoretical Oxygen Demand (ThOD). Concerning the three tests showing the biodegradation of EGBE in the presence of non-adapted inoculum (#2,4,5), they all show similar values after five days even for the one performed with artificial saltwater (#4), respectively 31, 29 and 26%.

Tests number 6 to 8 carried according to the OECD guideline 302B (Zahn-Wellens test) tend to demonstrate the inherent biodegradability of EGBE. Biodegradation percentages were > 70%

after 14 days in tests 6 (95% after 8 days) and 8 (100% degraded after 5 days) and reached 100% after 28 days in test 7. Each test was performed using a non-adapted inoculum from domestic sewage (#7 and 8) or industrial STP (#6).

All ready biodegradation tests (#9-13) have shown a biodegradation > 60%. Excepted test number 9 where the inoculum were adapted and came from an industrial waste water treatment plant, all other tests have used a non-adapted inoculum from a domestic sewage (as specified in the OECD guidelines n°301). Tests 11, 12 and 13 where EGBE biodegradation reaches, respectively, 57% after 10 days, 96% after 14 days and 47% after 5 days (70% after 15 days), tend to confirm that the 10 days time window criterion is fulfilled.

To conclude, according to standard tests on ready biodegradation and further experimental data which confirmed high biodegradation rates, EGBE can be regarded as readily biodegradable.

As no result from biodegradation simulation tests in STP, surface freshwater, surface saltwater and soil is available, the degradation rates have to be estimated based on the "ready biodegradability" classification and the partition behaviour of EGBE according to the method described in the Technical Guidance Document for risk assessment of new and existing chemicals (TGD - EC, 2003). Results of this estimation are presented in **Table 3.2**.

Table 3.2 Estimated biodegradation rate constants for EGBE in WWTP, surface water, soil and sediment

Compartment / medium	Biodegradation rate	Half-life
Activated sludge (WWTP)	K_{STP} = 1 h^{-1}	0.7 hour
Surface water	$K_{freshwater}$ = 4.7.10^{-2} d^{-1}	15 days
Marine water	$K_{saltwater}$ = 1.4.10^{-2} d^{-1}	50 days
Soil*	K_{soil} = 2.3.10^{-2} d^{-1}	30 days
Marine and freshwater sediments **	K_{sed} = 2.3.10^{-3} d^{-1}	300 days

* Biodegradation rates in sediment and soil take account of adsorption to solid matter (K_{oc}=27.3 L/kg, see below)
** Biodegradation rate in sediment takes account of the aerobic fraction of this compartment (0.1)

3.1.1.2 Distribution

Volatilisation

Based on the measured Henry's law constant of 0.08 Pa.m^3/mol at 25°C (Kim et al., 2000), the air-water partitioning coefficient ($K_{air\text{-}water}$) can be calculated. $K_{air\text{-}water}$ of 3.23.10^{-5} indicates that volatilisation of EGBE from surface water and moist soil is expected to be very low.

Adsorption/desorption

Using the log K_{ow} of 0.8 and according to the TGD (EC, 2003) (QSAR for soil and sediment sorption for nonhydrophobic chemicals) a K_{oc} of 27.3 L/kg can be estimated. The solid/water partition coefficients in each compartment can be calculated as supplied in TGD (EC, 2003) (see **Table 3.3**).

Table 3.3 Estimated solids / water partition coefficients

Water / Compartment	OC fraction in solid phase (F_{OCcomp})	Solids / water partition coefficients	Total compartment water partition coefficient
Soil / water	0.02	K_{p_soil} = 0.55 L/kg	$K_{soil\text{-}water}$ = 1.03 m^3/m^3
Sediment / water	0.05	K_{p_sed} = 1.37 L/kg	$K_{sed\text{-}water}$ = 1.49 m^3/m^3
Suspended matter / water	0.1	K_{p_susp} = 2.73 L/kg	$K_{susp\text{-}water}$ = 1.58 m^3/m^3

Distribution in the environment

The following theoretical distribution in the environment has been calculated using the multimedia fugacity model EQC (MacKay level I) and the physico-chemical properties given in Section 1.3.

Table 3.4 Calculated distribution of EGBE in the different compartments of the environment

Compartment	% EGBE
Air	0.24
Water	99.20
Soil	0.55
Sediment	0.01

Regarding these results, the hydrosphere is the preferential target of the substance in the environment.

Distribution in STPs

Based on physical chemical properties discussed in this study (log H = -1.1 and K_{oc} = 27.3 L/kg) as well as the biodegradation rate of 1 h^{-1} in STP, the elimination through biodegradation can be estimated with the model SIMPLETREAT in **Table 3.5**.

Table 3.5 Estimated distribution in a STP (SIMPLETREAT)

Designation	%
Air	$9.54.10^{-2}$
Water	12.6
Sludge	$2.57.10^{-1}$
% degraded	87.0
% removal	87.4

3.1.1.3 Accumulation

No experimental data on bioaccumulation is available. Therefore, BCF-values for fish and earthworm are estimated using the log Kow of 0.8. The estimated BCF-values amount to 0.97 and 1.6 for, respectively, fish and worm. Another calculated value is quoted: a BCF of 2.5 has been estimated by Syracuse program (US EPA and Syracuse Research Corporation, 2001).

In view of these BCF, EGBE is expected to have a low bioaccumulation potential.

3.1.2 Environmental releases

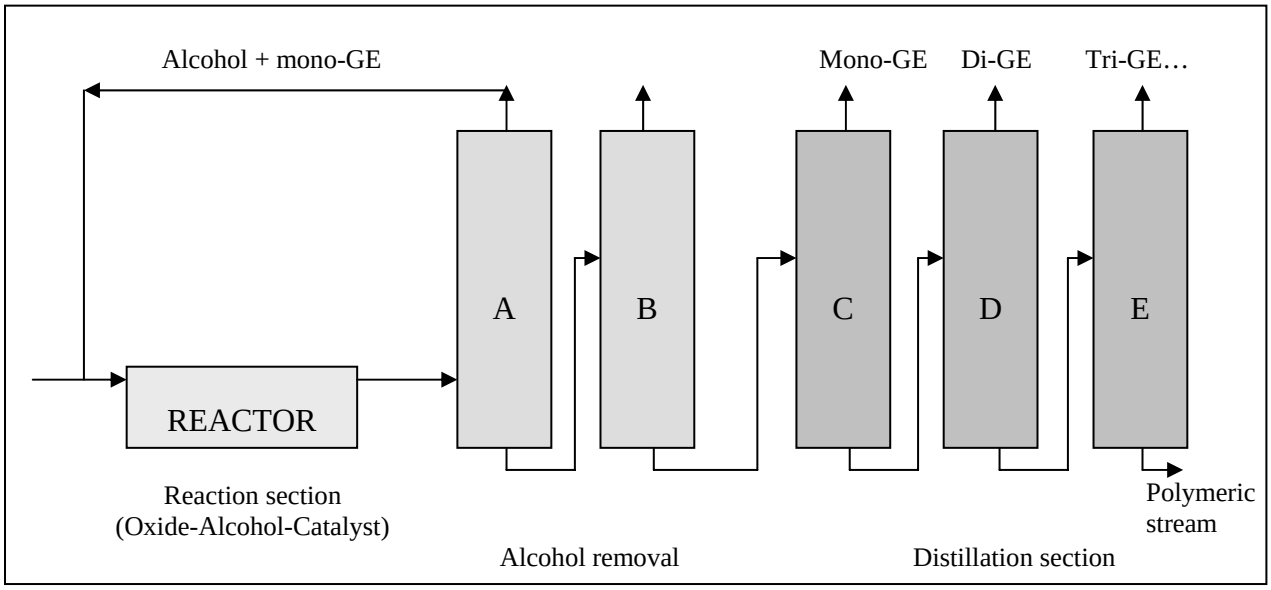
The regional and continental releases include all relevant life cycle stages of EGBE. For production, it is assumed that there is only one production site in the region. The exposure assessment is based on the EU Technical Guidance Documents (TGD - EC, 2003) applying the European Union System for the Evaluation of Substances, EUSES (EC, 2004).

3.1.2.1 Release from production

Ethylene oxide and anhydrous[5] alcohol (n-butanol for the production of EGBE) are added continuously to a reactor containing catalyst that operates at elevated temperature and pressure. To keep the proportion of lower molecular weight products as a high percentage of total production, a large excess of alcohol is used. Unreacted excess alcohol is recycled using an alcohol recovery section (A and B parts in **Figure 3.1**). Some of the EGBE is also recycled as part of the production control process. The remaining reactor stream is drawn off for purification using a train of distillation columns (C, D and E parts in **Figure 3.1**). Relatively pure mono and diethylene glycol alkyl ether streams are drawn off along with a heavy stream (mainly tri but also containing penta and tetraethylene glycol alkyl ether). The remaining low volume polymeric residue stream is either burnt or sold.

During normal operations, the process is entirely closed (due to physical properties of ethylene oxide: boiling point of 10.7°C) with little opportunity for environmental release. Such exposure is only likely during sampling, filling or loading operations along with campaign changes (most of producers revealed periodic campaigns of EGBE synthesis), when the plant is switched to a different glycol ether (different alcohol or using propylene rather than ethylene oxide).

Figure 3.1 Basic schematic of the EGBE production units (OSPA)



[5] Presence of water during the reaction is not expected as this reacts violently with ethylene oxide under the prevailing conditions.

Releases to water

Data of releases to water for EGBE production sites in Europe (see **Table 2.2**) are presented in **Table 3.6** (Note: the site numbers in **Table 3.6** do not directly correspond to the order of companies in **Table 2.2**).

As there are only five EGBE production sites in Europe, the regional production will not be set at 10% of total EGBE production (TGD default) but at the maximum volume produced at one site. The amount remaining corresponds to the continental production. Fraction releases and number of days of emission are taken from Table A1.1 and Table B1.5 (TGD - EC, 2003; Chapter 3 – Appendix I), with Main Category equal to Ib (substances produced in a continuous production process) for EGBE is produced in a continuous production process.

All EGBE producers reported that releases to water enter a sewage treatment plant. During this step, 87.4% of EGBE is expected to be removed by degradation processes. When more precise data were available (EGBE concentration in STP effluents) they were also used.

Relevant data for the calculation of Predicted Environmental Concentrations (PEC) in a STP and local concentrations in the aquatic compartment are presented in **Table 3.6**. When such data was not available the effluent concentration leaving the STP (PEC_{STP}) has been calculated according to **Equation 3.1**. This PEC_{STP} is divided by a dilution factor so as to obtain the local concentration in surface water - $Clocal_{aqua}$ (see **Equation 3.2**).

Equation 3.1 Calculation of PEC_{STP}

$$PEC_{STP} = \frac{Elocal_{water} \times F_{STPwater} \times 10^6}{EFFLUENT_{STP}}$$

$Elocal_{water}$	local release to waste water during episode	[kg/d]
$F_{STPwater}$	fraction of emission directed to water by STP	0.126
$EFFLUENT_{STP}$	effluent discharge rate of STP	[L/day]
PEC_{STP}	EGBE concentration in the STP effluent	[mg/L]

Equation 3.2 Calculation of $Clocal_{aqua}$

$$Clocal_{aqua} = \frac{PEC_{STP}}{DILUTION}$$

PEC_{STP}	concentration of the substance in the STP effluent	[mg/L]
$Clocal_{aqua}$	local concentration in surface water during emission episode	[mg/L]
$DILUTION$	(STP flow + river flow) / STP flow	[-]

The calculation of $Clocal_{aqua}$ is reduced to this equation due to the low adsorption of EGBE on suspended matter (Kp_{susp} = 2.73 L/kg).

Table 3.6 Aquatic emission data from production sites of EGBE in EU

Site #	Emission in water
1	Reference years: 2001 and 2002 Release to wastewater treatment plant: 10* kg/day (300* days of production). Assuming 87.4% removal in STP: 1.3* kg/day in STP effluent. Before discharge to receiving waters, the effluent undergoes a dilution by a factor of 100 (mixing with seawater). Receiving water is the sea. The real dilution for marine environment is unknown. The releases of EGBE occur in a region where the tidal influences are really low. For those particular seas it is proposed in the TGD to use only a dilution factor of 10 instead of 100. Flow of STP = 7,000* m^3/day. PEC_{STP} = 0.18* mg/L and $Clocal_{aqua}$ = $1.8.10^{-4}$* mg/L
2	Reference year: 1996 (production and processing volumes in 2000 were however comparable) Release to water: 1,680* kg/day in wastewater treatment plant influent and 8.7* kg/day in effluent (such values give 99.5*% removal of EGBE in STP). Emissions are calculated as a worst case using 90th percentile of daily measured concentrations in effluent, average STP flow and 365* days/year. Both production and processing releases are included. Flow of STP = 5* m^3/s; 10th percentile of receiving water flow = 734* m^3/s; dilution in receiving water = 150*. PEC_{STP} = 2.10^{-2}* mg/L ; $Clocal_{aqua}$ = $1.3.10^{-4}$* mg/L Assuming 87.4% removal in STP: PEC_{STP} = 0.49 mg/L ; $Clocal_{aqua}$ = $3.3.10^{-3}$ mg/L
3	Reference year: 2000 Release to water: 21.9* kg/day in STP influent (365* d/y). Assuming 87.4% removal in STP: 2.8 kg/day in STP effluent. Flow of STP = 0.83* m^3/s; 10^{th} percentile of receiving water flow = 18.4* m^3/s; dilution in receiving water = 23*. PEC_{STP} = $3.9.10^{-2}$ mg/L ; $Clocal_{aqua}$ = $1.7.10^{-3}$ mg/L
4	Reference year: 2000 Release to water: 7.1* kg/d in STP influent (140* d/y). Assuming 87.4% removal in STP: 0.9 kg/d in STP effluent. Concentration in STP influent = 0.2* mg/L; concentration in STP effluent = 1.10^{-3}* mg/L. PEC_{STP} = $8.9.10^{-2}$ mg/L ; $Clocal_{aqua}$ = $2.2.10^{-3}$ mg/L (assuming a dilution factor of 40 and a STP flow of 10,000 m^3/day – default value for chemical industry, Cf. emission scenario documents, Chapter 7 of the TGD, E.C., 2003)
5	No data but total annual site production for EGBE < 1kt. No process water discharge is reported

* Original data provided by industry or calculated with original data. Other data is calculated using default TGD values

Releases to air

Release data to air for EGBE production sites in EU are presented in **Table 3.7**. Local concentrations in air have been calculated based on information submitted by the producers, except for site 5.

Table 3.7 Atmospheric emissions of EGBE from European producers

Site #	Release to air (kg/d)	$Clocal_{air,ann}$* (mg/m^3)	Year
1	1.9 including emissions from plant and fugitive emissions (300 days/year)	$4.34.10^{-4}$	2002
2	0.6 at production and 5.4 at processing (365 days/year) Total (production + processing) 6	$1.67.10^{-4}$ mg/m³ at production and $1.50.10^{-3}$ at processing Total (production + processing) $1.67.10^{-3}$	1996 (production and processing volumes in 2000 were however comparable)
3	~0.1 (365 days/year)	$2.78.10^{-5}$	2000
4	~0.4 (140 days/year)	$4.27.10^{-5}$	2000
5	No data but total annual site production for EGBE < 1kt.		

* EGBE concentration in air calculated at a 100 m distance from the source (annual average)

Releases to soil

Release data to soil for EGBE production sites in EU are presented in **Table 3.8**. Local concentrations in soil have been calculated based on information submitted by the producers, except for site 5.

Table 3.8 Emissions of EGBE to soil from European producers

Site #	Total deposition flux during emission episode ($\mu g.m^{-2}.j^{-1}$)	Annual average deposition flux ($\mu g.m^{-2}.j^{-1}$)	Local concentration in agricultural soil after 30 days (µg/kg ww)	Local concentration in agricultural soil after 180 days (µg/kg ww)	Local concentration in grassland after 180 days (µg/kg ww)
1	0.76	0.63	33.40	10.30	3.84
2*	2.40	2.40	0.27	0.27	0.50
3*	0.04	0.04	0 (5.10^{-3})	0 (5.10^{-3})	0 (8.10^{-3})
4*	0.16	0.06	0 (7.10^{-3})	0 (7.10^{-3})	0.01
5	No data but total annual site production for EGBE < 1kt.				

* At this production site STP sludge is incinerated

3.1.2.2 Release from formulation, processing and private use

Generic exposure scenarios are used to estimate the releases from formulation, processing and private use of EGBE, as no actual data are available. The scenarios are based on the different use categories of EGBE (see Section 2.2). An overview of the various environmental exposure scenarios for formulation, processing and private use of EGBE is given in **Table 3.9**.

Table 3.9 Environmental exposure scenarios for formulation, processing and private uses of EGBE

Scenario names	Designation	Life cycle step
Paints I^F / Paints I^P ¤	General industrial coatings (water-based)	Formulation/Processing
Paints II^F / Paints II^P ¤	Decorative trade coatings (water-based)	Formulation/Processing
Paints III^F / Paints III^U	Decorative retail coatings (water-based)	Formulation/Private use
Paints IV^F / Paints IV^P ¤	Can coatings	Formulation/Processing

Table 3.9 continued overleaf

Table 3.9 continued Environmental exposure scenarios for formulation, processing and private uses of EGBE

Scenario names	Designation	Life cycle step
Paints V^F / Paints V^P ¤	Coil coatings	Formulation/Processing
Paints VI^F / Paints VI^P ¤	Automotive OEM coatings (water-based)	Formulation/Processing
Paints VII^F / Paints VII^P ¤	Anticorrosion coatings	Formulation/Processing
Paints $VIII^F$ / Paints $VIII^P$ ¤	Automotive OEM coatings	Formulation/Processing
Paints IX^F / Paints IX^P ¤	Wood coatings (water-based)	Formulation/Processing
Detergents I^F / Detergents I^P	Industrial detergents	Formulation/Processing
Detergents II^F / Detergents II^U	Domestic detergents	Formulation/Private use
Intermediates I^P	Intermediate for chemicals synthesis	Processing
Inks I^F / Inks I^P ¤	Printing inks	Formulation/Processing
Oilfield I^F / Oilfield I^P ¤	Oilfield chemicals	Formulation/Processing
Metal I^F / Metal I^P ¤	Metal cleaning	Formulation/Processing
Elec I^P	Electronic industry	Processing
Leather I^P	Leather treatment operations	Processing
Pharm I^P	Pharmaceuticals	Processing
Adhesives I^F / Adhesives I^P	Adhesives	Formulation/Processing
Cosmet I^F / Cosmet I^U	Cosmetics / Personal care	Formulation/Private use
Agri I^F / Agri I^P	Agricultural products	Formulation/Processing
Fire I^F / Fire I^P	Fire foams	Formulation/Processing

P Processing
F Formulation
U Private use
¤ For these end uses there is a possibility that formulation and processing steps take place at a same site. These cases will be treated during risk characterisation.

3.1.2.2.1 Continental and regional releases

The total continental and regional EGBE emissions from formulation, processing and private uses are given in **Table 3.10**. These results come from the calculation program EUSES (EC, 2004) and do not integrate the emissions due to the scenario "Agri I" because of the scenario number limitations of the software. Nevertheless, the calculations have been made for this scenario in a separate file and it appears that the impact of emissions volumes for this scenario on total continental and regional emissions is negligible.

Table 3.10 Total continental and regional EGBE emissions

	Air	Water (total / waste water*)	Soil
Continental	$9.69.10^4$ kg/day	$4.90.10^4$ kg/day/$3.92.10^4$ kg/day	$6.81.10^3$ kg/day
Regional	$1.08.10^4$ kg/day	$5.56.10^3$ kg/day/$4.45.10^3$ kg/day	$7.59.10^2$ kg/day

* It is assumed that 80% of the waste water is treated in a biological STP and the remaining 20% released directly into surface waters

3.1.2.2.2 Local releases: EGBE used in leather finishing operations

For the industrial use of EGBE in leather industry the dedicated scenario "leather processing industry' has been used (for water releases). The emission of EGBE to waste water can be calculated using **Equation 3.3**. Emissions to air and soil were estimated using A and B tables from Appendix 1 of the TGD (EC, 2003).

Equation 3.3 Calculation of waste water emission in leather processing industry

$$E_{local\_water} = Q_{product} \times Q_{chemical} \times (1 - F_{fix})$$

E_{local_water}	daily amount released to waste water	[kg/d]
$Q_{product}$	mass of processed raw hide per day	[15 t/d - default]
$Q_{chemical}$	mass of substance used per mass of raw hide	[3 kg/t[6]– personal communication from the European Leather Industry]
F_{fix}	proportion of the substance chemically concerted or fixed to the raw hide during processing	[0.01- Table A3.6]

3.1.2.2.3 Local releases: EGBE used for metal cleaning operations (processing)

During the various steps of the production process, metal parts must be cleaned of oils, fluxes and grease. Due to the properties of EGBE it is used in cleaning formulations as a wetting agent and dispersant. Typical cleaning formulations contained 4% of this kind of additives (cleaning formulations used for soak). In the calculation of EGBE daily releases to wastewater it will be assumed that the metal parts are cleaned by a static soak process. During this step of the process, losses of substance will be by drag out into the rinse bath and subsequent release of the rinse water. Here, as a worst case, the following assumptions are made:

- it is considered that there is no return of the rinse water ($F_{recycle} = 0$)
- the amount of solution removed from treatment bath due to drag out is taken as 0.3 L/m² as a worst case
- is a same way, the surface area of metal processed is taken at 40 m²/hr

If we consider a fraction of 0.04 for EGBE in the cleaning formulation, then, using a typical concentration of the formulation in the cleaning bath of 25-75 g/L, the concentration of substance in the treatment bath (C_{bath}) can be calculated as follow: 4% · 75 g/L = 3 g/L.

Equation 3.4 is used to calculate the daily emission to wastewater:

[6] This scenario only takes into account the use of EGBE in aqueous degreasing systems for leather. The other use of EGBE in the leather processing can be put in the category of "coating" operations and consequently is already covered by the dedicated scenarios for paints.

Equation 3.4 Daily emission to wastewater during metal cleaning operations

$$Elocal_{process,water} = Q_{dragout,type} \cdot AREA_{process,metal} \cdot C_{bath} \cdot 10^{-3} \cdot (1 - F_{recycle}) \cdot T_{process}$$

$$Elocal_{process,water} = 0.3 \cdot 40 \cdot 3 \cdot 10^{-3} \cdot (1 - 0) \cdot 22 = 0.792 kg / d$$

$Elocal_{process,water}$	emission from process to water per day	[kg/d]
$Q_{dragout,type}$	amount of solution removed from treatment bath per unit area	[0.3 L/m²]
$AREA_{process,metal}$	surface area of metal processed per hour	[40 m²/hr]
C_{bath}	concentration of substance in treatment bath	[3 g/L]
10^{-3}	conversion factor for g to kg	[kg/g]
$F_{recycle}$	fraction of drag out returned to treatment bath	[0]
$T_{process}$	number of hours worked per day	[22, worst case]

In addition, it will be considered that the cleaning bath is disposed of every 4-8 weeks. Assuming a bath capacity of 1,000 L, the amount of substance released will be 3 g/L · 1,000 L = 3 kg – source OECD, 2004. This release will be considered an intermittent emission.

Results are shown in **Table 3.15** to **Table 3.23**.

3.1.2.2.4 Local releases: EGBE used in oilfield chemicals (processing)

a) Estimation of exposure concentrations using the CHARM model

EGBE has a wide range of uses as a solvent in paints and surface coatings, detergents and surface cleaners, inks or dyes. One of the reported end use is as oilfield chemicals used on offshore platforms. Oilfield chemicals are in general brines and brine components used as completion, workover and drill-in base fluids. A generic scenario has been applied in order to cover the use of EGBE in oilfield chemicals onshore (see Section 3.1.2.2.6).

The CHARM model (Thatcher et al., 2004) takes into account four different types of application, since they might be introduced into the environment in a different way. Application groups are:

- production chemicals (with injection chemicals and surfactants as special cases)
- drilling chemicals (water based mud only)
- cementing chemicals (i.e., spacer and mixwater)
- completion and workover chemicals including well squeeze treatments and also pipeline hydrotest and preservation treating chemicals.

Due to the range of usual uses of EGBE, the application group, to which it belongs, seems to be “completion and workover chemicals”.

Two different scenarios are available depending on the use of the chemicals during those operations. A distinction has been made between surface/well cleaning and the other operations (other completion, workover, squeeze treatment and hydrotest chemicals). This is due to the fact that during cleaning operations, as noted in the CHARM manual, discharge is considered to be

100% of the amount used, while for all other operations a fraction of the chemical is retained in the formation by, for example, adsorption to the formation matrix during the operation.

The CHARM model considers that completion and workover chemicals are discharged in batches. Therefore a specific dilution factor has to be applied accounting for the discharge volumes. These releases should be considered as intermittent and compared to the appropriate PNEC for the risk characterisation.

The equations used for the calculation of PEC_{water} for completion, workover squeeze treatment and hydrotest chemicals are presented below.

For surface and well cleaning chemicals, the PEC is calculated using:

$$PEC_{water} = C_{i,cleaning} \times D_{batch,\ cleaning}$$

With $C_{i,\ cleaning}$ = initial concentration of chemical in cleaning fluid (dosage, $mg.L^{-1}$)

$D_{batch,\ cleaning}$ = batchwise dilution factor for cleaning fluids

For other completion, workover, squeeze treatment and hydrotest chemicals, the PEC is calculated using:

$$PEC_{water} = f_r \times C_{i,\ completion} \times D_{batch,\ completion}$$

With f_r = fraction released - chemical

$C_{i,\ completion}$ = initial concentration of chemical in completion and workover including squeeze treatments and hydrotest fluids (dosage, $mg.L^{-1}$)

$D_{batch,\ completion}$ = batchwise dilution factor for completion and workover including squeeze treatments and hydrotest fluids

Default values for the fraction released-chemical (f_r) and the batchwise dilution factor (D_{batch}) are reported in **Table 3.11** as proposed in the CHARM model. For cleaning chemicals, the fraction released should be set at 1 as the model assumes that the chemical is entirely released.

Table 3.11 Default values to be used for hazard assessment of completion and workover chemicals (specified as 'cleaning chemicals', other chemicals', 'squeeze treatments' and 'hydrotest chemicals') (Thatcher et al., 2004).

Parameter	Symbol	Cleaning chemicals	Other chemicals
Fraction released-chemical	f_r	n. r.	0.1
Dilution factor at 500 m	D_{batch}	$7.7\ 10^{-5}$ (1:13,000)	$7.1\ 10^{-5}$ (1:14,000)
Parameter	**Symbol**	**Squeeze treatments**	**Hydrotest chemicals**
Fraction released-chemical	f_r	0.33	1
Dilution factor at 500 m	D_{batch}	$7.1\ 10^{-5}$ (1:14,000)	0.001 (1:1,000)

EGBE has been reported in a manufactured product at concentrations between 60 and 100%. Different dilution ratios are suggested for this product, depending on the type of use. The lowest dilution ratio (1:10) applies to the cleaning of drilling and construction equipment. This ratio will be used as a worst case and gives a concentration of EGBE in cleaning fluids of 100 g/L. With this figure, the different scenarios can be used. Results are presented in **Table 3.12**.

Table 3.12 $PEC_{intermittent}$ for the use of oilfield chemicals containing EGBE on offshore platforms (marine compartment)

	$PEC_{seawater}$ (mg/L)
Surface and well cleaning chemicals	7.7
Squeeze treatments	2.3
Hydrotest chemicals	100

b) Regulations on oilfield chemicals

In June 2000, OSPAR introduced Decision 2000/2 on a Harmonised Mandatory Control System for the Use and Reduction of the Discharge of Offshore Chemicals. At the heart of OSPAR Decision 2000/2 are two Recommendations: Recommendation 2000/4 on a Harmonised Pre-Screening Scheme for Offshore Chemicals, which facilitates the substitution of chemicals with certain characteristics by less hazardous alternatives and Recommendation 2000/5.

Decision 2000/2 and its supporting Recommendations entered into force on 16 January 2001. The Decision requires offshore chemicals to be ranked according to their calculated Hazard Quotients (HQ - ratio of Predicted Environmental Concentration (PEC) to Predicted No Effect Concentration (PNEC). It also obliges authorities to use the CHARM "hazard assessment" module as the primary tool for ranking.

Details on the national regulations put in place in order to comply with OSPAR recommendations have been found for two countries (United Kingdom and Norway). However other countries which have offshore oil and gas installations[7] may have similar regulations in place following harmonisation of such schemes in 1996.

– Norway

The Norwegian State Pollution Control Authority (SFT) regulates the use of drilling fluids/muds through discharge permits. Water based muds are tested under OSPAR formats for bio-accumulation potential and bio-degradability and given a discharge permit if judged to be environmentally friendly. Synthetic muds are similarly evaluated and can be given a discharge permit according to their properties. All oil-based muds are injected or taken to shore for treatment. The discharge of solids containing more than 1% oil, by weight, is forbidden - whether the drilling fluid is water-, oil- or synthetic-based. The regulations also deal explicitly with well testing, workover and cementing.

Norwegian regulations:

1. Norwegian State Pollution Control Authority (SFT). 1998. Requirements for Ecotoxicological Testing and Environmental Assessment of Offshore Chemicals and Drilling Fluids. SFT, Oslo

2. Norwegian State Pollution Control Authority (SFT). 1999a. Environmental Monitoring of Petroleum Activities on the Norwegian Shelf; Guidelines 99:01. SFT, Oslo

3. Norwegian State Pollution Control Authority (SFT). 1999b. Pollution Control Act, 1981. SFT, Oslo)

[7] In some countries there are no offshore oil and gas installations in the OSPAR maritime area under the jurisdiction of the Contracting Parties (i.e. Belgium, Finland, France, Portugal, Sweden).

Norway uses the standard OSPAR "A" and "B" lists for offshore chemicals and requires that discharge of these "shall be reduced as much as possible, e.g., through recycling". Operators are required to ensure the purity of the substances they use, with minimum contamination by other chemicals. Discharge of unused chemicals into the sea is expressly forbidden, even if they are on list A or B and their toxicity is therefore well known. All discharges must have a permit and chemicals not on the lists must be separately tested and notified.

– United Kingdom

The Offshore Chemical Notification Scheme (OCNS) was originally introduced in 1979. In 1993, the UK Government introduced a revised scheme, which classified chemicals using test protocols approved by the Oslo and Paris Commissions (OSPAR). This was modified in detail, in early 1996, to meet the requirements of the OSPAR Harmonised Offshore Chemical Notification Format (HOCNF) which co-ordinates the testing requirements for oilfield chemicals throughout the NE Atlantic sector.

In June 2000, OSPAR introduced Decision 2000/2 on a Harmonised Mandatory Control System for the Use and Reduction of the Discharge of Offshore Chemicals. In the UK this is to be administered under the Offshore Chemical Regulations 2002 (OCR 2002) which came into force on 15th May 2002.

The UK operates the Offshore Chemical Notification Scheme (OCNS[8]). This is operated by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) on behalf of the Department for Trade and Industry (DTI). CEFAS is an Executive Agency of the UK Government's Department for Environment, Food and Rural Affairs (DEFRA). The OCNS requires the registration of all chemicals used in the actual exploration, exploitation and associated offshore processing of petroleum on the UK Continental Shelf. Dossiers on individual products rather than substances have to be notified. The required dossiers must include acute toxicity data on marine species from the three tropic levels as well as other data. Because individual products are notified, detailed information on usage rates and composition are available allowing more precise risk characterisations to be performed using the CHARM model and the products ranked according to the hazard they present. However, for reasons of commercial confidentiality, the toxicity data, usage and composition information is not published.

EGBE has been registered by a number of suppliers to the industry under the name 'EGMBE' (ethylene glycol mono butyl ether), as shown below.

Table 3.13 Examples of EGBE containing products registered in UK

Product type or function	Notifier	Rating
Carrier solvent	Baker Petrolite	Silver
Drilling fluid	Halliburton	Gold
Well stimulant (30% EGBE)	Baker Petrolite	Gold

Gold is the highest possible rating out of 6 and is for products where the PEC:PNEC is less than one. Silver, the next highest, is for substances where the model PEC:PNEC is between 1 and 30. EGBE is also listed as a hydrotest chemical, but under a category of products where CHARM is

[8] http://www.cefas.co.uk/ocns/index.htm

not regarded as suitable for assessing risk of use (not all uses are regarded as suitable for risk characterisation using CHARM). For this reason, it is only rated for hazard properties and falls into group E, the lowest hazard category available. It is also possible that EGBE and EGBE containing products may be registered under trade names (OSPA, personal communication).

Because they use detailed information supplied under confidentiality terms, specific assessments performed under such regulations should be regarded as more precise than those carried out in this assessment. For instance, detailed information is provided by the manufactures on actual usage and emission rates.

3.1.2.2.5 Local releases: EGBE used in oil spill dispersants (marine compartment)

The development of oil spill dispersants has started in the beginning of the 70's with the increase in oil transportation and as a consequence the increase in number of accidents. A summary of dispersants used today is provided in **Table 3.14** and a list of agreed dispersants according to the Bonn Agreement is also available[9].

Table 3.14 Dispersants used today, application methods and dosages (Bonn Agreement, 2001)

Standard name	Generation	Type	Application method	Solvent	Dosage (dispersant / oil)
Conventional dispersants	Second	1	Not diluted on ships	No aromatic hydrocarbons	30 –100%
Concentrated dispersants	Third	2	Diluted on ships	Water-based (e.g., glycol ether)	5 – 15% (concentrated products)
		3	Not diluted on ships or airplanes	Hydrocarbon-based	

It is difficult to know how often dispersants are used against oil spills. A survey of international uses of dispersants was performed by Lindgren et al., 2001. From this survey it seems clear that most of the time mechanical actions are recommended for combating oil spills and that the use of chemicals such as dispersants should be avoided as long as possible. This is particularly the case in the Baltic area, which falls under the HELCOM's recommendations (Helsinki Commission; Denmark, Finland, Germany, Latvia, Lithuania, Poland, Sweden and the Russian Federation).

In specific situations dispersants can nevertheless be used if the national authority approves it. This is also the case in the USA and Canada where mechanical methods are used unless weather prevents the use of these methods.

In Belgium, France, United Kingdom and Germany, dispersants are used, particularly in England where the total number of approved dispersants is high in comparison with other countries. However dispersants in these different countries are applied under specific rules and recommendations and usually after approval by the government or the competent authority in this field. Apart from these theoretical considerations, it seems that dispersants are nevertheless used in a lot of situations because it is often the easiest way to manage an oil spill (personal com. Cedre). Therefore small oil spills resulting from usual practice are most of the time treated with dispersants when of course this is possible.

[9] This list of agreed dispersants will be updated in 2005.

Lessard and Demarco, 2000 report that the rate of usage of dispersants has gone up over the past 30 years and that dispersants have been successfully applied in half as many major spills during the 1990s as during the prior 25 years.

Among all reported dispersants some contain EGBE in non-negligible concentrations. This is the case for example of Wellchem Wellaid 3315 (5% EGBE) and Corexit 9527[10] (38% EGBE). It was not possible to have the composition of all the agreed dispersants on the lists of the Bonn Agreement nevertheless from the two above examples it is clear that some of them contain high amounts of EGBE. Application rates of dispersants depend on the amount of oil to be treated. Usual application rates seem to be around 5 to 15% from information compiled on product fact-sheets (Cedre, 2004).

EGBE sales for oil spill dispersant use have been reported in 2001 (191 tonnes). However, for the following years (2002 and 2003) no sale was reported. This is due to the mode of use of oil spill dispersant that required some stocks to be available. Indeed, oil spill dispersants are only used on occasions when an oil spill occurs in the environment and so may be used locally in relatively large amounts on very infrequent occasions. This should be consequently treated as an intermittent release.

Figures on the number of oil spills detected are available for the Baltic Sea. Von Viebahn, 2002 reports that 250 to 650 oil spills are detected each year. It is also stated in the same report that the number of oil spills in the North Sea is higher, however exact figures are not available. More information on annual oil spills would be useful in order to propose a regional scenario. However due to a lack of time this is not possible at present.

In the same way, specific data (amount of oil released, dispersant used, etc.) are available on different oil spills, major as well as minor spills, in different locations. It is possible from the data available to construct worst case scenarios and assess local PEC, however this implies the development of specific and detailed emission scenario for major and minor spills. This could become difficult considering that a “typical oil spill” cannot be defined. Each presents unique circumstances, depending on location, quantity and type of oil, prevailing weather conditions etc.

It has also to be taken into consideration that the decision whether to use a dispersant is made with the regulatory authorities case-by-case and on a risk benefit basis. It is generally accepted that dispersants create adverse effects but their use is intended to mitigate the even worse potential effects of environmentally hazardous oil products. For this reason, even if detailed composition information was available, it would still not be possible to carry out such a risk benefit analysis within the framework of the Existing Substances risk evaluation process.

3.1.2.2.6 Local releases for other uses

Note: Tables used for local release estimates refer to TGD (EC, 2003), Tables A and B, Chapter 3, Appendix I; other Tables, Chapter 7.

The local release estimates for formulation, processing and use of EGBE are given in **Table 3.15** to **Table 3.20**.

[10] This dispersant has been removed from the UK list of approved dispersant on 30/07/98 because it did not pass the Rocky Shore Test. However existing stock may have been used away from rocky shorelines in appropriate conditions.

Table 3.15 Local releases of EGBE from formulation of paints

Scenario	Paints I_F	Paints II_F and Paints III_F	Paints IV_F	Paints V_F
Main category	Multi-purpose equipment	Multi-purpose equipment	Multi-purpose equipment	Multi-purpose equipment
Total fraction and connected tonnage	0.1411/13,700 tonnes	0.2175 / 21,100 tonnes	0.0756/ 7,340 tonnes	0.0546 / 5,300 tonnes
Regional tonnage	1,370 tonnes	2,110 tonnes	734 tonnes	530 tonnes
Typical max. % of EGBE	3	3	7	7
Number of days / Fraction of main source	(Table B2.3) 300 / 0.6	(Table B2.3) 300 / 0.4	(Table B2.3) 300 / 0.7	(Table B2.3) 300 / 0.8
Release estimates (fraction)	(Table A2.1)	(Table A2.1)	(Table 3.6)	(Table 3.5)
- air	0.005	0.005	0.03	0.01
- waste water	0.003	0.003	0	0
- soil	0.0001	0.0001	0	0
Amount released (kg/d)				
- air	13.7	14.1	51.4	14.1
- waste water	8.2	8.4	0	0
- soil	0.3	0.3	0	0

Table 3.16 Local releases of EGBE from formulation of paints (continue)

Scenario	Paints VI_F	Paints VII_F	Paints $VIII_F$	Paints IX_F
Main category	Multi-purpose equipment	Multi-purpose equipment	Multi-purpose equipment	Multi-purpose equipment
Total fraction and connected tonnage	0.0546 / 5,300 tonnes	0.0218 / 2,120 tonnes	0.0109 / 1,060 tonnes	0.0109 / 1,060 tonnes
Regional tonnage	530 tonnes	212 tonnes	106 tonnes	106 tonnes
Typical max. % of EGBE	8	1	2	2
Number of days / Fraction of main source	(Table B2.3) 300 / 0.8	(Table B2.3) 300 / 0.7	(Table B2.3) 300 / 0.8	(Table B2.3) 300 / 0.8
Release estimates (fraction)	(Table A2.1)	(Table A2.1)	(Table 3.15)	(Table 3.19)
- air	0.005	0.005	0.03	0.02
- waste water	0.02	0.02	0	0.01
- soil	0.0001	0.0001	0	0
Amount released (kg/d)				
- air	7.1	2.5	8.5	5.7
- waste water	28.3	9.9	0	2.8
- soil	0.1	0	0	0

Table 3.17 Local releases of EGBE from processing of paints

Scenario	Paints I_P	Paints II_P and Paints III_U*	Paints IV_P	Paints V_P
Main category	Non dispersive use	Wide dispersive use	Non dispersive use	Non dispersive use
Total fraction and connected tonnage	0.1411 / 13,700 tonnes	0.2175 / 21,100 tonnes	0.0756 / 7,340 tonnes	0.0546 / 5,300 tonnes
Regional tonnage	1,370 tonnes	2,110 tonnes	734 tonnes	530 tonnes
Typical max. % of EGBE	3	3	7	7
Number of days / Fraction of main source	(Table B3.13) 300 / 0.05	(Table B4.4) 300 / 0.002	(Table B3.13) 300 / 0.1	(Table B3.13) 300 / 0.1
Release estimates (fraction)	(Table A3.15 water based)	(Table A4.5 water based)	(Table 3.6)	(Table 3.5)
- air	0.8	0.8	0.94	0.01**
- waste water	0.1	0.15	0	0.01
- soil	0.001	0.01	0.03	0
Amount released (kg/d)				
- air	182.7	11.3	230.0	1.8
- waste water	22.8	2.1	0	1.8
- soil	0.2	0.1	7.3	0

* It has been confirmed that application conditions vary little between professional and retail decorative paints. The only difference is the classification of the persons applying the paints (professionals or private consumers). Consequently, these scenarios have been merged and the default emission factors for consumer products will be used (wide dispersive use: use outside of a factory).

** Assuming treatment of flue gases (98% if no treatment)

Table 3.18 Local releases of EGBE from processing and private use of paints

Scenario	Paints VI_P	Paints VII_P	Paints $VIII_P$	Paints IX_P
Main category	Non dispersive use	Non dispersive use	Non dispersive use	Non dispersive use
Total fraction and connected tonnage	0.0546 / 5,300 tonnes	0.0218 / 2,120 tonnes	0.0109 / 1,060 tonnes	0.0109 / 1,060 tonnes
Regional tonnage	530 tonnes	212 tonnes	106 tonnes	106 tonnes
Typical max. % of EGBE	8	1	2	2
Number of days / Fraction of main source	(Table B3.13) 300 / 0.1	(Table B3.13) 300 / 0.1	(Table B3.13) 300 / 0.1	(Table B3.13) 300 / 0.1
Release estimates (fraction)	(Table A3.15 water based)	(Table A3.15 solvent based)	(Table 3.15)	(Table 3.19)
- air			0.01**	0.96
- waste water	0.01**	0.9	0	0.01
- soil	0.1	0.02	0	0
	0.001	0.001		
Amount released (kg/d)				
- air	1.8	63.6	0.4	33.9
- waste water	17.7	1.4	0	0.4
- soil	0.2	0.1	0	0

** Assuming treatment of flue gases (98% if no treatment for solvent-based coatings and 80% for water-based coatings). Indeed, automotive producers operate VOC emission abatement systems to control air emissions

Table 3.19 Local releases of EGBE from formulation, processing and private use of detergents

Scenario	Detergents I_F	Detergents I_P	Detergents II_F	Detergents II_U
Main category	Multi-purpose equipment	Non dispersive use	Non dispersive use	Wide dispersive use
Total fraction and connected tonnage	0.0850 / 8,250 tonnes	0.0850 / 8,250 tonnes	0.0283 / 2,750 tonnes	0.0283 / 2,750 tonnes
Regional tonnage	825 tonnes	825 tonnes	275 tonnes	275 tonnes
Typical max. % of EGBE	10	10	10	10
Number of days / Fraction of main source	(Table B2.3) 300 / 0.8	(Table B3.3 for waste water only) 200 / 0.002	(Table B2.3) 300 / 1	(Table B4.1 for waste water only) 365 / 0.002
Release estimates (fraction)	(Table A2# - liquid)	(Table A3.5)	(Table A2# - liquid)	(Table A4.1)
- air	0.00002	0.0025	0.00002	0
- waste water	0.0009	0.9	0.0009	0.99
- soil	0.0032	0.05	0.0032	0.01
Amount released (kg/d)				
- air	0	-	0	-
- waste water	2.0	7.4	0.8	1.5
- soil	7.0	-	2.9	-

Table 3.20 Local releases of EGBE from formulation and processing of other uses

Scenario	Intermediates I_P	Inks I_F	Inks I_P	Pharm I_P	Elec I_P
Main category	Dedicated equipment	Multi-purpose equipment	Non dispersive use	Non dispersive use	Non dispersive use
Total fraction and connected tonnage	0.103/10,000 t. 0.0927/9,000 t. (captive use)	0.0257 / 2,500 t.	0.0257 / 2,500 t.	0.0051 / 500 t.	0.062 / 600 t.
Regional tonnage	10,000* t. 9,000* t. (captive use)	250 t.	250 t.	50 t.	60 t.
Typical max. % of EGBE	100 (default value)	20	20	100 (default value)	100 (default value)
Number of days / Fraction of main source	(Table B3.2) 300 / 0.25	(Table B2.3) 300 / 1	(Table B3.10 large companies) 300 / 0.333	(Table B3.2) 32 / 0.65	(Table B3.2) 12 / 0.5
Release estimates (fraction)	(Table A3.3)	(Table A2.1)	(Table A3.12 – printing and allied processes)	(Table A3.3)	(Table A3.4)
- air	0.00001	0.005	0.3	0.001	0.001
- waste water	0.007	0.02	0.001	0.02	0.005
- soil	0.0001	0.0001	0.0001	0.0001	0.01
Amount released (kg/d)					
- air	0.1 / 0.1**	4.2	83.3	1.0	2.5
- waste water	58.3 / 52.5**	16.7	0.3	20.3	12.5
- soil	0.8 / 0.8**	0.1	0	0	25.0

* The regional tonnage is the total tonnage (3 EGBEA producers in the EU)

** Captive use: these amounts will be added to the releases at production

Table 3.21 Local releases of EGBE from formulation and processing of other uses (continued)

Scenario	Leather l_P	Adhesives l_F	Adhesives l_P	Agri l_F	Agri l_P
Main category	Non dispersive use	Multi-purpose equipment	Non dispersive use	Multi-purpose equipment	Wide dispersive use
Total fraction and connected tonnage	0.0051 / 500 t.	0.0051 / 500 t.	0.0051 / 500 t.	0.0021 / 200 t.	0.0021 / 200 t.
Regional tonnage	50 t.	50 t.	50 t.	20 t.	20 t.
Typical max. % of EGBE	5	10	10	100 (default value)	1
Number of days / Fraction of main source	(Table B3.4 except for waste water release) 200 / 0.5	(Table B2.3) 300 / 1	(Table B3.3 only for waste water) 50 / 0.002	(Table B2.2) 300 / 1	(Table B3.1) 2 / 0.001
Release estimates (fraction)	(Table A3.6 except for waste water release)	(Table A2.1)	(Table A3.5)	(Table A2.1)	(Table A3.1)
- air	0	0.005	0.05	0.005	1
- waste water	specific scenario (see Section 3.1.2.2.2)	0.02	0.45	0.02	0
- soil	0.01	0.0001	0.45	0.0001	0
Amount released (kg/d)					
- air	-	0.8	-	0.3	10
- waste water	44.5	3.3	0.9	1.3	0
- soil	1.3	0	-	0	0

Table 3.22 Local releases of EGBE from formulation and processing of other uses (continued)

Scenario	Oilfield l_F	Oilfield l_P	Metal l_F	Metal l_P
Main category	Multi-purpose equipment	Wide dispersive use	Multi-purpose equipment	Non-dispersive use
Total fraction and connected tonnage	0.0257 / 2,500 t.	0.0257 / 2,500 t.	0.0206 / 2,000 t.	0.0206 / 2,000 t.
Regional tonnage	250 t.	250 t.	200 t.	200 t.
Typical max. % of EGBE	100	100	4	4
Number of days / Fraction of main source	(Table B2.3) 300 / 1	(Table B3.3 only for waste water) 50 / 0.002	(Table B2.34) 300 / 0.8	(Table B3.6) 300 / 0.7
Release estimates (fraction)	(Table A2.1)	(Table A3.5)	(Table A2.1)	(Table A3.7)
- air	0.005	0.05	0.01	0.25
- waste water	0.02	0.45	0.02	Specific scenario (see point 3.1.2.2.3)
- soil	0.0001	0.45	0.0001	0.05

Table 3.22 continued overleaf

Table 3.22 continued Local releases of EGBE from formulation and processing of other uses (continued)

Scenario	Oilfield l_F	Oilfield l_P	Metal l_F	Metal l_P
Main category	Multi-purpose equipment	Wide dispersive use	Multi-purpose equipment	Non-dispersive use
Amount released (kg/d)				
- air	4.2	-	5.3	116.7
- waste water	16.7	4.5 (specific scenario for the marine	10.7	0. 8 (3 kg – intermittent release)
- soil	0.1	compartment – see point 3.1.2.2.4)	0.1	
		-		23.3

Table 3.23 Local releases of EGBE from formulation and processing of other uses (continue)

Scenario	Cosmet l_F / Fire l_F	Cosmet l_U	Fire l_P
Main category	Multi-purpose equipment	-	Wide dispersive use
Total fraction and connected tonnage	0.0082 / 800 t.	0.0051 / 500 t.	0.0031 / 300 t.
Regional tonnage	80 t.	50 t.	30 t.
Typical max. % of EGBE	10 (cosmetic products) / 16 (fire foam)	10	0.5
Number of days / Fraction of main source	(Table B2.3) 300 / 1	(Table B4.1 only for waste water) 365 / 0.002	(Table B3.3 only for waste water) 50 / 0.002
Release estimates (fraction)	(Table A2.1)	(Table A4.1)	(Table A3.5)
- air	0.005	0	0.05
- waste water	0.02	0.8	0.45
- soil	0.0001	0.001	0.45
Amount released (kg/d)			
- air	1.3	-	-
- waste water	5.3	0.2	0.5
- soil	0	-	-

3.1.3 Continental and regional predicted environmental concentrations

Continental and regional computations are done by means of multimedia fate models based on the fugacity concept. The standardised continental and regional environments of the TGD (EC, 2003) are used. **Table 3.24** shows the calculated continental and regional PECs for air, water and soil using EUSES (EC, 2004). These results come from the calculation program EUSES (EC, 2004) and do not integrate the emissions due to the scenario "Agri I" because of the scenario number limitations of the software. Nevertheless, the calculations have been made for this scenario in a separate file and it appears that the impact of emissions volumes for this scenario on total continental and regional emissions is negligible.

Table 3.24 Regional PECs in air, water and soil (calculations made by EUSES – SIMPLEBOX model)

Compartment	PEC continental	PEC regional
Air	$1.10.10^{-5}$ mg/m³	$1.15.10^{-4}$ mg/m³
Water	$9.41.10^{-4}$ mg/L	$7.35.10^{-3}$ mg/L
Agricultural soil	$8.44.10^{-5}$ mg/kg (ww)	$8.74.10^{-4}$ mg/kg (ww)
Pore water of agricultural soils	$1.40.10^{-4}$ mg/L	$1.45.10^{-3}$ mg/L
Natural soil	$1.82.10^{-4}$ mg/kg (ww)	$1.89.10^{-3}$ mg/kg (ww)
Industrial soil	$4.39.10^{-3}$ mg/kg (ww)	$4.30.10^{-2}$ mg/kg (ww)
Sediment	$1.16.10^{-3}$ mg/kg (ww)	$9.08.10^{-3}$ mg/kg (ww)
Seawater	$6.09.10^{-7}$ mg/L	$6.12.10^{-4}$ mg/L
Marine sediment	$7.54.10^{-7}$ mg/kg (dw)	$7.57.10^{-4}$ mg/kg (ww)

3.1.4 Local predicted environmental concentrations (PEC_{local})

3.1.4.1 Aquatic compartment

3.1.4.1.1 PEC_{local} for production

PEC_{STP}

At production level, the local Predicted Environmental Concentration for micro-organisms in STP ranges from 0.02 to 0.49 mg/L (these values include emissions for EGBE processing, for on-site EGBEA production) (see **Table 3.6**)

PEC_{aqua}

Emissions at production and processing levels (EGBE captive use for EGBEA synthesis) result in a PEC for surface water ranging from 0.8 to 10.6 µg/L (including the PEC regional for the aquatic compartment). The PEC_{aqua} for the different production sites are presented in **Table 3.25**.

Table 3.25 Local PEC in water at production

Production sites	#1 ($PEClocal_{marine}$)	#2	#3	#4	#5
$PEClocal_{aqua}$ (µg/L)	0.8	10.6	9.0	9.6	-

3.1.4.1.2 Calculation of PEC_{local} for formulation, processing and private use

Concentrations of EGBE in water for formulation, processing and private use in the EU are estimated with a generic scenario which was carried out based on default values (TGD – EC, 2003) because no site specific data were available.

It is assumed that the amounts released to water will enter a sewage treatment plant. During sewage treatment, 87.4% of EGBE is expected to be removed (see **Table 3.5**). The default flow rate of the treatment plant is 2,000 m^3/day.

The effluent concentration leaving the STP (Predicted Environmental Concentration in a STP or PEC_{STP}) is calculated according to **Equation 3.1**. This PEC_{STP} is divided by a dilution factor (10: default value) to obtain the local PEC in surface water (see **Equation 3.2**). The daily amounts released for the generic scenarios are the basis for the calculation of the PECs. **Table 3.26** gives the PECs for the aquatic compartment. $PEC_{seawater}$ have been calculated with EUSES 2.0 (EC, 2004).

Table 3.26 Local PEC_{STP} and PEC_{aqua} for EGBE

Scenario	Daily release to waste water (kg/d)	PEC_{STP} (µg/L)	Local PEC_{aqua} (µg/L)	Total local PEC_{aqua}* (µg/L)	Total local $PEC_{seawater}$ ¤ (µg/L)
Paints I^F / Paints I^P	8.2 / 22.8	519 / 1,430	52 / 144	59 / 151	42 / 114
Paints II^F & III^F	8.4	533	54	61	43
Paints II^P and Paints III^U	2.1	132	13	21	11
Paints IV^F / Paints IV_P	0 / 0	- / -	- / -	7 / 7	1 / 1
Paints V^F / Paints V^P	0 / 1.8	- / 110	- / 11	7 / 18	1 / 9
Paints VI^F / Paints VI^P	28.3 / 17.7	1,780/1,090	179 / 109	186 / 116	142 / 87
Paints VII^F / Paints VII^P	9.9 / 1.4	623 / 87	63 / 9	70 / 16	50 / 8
Paints $VIII^F$ / Paints $VIII^P$	0 / 0	- / -	- / -	7 / 7	1 / 1
Paints IX^F / Paints IX^P	2.8 / 0.4	178 / 22	18 / 3	25 / 10	15 / 2
Detergents I^F / Detergents I^P	2.0 / 7.4	125 / 464	13 / 47	20 / 54	11 / 37
Detergents II^F / Detergents II^U	0.8 / 1.5	52 / 93	6 / 10	13 / 17	5 / 8
Intermediates** I^P ***	58.3	736	19	26	59
Inks I^F / Inks I^P	16.7 / 0.3	1,050 / 17	105 / 2	112 / 9	84 / 2
Pharm I^P ***	20.3	254	7	14	21
Elec I^P	12.5	791	80	87	63
Leather I^P	44.5	2810	281	288	223
Adhesives I^F / Adhesives I^P	3.3 / 0.9	167 / 55	17 / 6	24 / 13	14 / 5
Agri I^F / Agri I^P	1.3 / 0	82 / -	8 / -	15 / 7	7 / 1
Oilfield I^F / Oilfield I^P	16.7 / 4.5	1,050 / 276	105 / 28	112 / 35	84 / 23
Surface and well cleaning	-	-	-	-	7,700
Squeeze treatments	-	-	-	-	2,300
Hydrotest chemicals	-	-	-	-	100,000
Metal I^F / Metal I^P	10.7 / 0.8	673 / 51	68 / 5	75 / 12	54 / 5
Metal intermittent	3	1500	19	26	16

Table 3.26 continued overleaf

Table 3.26 continued Local PEC_{STP} and PEC_{aqua} for EGBE

Scenario	Daily release to waste water (kg/d)	PEC_{STP} (µg/L)	Local PEC_{aqua} (µg/L)	Total local PEC_{aqua}* (µg/L)	Total local $PEC_{seawater}$ ¤ (µg/L)
Cosmet I^F & Fire I^F/Cosmet I^U	5.3 / 0.2	335 / 14	34 / 2	41 / 9	27 / 3
Fire I^P	0.5	32	4	11	2

* Total local PEC_{aqua} = Local PEC_{aqua} + regional PEC_{aqua}
¤ Total local $PEC_{seawater}$ = Local $PEC_{seawater}$ + regional $PEC_{seawater}$
** Captive use not included
*** Dilution factor = 40 and $EFFLUENT_{STP}$ = 10,000 m^3/day (see scenario for IC3 chemicals used in synthesis)
P Processing
F Formulation
U Private use

3.1.4.1.3 Measured levels

Contaminated sites

Ground water sample collections were performed in February 1974 near the "Valley of Drums", Kentucky, USA, a contaminated site. EGBE was detected at a concentration of $2.3.10^{-2}$ mg/L in one sample out of seven (Stonebreaker and Smith, 1980).

In April 1980, EGBE was detected by GC/MS at concentrations of 1.31 and 5.68 mg/L in the water of the Hayashida River, Hyogo prefecture, Japan. In this surface water, EGBE was considered as a contaminant from leather industry effluents. The values represent respectively levels after steams and vacuum distillation (Yasuhara et al., 1981).

EGBE was measured in a municipal waste water treatment plant, in Göteborg, Sweden. Twenty-four hour flow proportional composite were taken in the influent (four samples during 1990 and eight samples in 1991) and the effluent (seven samples during 1989-1991). EGBE was separated by liquid – liquid extraction with dichloromethane and analysed by GC/MS. The range of influent concentrations goes from 0 (non detected) to $2.5.10^{-1}$ mg/L (average influent concentration in 1990 = $8.5.10^{-2}$ mg/L and in 1991 = $2.3.10^{-2}$ mg/L). The range of effluent concentrations was 0 (non detected) – 3.10^{-3} mg/L (Paxeus et al., 1992).

In France, EGBE was detected in the influent of a domestic STP located in northern suburban Paris, in 1991 (November, 26). Analysis was performed with purge and trap – high resolution capillary GC/MS. EGBE was detected at a concentration of $3.5.10^{-2}$ mg/L Nguyen et al., 1994).

Concentrations of EGBE have been measured with GC/MS in aqueous samples from a municipal and an industrial landfill in the United States. They were ranging from < 0.4 (detection limit) to 84 mg/L. The municipal landfill consisted mostly of municipal waste (four wells were analysed). There, EGBE concentrations were detected in wells 3 (18 mg/L) and 4 (84 mg/L) which were located in the landfill. EGBE has not been detected in wells 1 and 2 which were located downgrade from the landfill. Industrial landfill consisted mostly in industrial waste and EGBE concentrations were detected: 2.5 mg/L (well 8), 3 mg/L (wells 5 and 6). In well 7, EGBE has not been detected. Wells 5, 6 and 7 were located on the landfill whereas well 8 is a downgrade interceptor well located between the landfill and the treatment plant (Beihoffer and Ferguson, 1994).

3.1.4.1.4 Comparison between predicted and measured levels

Exposure during formulation, processing and private use

Measured concentrations are only available for some uses. In this section, they are compared to the concentrations predicted by exposure scenarios.

EGBE concentrations measured by Yasuhara et al., 1981 can be put in parallel with values calculated with scenario "Leather I_P". The measured values, ranging between 1.31 and 5.68 mg/L show factors 5 or 20 higher than the corresponding PEC calculated in surface water, 0.288 mg/L. But we must also consider that these data come from a 20 year-old study and measurements were performed in a place where EGBE contamination from the leather industry was expected. Therefore, these results should be considered as a highly worst-case.

EGBE was also measured in municipal waste water effluents. In the study by Paxeus et al., 1992 EGBE concentrations reach 3.10^{-3} mg/L. In a first time this value can be compared to PEC_{STP} presented in **Table 3.26**. These concentrations range between 0.014 and 1.78 mg/L.

3.1.4.2 Terrestrial compartment

Different PECs can be determined to assess the exposure level in terrestrial compartment. The local PEC in soil is calculated according to the following equation:

Equation 3.5 Calculation of $PEClocal_{soil}$

$$PEClocal_{soil} = Clocal_{soil} + PECregional_{natural\_soil}$$

3.1.4.2.1 PEC_{local} for production

The different PEC_{local} in soil at production and processing (EGBE captive use for on-site EGBEA production) levels are presented in **Table 3.27**.

Table 3.27 $PEClocal_{soil}$ at production and *in situ* processing (according to EUSES)

Site #	Local PEC in agricultural soil averaged over 30 days (µg/kg ww)	Local PEC in agricultural soil averaged over 180 days (µg/kg ww)	Local PEC in grassland averaged over 180 days (µg/kg ww)
1	34.25	11.15	4.72
2*	1.15	1.15	1.38
3*	0.88	0.88	0.89
4*	0.89	0.89	0.89
5	No data but total annual site production for EGBE < 1kt.		

* At this production site STP sludge is incinerated

3.1.4.2.2 Calculation of PEC_{local} for formulation, processing and private use

The EUSES models (EC, 2004) take into account both the application of STP sludge on agricultural soil and deposition from air for the calculation of EGBE concentrations in the

terrestrial compartment. **Table 3.298** gives the terrestrial PECs at local scale for the various generic scenarios.

Table 3.28 Local PEC_{soil} for EGBE

Scenario	PEC_{soil} - average concentration in agricultural soil over 30 days (µg/kg ww)	PEC_{soil} - average concentration in agricultural soil over 180 days (µg/kg ww)	PEC_{soil} - average concentration in grassland over 180 days (µg/kg ww)
Paints I^F / Paints I^P	30 / 84	11 / 32	6 / 23
Paints II^F & III^F	31	11	6
Paints II^P and Paints III^U	9	4	3
Paints IV^F / Paints IV^P	4 / 10	4 / 10	5 / 17
Paints V^F / Paints V^P	2 / 8	2 / 4	3 / 3
Paints VI^F / Paints VI^P	96 / 59	31 / 20	13 / 8
Paints VII^F / Paints VII^P	5 / 9	12 / 6	6 / 7
Paints $VIII^F$ / Paints $VIII^P$	2 / 2	2 / 2	2 / 2
Paints IX^F / Paints IX^P	12 / 4	5 / 3	3 / 4
Detergents I^F / Detergents I^P	9 / 26	4 / 9	3 / 5
Detergents II^F / Detergents II^U	5 / 7	3 / 3	2 / 2
Intermediates** I^P	41	14	6
Inks I^F / Inks I^P	57 / 6	19 / 5	8 / 8
Pharm I^P	15	6	3
Elec I^P	44	15	7
Leather I^P	150	47	19
Adhesives I^F / Adhesives I^P	11 / 5	5 / 3	3 / 2
Agri I^F / Agri I^P	7 / 3	3.8 / 2.5	3.0 / 2.5
Oilfield I^F / Oilfield I^P	57 / 17	19 / 6	8 / 4
Metal I^F / Metal I^P	38 / 9	13 / 7	6 / 10
Cosmet I^F & Fire I^F / Cosmet I^U	20 / 3	7 / 2	4 / 2
Fire I^P	4	2	2

* Captive use not included
\- $PECregional_{natural\_soil}$ ~ 1.89 µg/kg (ww)Atmosphere
P Processing
F Formulation
U Private use

3.1.4.3 Atmosphere

3.1.4.3.1 PEC_{local} for production

Emissions of EGBE in air, both at production and processing (on-site EGBEA production), result in an average annual concentration in air ($Clocal_{air,ann}$) of 1.67 µg/m^3 in the worst case (site specific information for site #2). This results in a $PEClocal_{air,ann}$[11] of 1.77 µg/m^3.

3.1.4.3.2 Calculation of PEC_{local} for formulation, processing and private use

The calculated annual average EGBE concentrations in air are presented in **Table 3.29** for the different use patterns.

Table 3.29 Local PEC_{air} for EGBE

Scenario	Concentration during emission (µg/m³)	$C_{local\_air,ann}$ 100m from source (µg/m³)	Annual deposition (µg/m²/d)	$PEC_{local\_air,ann}$* (µg/m³)
Paints I^F / Paints I^P	3.81 / 50.40	3.13 / 41.40	4.51 / 59.60	3.25 / 41.50
Paints II^F & III^F	3.91	3.22	4.63	3.33
Paints II^P and Paints III^U	3.11	2.55	3.67	2.67
Paints IV^F / Paints IV^P	14.30 / 62.00	11.70 / 51.00	16.90 / 73.40	11.90 / 51.10
Paints V^F / Paints V^P	3.93 / 0.49	3.23 / 0.40	4.65 / 0.58	3.35 / 0.51
Paints VI^F / Paints VI^P	1.97 / 0.48	1.62 / 0.39	2.33 / 0.57	1.73 / 0.51
Paints VII^F / Paints VII^P	0.69 / 17.20	0.56 / 14.10	0.81 / 20.40	0.68 / 14.30
Paints VIII^F / Paints VIII^P	2.35 / 0.10	1.93 / 0.08	2.78 / 0.11	2.05 / 0.19
Paints IX^F / Paints IX^P	1.57 / 9.13	1.29 / 7.51	1.86 / 10.80	1.40 / 7.62
Detergents I^F / Detergents I^P	$0.12 / 7.12.10^{-4}$	$0.10 / 3.90.10^{-4}$	$0.14 / 5.62.10^{-4}$	0.22 / 0.12
Detergents II^F / Detergents II^U	$5.09.10^{-3} / 1.43.10^{-4}$	$4.19.10^{-3} / 1.43.10^{-4}$	$6.12.10^{-3} / 2.06.10^{-4}$	0.16 / 0.12
Intermediates** I^P	0.02	0.02	0.03	0.13
Inks I^F / Inks I^P	1.16 / 22.50	1.00 / 18.50	1.37 / 26.60	1.07 / 18.60
Pharm I^P	0.30	0.02	0.04	0.14
Elec I^P	0.70	0.02	0.03	0.14
Leather I^P	$4.29.10^{-3}$	$2.33.10^{-3}$	$3.35.10^{-3}$	0.12
Adhesives I^F / Adhesives I^P	$0.23 / 8.37.10^{-5}$	$0.19 / 1.15.10^{-5}$	$0.27 / 1.65.10^{-5}$	0.27 / 0.12
Agri I^F / Agri I^P	0.09 / 27.6	0.08 / 0.15	0.10 / 0.22	0.23 / 0.30
Oilfield I^F / Oilfield I^P	$1.16 / 4.22.10^{-4}$	$0.95 / 5.78.10^{-5}$	$1.37 / 8.31.10^{-5}$	1.07 / 0.12

Table 3.29 continued overleaf

[11] $PEClocal_{air,ann} = Clocal_{air,ann} + PECregional_{air}$

Table 3.29 continued Local PEC_{air} for EGBE

Scenario	Concentration during emission (µg/m³)	$C_{local\_air,ann}$ 100m from source (µg/m³)	Annual deposition (µg/m²/d)	$PEC_{local\_air,ann}$* (µg/m³)
Metal I^F / Metal I^P	0.93 / 32.4	0.76 / 26.7	1.10 / 38.4	1.33 / 26.80
Cosmet I^F & Fire I^F / Cosmet I^U	0.37 / $2.06.10^{-5}$	0.30 / $2.06.10^{-5}$	0.44 / $2.96.10^{-5}$	0.42 / 0.12
Fire I^P	$4.82.10^{-5}$	$6.60.10^{-6}$	$9.49.10^{-6}$	0.12

* $PEC_{local\_air,ann} = C_{local\_air,ann}$ + regional PEC_{air}
** Captive use not included
P Processing
F Formulation
U Private use

3.1.4.3.3 Measured levels

EGBE was detected at 8 µg/m^3 in one out of six samples by GC/MS. Indoor air samples were collected from 14 homes and a small office in Italy (De Bortoli et al., 1986).

Concentrations of EGBE have been included in the Environmental Protection Agency's volatile organic compounds national ambient database. This database compiles data on indoor air but not industrial space. The average EGBE concentration for 14 samples was 1.05 µg/m^3 (median = 0.37 µg/m^3; lower quartile = 0.15 µg/m^3; upper quartile = 1.77 µg/m^3) (Shah and Singh, 1988).

EGBE was also detected in the clean flue gas of a municipal waste incinerator in Germany, in 1995. It has been measured using the technique of adsorption/thermodesorption and adsorption/elution on selected XAD resins and then detected with FID. EGBE concentration was 0.23 µg/m^3 (Jay and Stieglitz, 1995).

EGBE background levels have been measured by Ciccioli et al., 1993 and Ciccioli, 1996 and Ciccioli et al., 1996, in Northern and Southern Europe, in Himalayas and in Antarctica. In the first study, concentrations ranged between 0.1 and 1.59 µg/m^3 and in the second between 1.26 and 14.85 µg/m^3. Industrial or domestic emissions of EGBE were avoided in both studies. Major sources seemed to be vegetation in the first case and in the second, marine emission are proposed by the authors to explain the measurements.

3.1.4.3.4 Comparison between predicted and measured levels

The highest predicted EGBE concentrations in air take place during industrial uses of paints. For example, $PEC_{local\_air,ann}$ reaches 51 µg/m^3 for the scenario "Paints IV_P". No measured data is available in the neighbourhood of plants using paints at high scale so comparison is not possible. EGBE emissions in air are often a ten order less at formulation than at processing with most of the scenarios used for paints ("Paints I-II/VI-IX") and printing inks. For other uses, emissions to air are reduced: a maximum of ~ 41 µg/m^3 occurred for the processing of general industrial coatings. Some indoor measured concentrations of EGBE are only presented to get an order of magnitude.

EGBE background levels have been measured in appropriate places in order to avoid industrial and domestic sources (by Ciccioli et al., 1993 and Ciccioli, 1996). These studies show a natural

production of EGBE by plants and the second study particularly underlined a possible marine source of EGBE.

3.1.4.4 Secondary poisoning

The bioconcentration factor for fish is very low, so it is not expected that there is a significant exposure for humans or predators via the local environment. Moreover, as EGBE is not classified as Very Toxic (T+), Toxic (T) or Harmful (Xn and R48), it is assumed that there is a low potential for the substance to cause toxic effects if accumulated in higher organisms.

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT ASSESSMENT)

Studies are classified as valid if they fully describe the test material used, the test organism, the test method and conditions and if the endpoint concentration is based upon measured levels. Where only some of these criteria are described the tests may be used with care or considered not valid. Moreover for some studies or results, some data are lacking, i.e. the original paper is not available but only a citation.

3.2.1 Aquatic compartment (incl. sediment)

3.2.1.1 Fish

Acute toxicity

EGBE short term toxicity studies for fish are summarised in **Table 3.30**.

Table 3.30 Short term fish toxicity data for EGBE

Test #	Species	Duration	Endpoint	Result (mg/L)	Method	References	Validity
1	*Poecilia reticulata*	7 days	LC_{50}	983	Not specified	Konemann, 1981	Valid with restrictions
2	*Menidia beryllina**	96 hours	LC_{50}	1,125	Not specified	Dawson et al., 1975	Valid with restrictions
3	*Lebistes reticulatus*	96 hours	LC_0 LC_{50} LC_{100}	1,000 1,400 2,000	Not specified	Knappe and Popp, 1974	Invalid
4	*Oncorhynchus mykiss*	96 hours	LC_{50}	1,474	OECD guideline 203, 1999	INERIS, 1999b	Valid
5	*Lepomis macrochirus*	96 hours	LC_{50}	1,490	Not specified	Dawson et al., 1975	Valid with restrictions
6	*Leuciscus idus melanotus*	48 hours	LC_0 LC_{50} LC_{100}	1,170-1,350 1,395-1,575 1,490-1,620	DIN 38412 part 15	Juhnke and Lüdemann, 1978	Valid with restrictions
7	*Carassius auratus*	24 hours	LC_{50}	1,700	APHA n°231	Bridié et al., 1979b	Valid with restrictions
8	*Leuciscus idus*	48 hours	LC_{50}	1,880	DIN 38412 part 15	Huels, 1982	Invalid
9	*Pimephales promelas*	96 hours	LC_{50}	2,137	US EPA	Dow Chemical Co, 1979	Valid with restrictions
10	*Lepomis macrochirus*	96 hours	LC_{50}	2,950	Not specified	Dow Chemical Co, 1981, cited in Neely, 1984	Invalid

Table 3.30 continued overleaf

Table 3.30 continued Short term fish toxicity data for EGBE

Test #	Species	Duration	Endpoint	Result (mg/L)	Method	References	Validity
-	*Fundulus heteroclitus**	96 hours	NOEC LC_{50}	4 6.7	Stephan, 1974	Biospherics Inc., 1981b	Invalid**
-	*Cyprinodon variegatus**	96 hours	LC_{50}	116	US EPA, 1975	Welchem Inc, 1984	Invalid**
-	*Lepomis macrochirus*	96 hours	LC_{50}	127	Stephan, 1974	CIBA-GEIGY, 1976	Invalid**

* Marine and estuarine species
** Test performed with a preparation containing EGBE

Konemann, 1981 studied the effect of EGBE on a fresh water fish species: *Poecilia reticulata* (#1). This test lasted seven days in semi static conditions. The guideline followed was not recorded and the purity of EGBE was unknown. Groups of eight 2-3 month-old fish were exposed to each concentration tested in following conditions: 22 +/- 1°C, oxygen content > 5 mg/L, water hardness = 25 mg/L ($CaCO_3$). Test solutions were renewed daily and a solvent vehicle was also employed (acetone or 2-propanol). A log EC_{50} was measured at 3.92 µmol/L (983 mg/L).

In test #2, performed by Dawson et al., 1975, a chemically pure grade EGBE was tested on the marine fish *Menidia beryllina*. Test was conducted in synthetic seawater, at 20°C with mild aeration according to the oxygen demand, water hardness was 55 mg/L ($CaCO_3$). For other test conditions see test #5 description. A LC_{50} of 1125 mg/L was obtained after 96 hours.

The toxicity of EGBE has been tested on the fish *Oncorhynchus mykiss* by INERIS, 1999b - test #4, according to the OECD guideline No 203. A LC_{50} of 1,474 mg/L was obtained after 96 hours (95% confidence interval = 1,145-1,840 mg/L). Seven fish were disposed per aquarium, in 15 L test medium with continuous aeration. Water hardness was 250 mg/L ($CaCO_3$), the range of pH during the test was 7.6-8.0 (measured before and after the test), DO between 8.6 and 10.1 and temperature was 16 +/- 1°C. Nominal concentrations tested in duplicates were as follow: 0, 100, 500, 1,000, 2,000 and 4,000 mg/L. The loss of test substance during the test was between 3 and 13%.

The test #5 was also performed by Dawson et al., 1975 on the fish species *Lepomis macrochirus* and a LC_{50} of 1,490 mg/L was obtained after 96 hours. The test was conducted in well water, at 23°C with mild aeration supplied after 24 hours according to the oxygen demand measured daily. A range of pH has been measured between the beginning and the end of the test: 7.6-7.9. Fish have gone through an acclimation period of 14 days and have not been fed 48 hours prior to testing. A minimum of 1 L of test medium was used per gram of fish. No analytical monitoring was performed. Moreover, it is reported that some test substances in the study were diluted with distilled water or a solvent described as having relatively low toxicity but most samples did not required dilution (it can be expected that EGBE did not undergo dilution for its high water solubility).

Test #6 is a static test performed on the fish *Leuciscus idus melanotus* in parallel in two different laboratories by Juhnke and Lüdemann, 1978. A continuous aeration is mentioned (mild bubbling) but no data on other test conditions is available. Different Lethal Concentrations were measured after 48 hours. Results for both laboratories are presented in **Table 3.30**.

Standard methods for the examination of water and wastewater, APHA n°231: static tank acute toxicity test, have been used by Bridié et al., 1979b so as to measure the toxicity of EGBE (#7). A LC_{50} of 1,700 mg/L has been obtained after 24 hours. Ten fish (*Carassius auratus*) were used for each concentration, in 25 L test medium, at 20°C, pH 7 and solutions were aerated during the test period. Concentrations of EGBE in the test medium were determined using a total organic carbon analyser or by extraction and GC both at the beginning and at the end of the test but data are not available.

Test #9, conducted with a fresh water fish, *Pimephales promelas*, by Dow Chemical Co, 1979 gives a LC_{50} of 2,137 mg/L after 96 hours (95% confidence interval = 2,022-2,263 mg/L – moving average method). The method followed came from the US EPA. A static open system was used with raw lake water dechlorinated with activated carbon used in test aquariums containing 10 fish. Other test conditions were as follow: temperature = 12°C, pH between 7.6 and 7.8, water hardness 101 mg/L ($CaCO_3$), three test concentrations (1,550, 2,100, 2,800 mg/L) plus control. A water temperature between 20 and 24°C is recommended for *Pimephales promelas* in the directive 92/CEE, method C1 "acute toxicity to fish". The low water temperature employed in this test is thus a reason to restrict its validity.

Tests number 8 and 10 must be considered as invalid for available data are reduced to minimum and, in some cases, reports are not available but only a quotation. Test number 3 is also classified as invalid for there were both insufficient data on test conditions and an insufficient number of fishes per test concentration (5 fish).

Long-term toxicity

A chronic toxicity test for EGBE is presented in **Table 3.31**.

Table 3.31 Long term fish toxicity data for EGBE

Test #	Species	Duration	Endpoint	Result (mg/L)	Method	References	Validity
1	*Brachydanio rario*	21 days	NOEC	> 100	Project of OECD guideline	INERIS, 2001	Valid

Test conditions for this long term fish toxicity data are detailed in Section 3.2.1.5 of this study (endocrine disruption). Neither mortality nor endocrine disruption effect was observed at any test concentration. Thus a No Observed Effect Concentration can be set at > 100 mg/L, the highest tested concentration.

3.2.1.2 Aquatic invertebrates

Acute toxicity

Short term toxicity tests for EGBE are presented in **Table 3.32**.

Table 3.32 Short term invertebrate toxicity data for EGBE

Test #	Species	Duration	Endpoint	Result (mg/L)	Method	References	Validity
1	*Hydra attenuata* or *H. vulgaris* (cnideria)	72 hours	EC_{50}	540 690	Regeneration potential test Morphological characteristics	Bowden et al., 1995	Valid with restrictions
2	*Crangon crangon* (crustacea)	96 hours	LC_{50}	698	Not specified	Blackman, 1974	Lack of data
3	*Daphnia magna*	48 hours	EC_{50}	835	Not specified	Dow Chemical Co, 1979	Valid with restrictions
4	*Artemia salina** (crustacea)	24 hours	EC_{50}	1,000	Not specified	Price et al., 1974	Valid with restrictions
5	*Daphnia magna*	24 hours	EC_0 EC_{50} EC_{100}	1,140 1,720 2,500	DIN 38412 part 11	Bringmann and Kühn, 1977	Valid with restrictions
6	*Daphnia magna*	24 hours	EC_0 EC_{50} EC_{100}	1,283 1,815 2,500	DIN 38412 part 11	Bringmann and Kuhn, 1982	Valid with restrictions
7	*Daphnia magna*	48 hours	EC_{50}	1,550	OECD guideline n°202	INERIS, 1999a	Valid
8	*Daphnia magna*	24 hours	EC_{50}	5,000	DIN 38412 part 11	Hoechst, 1982	Lack of data
-	*Palaemonetes pugio** *(crustacea)*	96 hours	NOEC EC_{50}	2 5.4	US EPA, 1975	Biospherics Inc., 1981a	Invalid**
-	*Crassostrea virginica* (oyster)	96 hours	LC_{50}	80.5	US EPA, 1975	Welchem Inc, 1984	Invalid**
-	*Panaeus setiferus* (white shrimp)	96 hours	LC_{50}	117	US EPA, 1975	Welchem Inc, 1984	Invalid**

* Marine species
** Test performed with a preparation containing EGBE

Bowden et al., 1995 have conducted their EGBE toxicity test on *Hydra attenuata* – test #1. The aim of this test was to measure the regeneration potential of this organism. Test conditions were as follow: 10 isolated digestive regions per test concentration, five test concentrations (90, 180, 370, 740, 920 mg/L) plus one control, test solutions were renewed every 24 hours. At up to 370 mg/L, the digestive region had regenerated some tentacles and in some cases the basal disc. Only normal wound healing was observed at 740 mg/L while at 920 mg/L the wounds were healed but the region expanded. A 72-hour EC_{50} was derived from these results: 540 mg/L. It should be noticed that there was no analytical monitoring and that the endpoint was unusual. Moreover no clear effect was shown in presence of the test substance. On the other side, another test performed with the same organism by the same team gives a 72-hour EC_{50} of 690 mg/L. Here, the endpoint was based on morphological characteristics. Test conditions were similar and 10 adult polyps were used per test concentrations. Varying degrees of shortened tentacles and body were seen at 740 mg/L and at 920 mg/L polyps were totally contracted.

Blackman, 1974 conducted a EGBE toxicity test on *Crangon crangon* and determined a LC_{50} of 698 mg/L after 96 hours (test #2). 20 animals were used per test concentration and test solutions were renewed every 6, 12 or 24 hours. It should be noticed that the report is poorly documented.

Dow Chemical Co, 1979 has conducted a test on *Daphnia magna*. A 48-hour EC_{50} was obtained: 835 mg/L (95% confidence interval = 640-1,051 mg/L) – test #3. A static open system was used. Aquariums were filled with ten daphnids and raw lake water dechlorinated with activated carbon. Five test concentrations (370, 650, 1,000, 1,500, 2,800 mg/L) plus one control were tested in three replicates. Temperature was around 20°C, pH between 7.6 and 7.8 and water hardness was 101 mg/L ($CaCO_3$).

Test #4 has been performed with a crustacean, *Artemia salina,* by Price et al., 1974 and a 24-hour EC_{50} of 1,000 mg/L has been determined. The test medium was constituted by artificial seawater, at 24.5°C. 30 to 50 shrimp / mL were disposed at the beginning of the test in 100 mL test solution. The following EGBE concentrations were tested: 100, 1,000, 10,000 mg/L. At the end of the test, the number of alive shrimp was determined with a colony counter. The lack of analytical monitoring and the insufficient number of test concentrations have to be deplored.

In test #5, Bringmann and Kühn, 1977 have tested EGBE toxicity on *Daphnia magna*, according to the method DIN 38412 part 11: immobilisation in artificial fresh water. They noticed a 24-hour EC_{50} of 1,720 mg/L. The test was conducted in an open system with 24 hour-old daphnids (wild species) in tap water. Temperature ranged between 20 and 22°C and pH was about 7.6-7.7. Test solution was saturated with oxygen at the beginning of the essay but no mention of O_2 concentration at the end of the test was made. Three replicates were conducted for each concentration.

Test #6 has also been performed by Bringmann and Kuhn, 1982 on 24 hour-old *Daphnia magna* (IRCHA clone) with the same method as above. The EC_{50} after 24 hours was 1,815 mg/L (95% confidence interval = 1,698-1,940 mg/L). Other test conditions were as follow: open system, 20°C, pH = 8 +/- 0.2, water hardness was 250 mg/L ($CaCO_3$) and DO was measured at the end of the study to check that it was within the range tolerated by daphnids (2mg/L). Concentrations were tested in duplicate.

INERIS, 1999a has tested the toxicity of EGBE on *Daphnia magna* according to OECD guideline 202 (test #7). Five daphnids were used per containers and four replicates were performed for each test concentration: 0, 650, 1,000, 1,500, 2,200, 3,350, 5,000 mg/L. Other test conditions were as follow: water hardness 250 mg/L ($CaCO_3$), pH = 7.8 +/- 0.2, DO = 5.3-6.8 mg/L. Less than 10% immobilisation was observed in the control after 48 hours and the analytical monitoring revealed a loss of substance during the test < 20%. A 48-hour EC_{50} of 1,550 mg/L (95% confidence interval = 1,390-1,720 mg/L) was observed.

We can notice another 24-hour EC_{50} result of 5,000 mg/L with *Daphnia* Hoechst, 1982) – test #8. But as no further data is available, this test is classified as invalid.

Long-term toxicity

Chronic toxicity tests for EGBE are presented in **Table 3.33**.

Table 3.33 Long term invertebrate toxicity data for EGBE

Test #	Species	Duration	Endpoint	Result (mg/L)	Method	References	Validity
1	*Brachionus calyciflorus*	48 hours	EC_{10} EC_{20} EC_{50}	7.2 14.3 164	AFNOR NF T 90-377	DeVillers et al., 2002a	Not useable for PNEC derivation
2	*Daphnia magna*	21 days	NOEC EC_{10} EC_{20} EC_{50}	100 134 175 297	OECD guideline n°211	INERIS, 1999d	Valid
3	*Ceriodaphnia dubia*	7 days	EC_{10}	134.9	Draft AFNOR NF T 90 – 376	DeVillers et al., 2003	Valid with restrictions

The chronic toxicity of EGBE on the rotifer *Brachionus calyciflorus* has been determined by DeVillers et al., 2002a according to the French norm AFNOR NF T 90-377 – test #1. Cyst hatching was initiated in moderately hard water, about 20 hours before the beginning of the test (at 25°C under a light intensity of 3,000 lux.). pH was adjusted to 7.5. After 18 hours of incubation, cysts were regularly checked to ensure the removal of test organisms within two hours of hatching. The assay was performed in a 48-well microplate (five concentrations plus one control with eight replicates). Test media consisted in synthetic fresh water solution with a suspension of the green alga *Chlorella vulgaris* as food source. One rotifer was disposed per well (newly hatched rotifer) and the incubation occurred at 25°C in darkness, in a covered microplate. After 48 hours, the total number of rotifers per well was counted and EC_{10}, EC_{20} and EC_{50} were determined by non-linear regression using a log logistic model. The respective confidence intervals for these endpoints were as follow: EC_{10} = 4.5-13.7 mg/L, EC_{20} = 8.8-37.8 mg/L and EC_{50} = 75-486 mg/L. No analytical monitoring was performed during the test.

Several points can be highlighted so as to assess the validity of this test performed on *Brachionus calyciflorus* and showing the highest sensitivity. The pros and cons for the validation of the test are compiled in **Table 3.34**.

Table 3.34 Pros and cons for the validation of the test performed with *Brachionus calyciflorus*

Issue	Pros	Cons
Analytical monitoring	The test compound is considered stable in similar testing conditions, during 48 hours. Under static conditions, the monitoring of test concentrations during test #7 for invertebrates (INERIS, 1999a) has revealed a loss of the substance less than 20%, after 48 hours. In order to prevent evaporation of the solvent or the test substance during the test (EGBE evaporates more slowly than water), the microplate was covered. Indeed, the French norm recommends that the incubation is carried out in a water saturated atmosphere in order to avoid losses of test solution by evaporation; for example, the microplate can be arranged in a closed dish at the bottom of which a film of water has been previously deposited. Furthermore, an interlaboratory test has been successfully performed with copper sulphate and no evaporation problem has been encountered.	No analytical monitoring has been performed during the test. Consequently, neither a loss of test substance nor a loss of solvent (water) during the test duration could not have been followed.

Table 3.34 continued overleaf

Table 3.34 continued Pros and cons for the validation of the test performed with *Brachionus calyciflorus*

Issue	Pros	Cons
Concentration / effect relationship	Glycol ethers often show non-conventional concentration/effect relationships. Such non-conventional curves have already been reported for *Xenopus* exposed to 2-methoxyethanol (Daston et al., 1991) or for *Hydra attenuata* exposed to EGBE (Bowden et al., 1995). DeVillers et al., 2002b has also reported the same difficulty in finding clear relationships between the tested concentrations and the endpoints studied for their tests with EGME/EGMA performed on algae, rotifers, molluscs, daphnids and fish (some of these tests where repeated two or more times so as to ensure that they reached their respective conditions of acceptance). The authors also report that similar conclusions can be drawn from an analysis of published papers in which the ranges of tested concentrations of EGME are given. These tests were performed with *Pimephales promelas* and *Drosophila melanogaster* (Daston et al., 1991), *D. melanogaster* again in Lynch and Toraason, 1996.	The test results show a non-conventional concentration/effect relationship (a factor of 23 can be calculated between EC50 and EC10). The studies from Daston et al., 1991 and Bowden et al., 1995 also show a conventional response for methoxyethanol with *Hydra* and, whilst the EGBE response is flat, it is still of conventional shape, which is not the case with the *Brachionus*. Even if there are indications that the non conventional dose-response curves may be a general reaction to glycol ethers, no explanation has been found to this phenomenon.
Normalisation	The test has been carried out following a French norm (NF T 90-377). An ISO norm is also in preparation.	The methodology used for the test with rotifers is not as well normalised as the one for standard tests usually used for effect assessments of chemicals.
Higher sensitivity		For EGBE, the *Brachionus* test shows the highest sensitivity among all species tested. A ratio of 18.6 can be calculated between the ECs10 for *Daphnia magna* and *Brachionus* indicating a greater sensitivity of *Brachionus* compared to *Daphnia magna*. This is not in accordance with the trend generally observed when sensitivities of both species are compared, giving a ration of 2 between *Brachionus* and *Daphnia* indicating that *Daphnia* would be twice as sensitive as *Brachionus* (RIVM, 2004).
Oxygenation of the test media	*Daphnia* 48-hour test can be taken for comparison with the 48-hour *Brachionus* test. In an acute daphnids test (INERIS, 1999a), the measured dissolved oxygen concentration at the end of the test was between 5.3 and 6.8 mg/L. It can be expected that a similar DO level was maintained during the rotifer test. Although test solution volumes are lower in the rotifer test, both tests show similar conditions and the same duration	Since no aeration took place and the test wells were covered during the test phase, it should have been reported how appropriate oxygen concentration was maintained during the whole test phase. This was not done. In fact, because EGBE is readily biodegradable, oxygen depletion is possible, which would mean that the low NOECs of the *Brachionus calyciflorus* studies were not caused by the toxic properties of EGBE. This may not occur to a significant degree over the timescale of the study but, with no oxygen data, it is not possible to be certain.

Considering all the elements from **Table 3.34** no clear reason can be found to fully invalidate the test. However, considering all the elements highlighted that have triggered off some concerns for the validation of this test, this study will be excluded from the PNEC derivation. This decision is also supported by the availability of standard toxicity test results such as the one performed with *Daphnia magna* (test #2).

Test #2 performed by INERIS, 1999d on *Daphnia magna* according to OECD guideline No 211, revealed a 21-day NOEC of 100 mg/L. Five concentrations (0.1, 1, 10, 100 and 500 mg/L + control) were tested and solutions were renewed three times a week. Other test conditions were as follows: pH = 7.5-7.8, temperature = 20 +/- 2°C, DO ranged between 6.9 and 8.2 mg/L and water hardness was 220 mg/L ($CaCO_3$). A partial analytical monitoring has been performed, i.e. only for concentrations of 100 and 500 mg/L, and loss of substance over test duration was ranging between 3 and 14%. The confidence intervals for the endpoints measured were as follow: EC_{10} = 110-140 mg/L, EC_{20} = 150-180 mg/L and EC_{50} = 282-300 mg/L.

DeVillers et al., 2003 – test #3 - have tested the toxicity of EGBE on *Ceriodaphnia dubia* according to the draft of the method AFNOR NF T90-376 (equivalent to OECD guideline No 211). An EC_{10} of 134.9 mg/L has been determined after seven days. One daphnid was disposed per container, in ten replicates. The parental mortality and the number of offspring per living parent were used as endpoints. Other test conditions are shown here: eight concentrations plus control were tested at a temperature of 23 +/- 1°C and pH = 8-9, DO = 8.1-8.3 mg/L and water hardness was 200 +/- 40 mg/L ($CaCO_3$). There was no analytical monitoring because preliminary studies showed that EGBE was stable at least during 24 hours, i.e. between two renewals. Furthermore, the dose – response curve was not clear and the 95% confidence interval for the EC_{10} ranged between 25.3 and 147.4 mg/L. A NOEC (55.6 mg/L) and an EC_{50} (138.2 mg/L) have also been calculated in another report (INERIS, 1999c) but methods used for calculation tend to give a preference to the EC_{10} value.

3.2.1.3 Algae

Toxicity tests for EGBE are summarised in **Table 3.35**.

Table 3.35 Algae toxicity data for EGBE

Test #	Species	Duration	Endpoint	Result (mg/L)	Method	References	Validity
1	*Microcystis aeruginosa*	8 days	EC_3 (biomass)	35	Cell multiplication inhibition test	Bringmann and Kühn, 1976	Not useable for PNEC derivation
2	*Pseudokirchneriella subcapitata*	72 hours	NOEC	286	OECD guideline n°201	INERIS, 1999e	Valid
3	*Selenastrum capricornutum*	7 days	NOEC LOEC EC_{50}	125 250 >1,000	US EPA 600/9-78-018	Dill and Milazzo, 1988	Not useable for PNEC derivation
4	*Scenedesmus quadricauda*	8 days	EC_3 (biomass)	900	Cell multiplication inhibition test	Bringmann and Kühn, 1978	Not useable for PNEC derivation

Note: in some tests performed by Bringmann, TGK (Toxische GrenzKonzentration) values were established. As the percentage effect at the TGK is 3 or 5% (EC3 or EC5) these values are regarded as NOEC values.

Bringmann and Kühn, 1976 have performed a EGBE toxicity test on the algae *Microcystis aeruginosa* – test #1. The test occurred in a closed static system and 14 concentrations were tested in duplicates (dilution with a pace of two from a stock solution). Agitation took place once a day and the test was conducted at 27°C in a neutral pH. The biomass was measured by spectrophotometry at 578 nm and an EC_3 of 35 mg/L was determined after eight days. This EC_3 can be considered as a NOEC. It should be noted that several drawbacks are associated with this

study. Indeed, the test should have been performed during the exponential growth phase of algal cells. However, algal cells could not have been maintained in this phase within the duration of the study (7 days). There is also a deviation from more standard test results when converting the endpoint measured in the study (EC_3) to a NOEC or EC_{10}. Finally, the result obtained in this study is based on biomass and is comparable with test #2 and #3 results for biomass measurements too. Nevertheless, growth rate measurement is usually preferred when assessing the toxicity towards algae. Therefore, this study will not be used for the PNEC calculation.

A NOEC of 286 mg/L was determined in test #2 (INERIS, 1999e) based on growth rate determination. This test was performed according to the OECD guideline No 201. Test conditions were as follow: 23 +/- 2°C, pH between 7.6 and 8.3, cell concentration = 10,000 cells/mL, illumination was set at 7,000 lux. and test medium was subject to agitation. Nominal concentrations are presented hereafter: 0, 88, 159, 286, 514, 926, 1,667 and 3,000 mg/L. There was an analytical monitoring and loss of substance over test duration ranged between 2 and 11%. Other endpoints have been measured during this test. A 72-hour NOEC of 88 mg/L, an EC_{10} of 308 mg/L and an EC_{50} of 911 mg/L were determined as far as biomass is concerned.

Test #3 has been performed by Dill and Milazzo, 1988 on the alga *Selenastrum capricornutum*. A 7-day NOEC of 125 mg/L has been determined according to a method from the US EPA: the *Selenastrum capricornutum* Printz algal assay EPA-600/9-78-018. Test conditions were as follow: pH was measured before and after the test and ranged between 7.4 and 7.8, temperature = 24 +/- 2°C, initial cell concentration = 10,000 cells/mL, illumination was set at 4,304 +/- 403 lux. Five concentrations (63, 125, 250, 500 and 1,000 mg/L) plus one control were tested in three replicates. Endpoints taken into account were the number of cells / mL and the total cell volume; two counts per replicate were performed with an electronic counter. It can be noticed that the highest concentration tested did not inhibit 50% growth. The comments made on test #1 for its validation apply also for this test.

Bringmann and Kühn, 1978 examined, in test #4, the toxicity of EGBE on *Scenedesmus quadricauda*. An EC_3 of 900 mg/L (equivalent to a NOEC) was determined according to biomass measurement by spectrometry at 578 nm. Test conditions are shown hereafter: closed static system, neutral pH, temperature = 27°C, agitation once a day. 14 concentrations were tested in duplicate but no analytical monitoring took place. The comments made on test #1 for its validation apply also for this test.

3.2.1.4 Micro-organisms

EGBE toxicity studies with micro-organisms are presented in **Table 3.36**.

Table 3.36 Micro-organisms toxicity data for EGBE

Test #	Species	Duration	Endpoint	Result (mg/L)	Method	References	Validity
1	*Entosiphon sulcatum** (protozoa)	72 hours	EC_5	91	Cell multiplication inhibition test	Bringmann, 1978	Valid with restrictions
2	*Uronema parduzci* (protozoa)	20 hours	EC_5	463	Cell multiplication inhibition test	Bringmann and Kühn, 1980	Valid with restrictions
3	*Pseudomonas putida* (bacteria)	16 hours	EC_3	700	Cell multiplication inhibition test	Bringmann and Kühn, 1976	Valid with restrictions
4	*Chilomonas paramecium** (protozoa)	48 hours	EC_5	911	Cell multiplication inhibition test	Bringmann et al., 1980	Valid with restrictions

* Unciliated

The toxicity of EGBE on protozoa has been evaluated in three tests (#1,2,4). To assess the toxicity of a chemical on micro-organisms tests conducted on ciliated protozoa are accepted but only one micro-organism is matching this characteristic: *Uronema parduzci* (#2), the others belong to unciliated protozoa. The cell multiplication inhibition was measured with an electronic cell counter. Tests were performed in a closed static system, at 25°C and neutral pH and 14 concentrations were tested in duplicate.

A test performed with the bacteria *Pseudomonas putida* (Bringmann and Kühn, 1976) is also quoted – test #3. The cell multiplication inhibition was retained as an endpoint (biomass measurement via extinction at 436 nm) and the test was performed in a closed static system during 16 hours, at 25°C and neutral pH. A range of 14 concentrations was tested in duplicate.

3.2.1.5 Endocrine disruption

A test has been conducted so as to identify a potential endocrine disrupting effect due to EGBE: INERIS, 2001. This study was performed according to an OECD guideline project based on the OECD guideline n°204. Fish (*Brachydanio rerio*) have been exposed during 21 days at three EGBE concentrations: 1, 10, 100 mg/L plus one control. No mortality was observed at any test concentration. Moreover the substance did not induce a higher vitellogenin synthesis (lower quantities than in the control were actually measured). Although a lower gonado-somatic index[12] was observed at the lowest concentration this was not the case for higher ones. After an exposure to the positive control substance (oestradiol, an oestrogenic chemical) the brachydanio female presented genito-urinary buds which can normally only be found on the male. The percentage of apparition of genito-urinary buds was less than 10% in all treatment groups compared to 40% in the group of fish in contact with oestradiol.

To conclude, EGBE has no potential for endocrine disruption.

[12] Ratio of weight of gonads compared to weight of total organism.

3.2.1.6 PNEC for the aquatic compartment

Three long term test results from three species representing three trophic levels will be used to derive the $PNEC_{aqua}$ for EGBE. These tests are gathered in **Table 3.37**.

Table 3.37 Toxicity tests retained for the derivation of $PNEC_{aqua}$

Species	Duration	Endpoint	Result (mg/L)	Reference	Lowest short term toxicity result for the same trophic level
Fish: *Brachydanio rerio*	21 days	NOEC	> 100	INERIS, 2001	*Poecilia reticulata* LC_{50} after 7 days = 983 mg/L (Konemann, 1981).
Invertebrates: *Daphnia magna*	21 days	NOEC	100	DeVillers et al., 2002a	*Hydra attenuata* EC_{50} after 72 hours =540 mg/L (Bowden et al., 1995) *Daphnia magna* EC_{50} after 48 hours = 835 mg/L (Dow Chemical Co, 1979)
Algae: *Pseudokirchneriella subcapitata*	72 hours	NOEC	286	INERIS, 1999e	*Pseudokirchneriella subcapitata* EC_{50} after 72 hours = 911 mg/L (INERIS, 1999e)

An assessment factor of 10 is applied to the lowest test result in order to derive the $PNEC_{aqua}$:

$$PNEC_{aqua} = 10 \text{ mg/L}$$

To support this assessment, we can add that EGBE is a non-polar narcotic (OECD, 1995) and acts with a non-specific mod of action in organisms. Although a smaller Assessment Factor can be used to derive a PNEC for non polar narcotics, a factor lower than 10 is not recommended by TGD (EC, 2003). Only additional studies, i.e. field data or model ecosystems, could result, after a review on a case by case basis, in a lower assessment factor. Nevertheless, the knowledge of a non-specific mode of action for EGBE seconds the $PNEC_{aqua}$ proposal.

Table 3.38 shows ecotoxicity results calculated from a log K_{ow} of 0.8 and a molar weight of 118.17 g/mol. The equations for non-polar narcotics given in Table 1 of Chapter 4, Part III of TGD (EC, 2003) were used to estimate QSAR ecotoxicity data.

Table 3.38 QSAR ecotoxicity data for EGBE

Species	Enpoint	Result (mg/L)
Fish: *Pimephales promelas*	96-hour LC_{50}	1,006
Fish: *Brachydanio rerio* or *Pimephales promelas*	28, 32-day NOEC	113
Daphnia: *Daphnia magna*	48-hour EC50	983
Daphnia: *Daphnia magna*	16-day NOEC	242
Algae: *Selenastrum capricornutum*	72, 96-hour EC_{50}	1,103

The close relationship between predicted ecotoxicity of EGBE and results from ecotoxicity tests confirms that EGBE does not act with a specific action manner.

3.2.1.6.1 Calculation of the intermittent PNEC for freshwater

For substances subject to intermittent release, long-term effects are not likely to occur. Consequently, the effect assessment for substances with intermittent release is based on the acute toxicity data set. For EGBE, these tests are gathered in **Table 3.39**.

Table 3.39 Toxicity tests retained for the derivation of the intermittent $PNEC_{aqua}$

Species	Duration	Endpoint	Result (mg/L)	Reference
Fish: *Poecilia reticulata*	7 days	LC_{50}	983	Konemann, 1981
Invertebrates: *Hydra attenuata*	72 hours	EC_{50}	540	Bowden et al., 1995
Algae: *Pseudokirchneriella subcapitata*	72 hours	EC_{50}	911	INERIS, 1999e

Usually an assessment factor of 100 applies to the lowest result of toxicity tests performed on three trophic levels. It has been established that EGBE can be classified as non-polar narcotic. This could be a rationale for a lowering of the assessment factor. Consequently: $PNEC_{aqua,inter}$ = 540 / 10 = 54 mg/L.

3.2.1.6.2 Calculation of the PNEC for the seawater compartment

Chronic toxicity data on three freshwater species representing three trophic levels are available. Only acute toxicity data on marine organisms (fish and invertebrates) are available. Nevertheless, the species tested do not show higher sensitivities than freshwater organisms. According to TGD (EC, 2003), both freshwater and seawater species are used to derive the PNEC for seawater. Thus the PNEC for marine organisms is determined from the lowest chronic test result (NOEC (21 days) = 100 mg/L on *Daphnia magna*) to which an assessment factor of 100 is applied as proposed in the TGD. This gives a $PNEC_{saltwater}$ of 1 mg/L.

3.2.1.6.3 Calculation of the intermittent PNEC for seawater

An assessment factor of 1,000 should be applied on the lowest acute toxicity test result in order to determine an intermittent PNEC for seawater. For the same reason explained above, this factor could be lowered. Consequently: $PNEC_{saltwater,inter}$ = 540/100 = 5.4 mg/L.

3.2.1.7 Calculation of a PNEC for the sediment compartment

As no specific data is available for this compartment, the $PNEC_{sed}$ will be calculated from the $PNEC_{aqua}$ using the equilibrium partitioning method.

Equation 3.6 Formula for the calculation of $PNEC_{sed}$ using the equilibrium partitioning approach

$$PNEC_{sed} = \frac{K_{susp-water}}{RHO_{susp}} \times PNEC_{aqua} \times 1000$$

$PNEC_{sed}$	Predicted No Effect Concentration in sediment	[mg/kg, wet weight]
$K_{susp-water}$	partition coefficient suspended matter / water	[~1.58 m^3/m^3]
RHO_{susp}	bulk density of wet suspended matter	[~1150 kg/m^3]
$PNEC_{aqua}$	Predicted No Effect Concentration in water	[10 mg/L]

This results in: $PNEC_{sed}$ = 13.7 mg/kg (ww)

3.2.1.7.1 Calculation of the PNEC for the marine sediment compartment

No test is available on sediment dwelling organisms exposed via sediment. The PNEC for organisms living in marine sediments may provisionally be calculated using the equilibrium partitioning method from the PNEC for the marine aquatic compartment ($PNEC_{saltwater}$).

Thus, the $PNEC_{marine\ sed}$ = 1.4 mg/kg wet weight of marine sediment.

3.2.1.8 PNEC for micro-organisms in STP

Four EGBE toxicity tests on micro-organisms are quoted. Three tests were conducted with protozoa and one with an individual bacteria species. Studies testing ciliated protozoa can be used for the determination of a $PNEC_{micro\text{-}organisms}$. That is why the test conducted on *Uronema parduzci* will be retained for the PNEC determination.

$PNEC_{micro\text{-}organisms}$ = 463 mg/L.

3.2.2 Terrestrial compartment

Since there are no EGBE toxicity data for terrestrial organisms, no $PNEC_{soil}$ can be derived directly. Therefore, this PNEC was estimated from the PNEC for aquatic organisms using the equilibrium partitioning approach.

Equation 3.7 Formula for the calculation of $PNEC_{soil}$ using the equilibrium partitioning approach

$$PNEC_{soil} = \frac{K_{soil-water}}{RHO_{soil}} \times PNEC_{aqua} \times 1000$$

$PNEC_{soil}$	Predicted No Effect Concentration in soil	[mg/kg, wet weight]
$K_{soil\text{-}water}$	partition coefficient soil water	[~1.025 m^3/m^3]
RHO_{soil}	bulk density of wet soil	[~1700 kg/m^3]
$PNEC_{aqua}$	Predicted No Effect Concentration in water	[10 mg/L]

This results in: $PNEC_{soil}$ = 6 mg/kg (ww)

3.2.3 Atmosphere

No data are available in order to correctly assess the effect of EGBE for species living in the environment and exposed via the air compartment. In a first attempt to quantify the risk for this compartment, inhalation toxicity data from the human risk assessment have been reported in this section.

In a repeat dose study with rats exposed by inhalation, a NOAEC value of 25 ppm (121 mg/m^3) has been identified from a sub-chronic study. During these studies, haemolysis was consistently observed and sometimes associated with hepatic effects. Effects on body weight gain, on the forestomach and on the WBC sub-populations (T limphocyte) were also observed. In a separate study a LOAEC of 31 ppm (150 mg/m^3) has been determined for mice and rats. Due to the closeness of the apparent LOAEC and NOAEC, it has been considered prudent to take the more conservative LOAEC of 31 ppm forward for the human health risk characterisation (with appropriate assessment factors). However, as the approach taken for the risk characterisation for the environmental section (atmospheric compartment) should be considered as a first tier, the NOAEC will be retained.

3.2.4 Secondary poisoning

No specific data available.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment (incl. sediment)

STP and surface water (including seawater)

Table 3.40 presents the calculated PEC / PNEC ratios for the aquatic compartment. PECs for STP and surface water appear in Section 3.1.4.1 of this study whereas the corresponding PNECs are determined in Section 3.2.1: 463 mg/L for the $PNEC_{STP}$ and 10 mg/L for $PNEC_{aqua}$ (1 mg/L for $PNEC_{saltwater}$).

Table 3.40 Risk characterisation for micro-organisms in STP and aquatic organisms

Scenario	RCR_{STP}	RCR_{aqua}	$RCR_{seawater}$
Production site #1	0 (3.10^{-5})	n. a.	0 (8.10^{-4})
Production site #2	0 (7.10^{-5})	0.001	n. a.
Production site #3	0 (6.10^{-6})	0.001	n. a.
Production site #4	0 (4.10^{-6})	0.001	n. a.
Paints I^F / Paints I^P	0 (7.10^{-5}) / 0 (2.10^{-4})	0.006 / 0.015	0.042 / 0.114
Paints II^F & III^F	0 (7.10^{-5})	0.006	0.043
Paints II^P and Paints III^U	0 (3.10^{-4})	0.002	0.011
Paints IV^F / Paints IV^P	- / -	0 (7.10^{-4}) / 0 (7.10^{-4})	0.001 / 0.001
Paints V^F / Paints V^P	- / 0 ($1.4.10^{-5}$)	0 (7.10^{-4}) / 0.002	0.001 / 0.009
Paints VI^F / Paints VI^P	0 (3.10^{-4}) / 0 ($1.4.10^{-4}$)	0.019 / 0.012	0.142 / 0.087
Paints VII^F / Paints VII^P	0 (7.10^{-5}) / 0 ($1.4.10^{-5}$)	0.007 / 0.002	0.050 / 0.008
Paints $VIII^F$ / Paints $VIII^P$	- / -	0 (7.10^{-4}) / 0 (7.10^{-4})	0.001 / 0.001
Paints IX^F / Paints IX^P	0 (3.10^{-5}) / 0 (4.10^{-6})	0.003 / 0.001	0.015 / 0.002
Detergents I^F / Detergents I^P	0 (2.10^{-5}) / 0 (7.10^{-5})	0.002 / 0.005	0.011 / 0.037
Detergents II^F / Detergents II^U	0 (7.10^{-6}) / 0 (1.10^{-5})	0.001 / 0.002	0.005 / 0.008
Intermediates** I^P	0 (1.10^{-4})	0.003	0.059
Inks I^F / Inks I^P	0 (1.10^{-4}) / 0 (3.10^{-6})	0.011 / 0 (9.10^{-4})	0.084 / 0.002
Pharm I^P	0 (4.10^{-5})	0.001	0.021
Elec I^P	0 (1.10^{-4})	0.009	0.063
Leather I^P	0 (4.10^{-4})	0.029	0.223
Adhesives I^F / Adhesives I^P	0 (3.10^{-5}) / 0 (7.10^{-6})	0.002 / 0.001	0.014 / 0.005
Agri I^F / Agri I^P	0 (1.10^{-5}) / -	0.002 / 0 (7.10^{-4})	0.007 / 0.001

Table 3.40 continued overleaf

Table 3.40 continued Risk characterisation for micro-organisms in STP and aquatic organisms

Scenario	RCR_{STP}	RCR_{aqua}	$RCR_{seawater}$
Oilfield I^F / Oilfield I^P	0 (1.10^{-4}) / 0 (4.10^{-5})	0.011 / 0.004	0.084 / 0.023
Metal I^F / Metal I^P	0.001 / 0 (1.10^{-4})	0.007 / 0.001	0.054 / 0.005
Metal intermittent	0.003	0 (5.10^{-4})	0 (5.10^{-4})
Cosmet I^F & Fire I^F / Cosmet I^U	0 (5.10^{-5}) / 0 (2.10^{-6})	0.004 / 0 (9.10^{-4})	0.027 / 0.003
Fire I_P	0 (5.10^{-6})	0.001	0.002

* Captive use not included
P Processing
F Formulation
U Private use

For the risk characterisation at production and processing taking place on-site, the worst case based on site-specific data gives a risk characterisation ratio of 0.001 for freshwater and 8.10^{-4} for seawater.

For some end uses, formulation and processing steps can be achieved at a same site. So, in order to characterise the total risk at such sites it is necessary to add the calculated risks for each step.

According to **Table 3.40** no risk is identified for all end uses even when both formulation and processing can be considered at a same site (dimmed lines of **Table 3.40**).

No risk assessment could have been performed concerning the use of EGBE in oil spill dispersants (see Section 3.1.2.2.5). Nevertheless, no conclusion will be drawn concerning this use in the EU risk assessment since it has to be acknowledged that the decision whether to use a dispersant is made with the regulatory authorities case-by-case and on a risk benefit basis. It is generally accepted that dispersants create adverse effects but their use is intended to mitigate the even worse potential effects of environmentally hazardous oil products. For this reason, even if detailed composition information was available, it would still not be possible to carry out such a risk benefit analysis within the framework of the Existing Substances risk evaluation process.

As far as the use of oilfield chemicals is concerned, specific regulations are in place in different EU countries. Because they use detailed information supplied under confidentiality terms, specific assessments performed under these regulations should be regarded as more precise than those carried out in this assessment. For instance, detailed information is provided by the manufactures on actual usage and emission rates. Consequently, in the knowledge that the use of such products is controlled by specific legislation, a **Conclusion (ii)** is appropriate.

EGBE is readily biodegradable and has a low potential for accumulation in biota. Consequently, this substance will not remain in the environment and secondary poisoning is not expected. Based on the risk assessment performed for freshwater and on the lack of specific hazard identified for the marine environment, no risk is expected in the marine compartment.

Sediment (freshwater and marine sediments)

As neither monitoring data on levels of EGBE in sediment nor ecotoxicity data for benthic organisms are available, no risk characterisation is conducted for this compartment. In addition, the partition coefficient between sediment and water for EGBE is low. So it can be assumed that the risk assessment for the sediment is covered by that for surface water (freshwater and seawater).

Conclusions to the risk assessment for the aquatic compartment

Conclusion (ii).

Conclusion (ii) is applied to all levels of the life cycle of EGBE: production, formulation, processing and private use.

3.3.2 Terrestrial compartment

Risk characterisation for the terrestrial compartment has been performed calculating PEC/PNEC ratios. PECs for soil have been estimated in Section 3.1.4.2 and the corresponding PNEC has been determined in Section 3.2.2 (6 mg/kg, wet weight). Results are shown in **Table 3.41**.

Table 3.41 Risk characterisation for terrestrial compartment

Scenario	$RCR_{agricultural\_soil\_over\_30\_days}$	$RCR_{grassland\_over\_180\_days}$
Production site #1	0.006	0 (8.10^{-4})
Production site #2	0 (2.10^{-4})	0 (3.10^{-4})
Production site #3	0 (2.10^{-4})	0 (2.10^{-4})
Production site #4	0 (2.10^{-4})	0 (2.10^{-4})
Paints I^F / Paints I^P	0.005 / 0.014	0 (9.10^{-4}) / 0.004
Paints II^F and III^F	0.005 / 0.013	0 (9.10^{-4}) / 0.003
Paints II^P and Paints III^U	0.002	0 (6.10^{-4})
Paints IV^F / Paints IV^P	0 (5.10^{-4}) / 0.002	0 (8.10^{-4}) / 0.003
Paints V^F / Paints V^P	0 (3.10^{-4}) / 0.001	0 (4.10^{-4}) / 0 (3.10^{-4})
Paints VI^F / Paints VI^P	0.016 / 0.011	0.002 / 0.003
Paints VII^F / Paints VII^P	0.006 / 0.001	0 (9.10^{-4}) / 0.001
Paints $VIII^F$ / Paints $VIII^P$	0 (2.10^{-4}) / 0 (4.10^{-4})	0 (3.10^{-4}) / 0 (6.10^{-4})
Paints IX^F / Paints IX^P	0.002 / 0 (6.10^{-4})	0 (4.10^{-4}) / 0 (6.10^{-4})
Detergents I^F / Detergents I^P	0.001 / 0.004	0 (3.10^{-4}) / 0 (7.10^{-4})
Detergents II^F / Detergents II^U	0 (7.10^{-4}) / 0.001	0 (2.10^{-4}) / 0 (3.10^{-4})
Intermediates** I^P	0.032	0.004
Inks I^F / Inks I^P	0.009 / 0 (9.10^{-4})	0.001 / 0.001
Pharm I^P	0.011	0.001
Elec I^P	0.007	0 (9.10^{-4})
Leather I^P	0.025	0.003
Adhesives I^F / Adhesives I^P	0.002 / 0 (7.10^{-4})	0 (4.10^{-4}) / 0 (2.10^{-4})
Agri I^F / Agri I^P	0.001 / 0 (4.10^{-4})	0 (5.10^{-4}) / 0 (4.10^{-4})
Oilfield I^F / Oilfield I^P	0.009 / 0.003	0.001 / 0 (4.10^{-4})

Table 3.41 continued overleaf

Table 3.41 continued Risk characterisation for terrestrial compartment

Scenario	$RCR_{agricultural\_soil\_over\_30\_days}$	$RCR_{grassland\_over\_180\_days}$
Metal I^F / Metal I^P	0.008 / 0.002	0.001 / 0 (4.10^{-4})
Cosmet I^F and Fire I^F / Cosmet I^U	0.003 / 0 (3.10^{-4})	0 (5.10^{-4}) / 0 (2.10^{-4})
Fire I^P	0 (4.10^{-4})	0 (2.10^{-4})

* Captive use not included
P Processing
F Formulation
U Private use

For the risk characterisation at production and processing taking place on-site it can be noticed that no risk is expected. According to the figures presented in **Table 3.41**, no risk is expected at formulation, processing or private use stages.

For some end uses, formulation and processing steps can be achieved at a same site (see **Table** 3.41). So, in order to characterise the total risk at such sites it is necessary to add the calculated risks for each step. According to **Table 3.41** no risk is identified for all end uses where both formulation and processing are considered.

Conclusions to the risk assessment for the terrestrial compartment:

Conclusion (ii).

Conclusion (ii) is applied to all levels of the life cycle of EGBE: production, formulation, processing and private use.

3.3.3 Atmosphere

No specific effect data are available in order to accurately assess the risk for the atmospheric compartment. However, due to the volatility of EGBE, direct emissions to air should not be overlooked. In a first attempt to quantify the risk for the air compartment, a NOAEC of 121 mg/m^3 will be compared to the PECs calculated for air. This NOAEC has been determined in a study where rats where exposed via inhalation.

The worst $PEC_{local\_air,ann}$ of 51 $\mu g/m^3$ has been calculated for the scenario Paint IV_P (use in can coatings).

Using the methodology described in the risk characterisation for consumers, a minimal margin of safety (MOS) can be calculated as follows:

Interspecies differences	0.1 (based on rationale described in the risk characterisation for workers part to account for relative species sensitivity to critical end point)
Intraspecies differences	10 (default for consumers)
Type of effect	1
Confidence of the database	1
Minimal MOS required	1

The ratio between the threshold retained in the effect assessment and this worst case exposure is about a factor of 2,400. This rough risk characterisation for the air compartment leads to no concern by a sufficiently large margin that a more accurate assessment is not considered necessary.

Conclusions to the risk assessment for atmosphere

Conclusion (ii).

Conclusion (ii) is applied to all levels of the life cycle of EGBE: production, formulation, processing and private use.

3.3.4 Secondary poisoning

Conclusions to the risk assessment for secondary poisoning

Conclusion (ii).

Conclusion (ii) is applied to all levels of the life cycle of EGBE: production, formulation, processing and private use.

4 HUMAN HEALTH

(to be added later).

5 RESULTS

5.1 ENVIRONMENT

Conclusions to the risk assessment for the aquatic compartment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) is applied to all levels of the life cycle of EGBE: production, formulation, processing and private use.

Conclusions to the risk assessment for the terrestrial compartment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) is applied to all levels of the life cycle of EGBE: production, formulation, processing and private use.

Conclusions to the risk assessment for the atmospheric compartment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) is applied to all levels of the life cycle of EGBE: production, formulation, processing and private use.

Conclusions to the risk assessment for secondary poisoning

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) is applied to all levels of the life cycle of EGBE: production, formulation, processing and private use.

5.2 HUMAN HEALTH

(to be added later).

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
BOD	Biochemical Oxygen Demand
bw	body weight / *Bw*, *bw*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CEPE	European Committee for Paints and Inks
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 90 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]

EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
foc	Organic carbon factor (compartment depending)
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 tonnes/annum)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues

Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidising (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
OC	Organic Carbon content
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling

PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst-Case
S phrases	Safety phrases according to Annex IV of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SCHER	Scientific Committee on Health and Envionment Risks
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
ThOD	Theoritical Oxygen Demand
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations

UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
VOC	Volatile Organic Compound
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organisation
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

European Commission
DG Joint Research Centre, Institute of Health and Consumer Protection
European Chemicals Bureau

EUR 22501 EN **European Union Risk Assessment Report**
2-butoxyethanol (EGBE) – Part I – Environment, Volume 68

Editors: S.J. Munn, K. Aschberger, O. Cosgrove, S. Pakalin, A. Paya-Perez, B. Schwarz-Schulz, S. Vegro

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The report provides the comprehensive risk assessment of the substance 2-butoxyethanol (EGBE). It has been prepared by France in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans and the environment, laid down in Commission Regulation (EC) No. 1488/94.

Part I – Environment

The evaluation considers the emissions and the resulting exposure to the environment in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined.

The environmental risk assessment for 2-butoxyethanol (EGBE) concludes that there is at present no concern for the atmosphere, the aquatic ecosystem, the terrestrial ecosystem or for microorganisms in the sewage treatment plant. There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Part II – Human Health

This part of the evaluation considers the emissions and the resulting exposure to human populations in all life cycle steps. The scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

This part of the evaluation will be added later.

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European Commission – Joint Research Centre
Institute for Health and Consumer Protection
European Chemicals Bureau (ECB)

European Union Risk Assessment Report

2-butoxyethanol (EGBE)
Part I – environment

CAS No: 111-76-2 EINECS No: 203-905-0

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European Union Risk Assessment Report

2-BUTOXYETHANOL

Part II – Human Health

CAS No: 111-76-2

EINECS No: 203-905-0

RISK ASSESSMENT

FINAL APPROVED VERSION

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Part II – Human Health

CAS No: 111-76-2

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RISK ASSESSMENT

FINAL APPROVED VERSION

August 2008

France

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Date of Last Literature Search : **2003**
Review of report by MS Technical Experts finalised: **07-2007**
Final report: **2008**

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.
The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.
The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.
If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.
The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.
This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

[1] O.J. No L 084, 05/04/199 p.0001 – 0075

[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011

[3] Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

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0 OVERALL RESULTS OF THE RISK ASSESSMENT[4]

CAS Number: 111-76-2
EINECS Number: 203-905-0
IUPAC Name: 2-butoxyethanol

Human health

Human health (toxicity)

Workers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Consumers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to all scenarios and all toxicological end-points

Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Human health (physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

4 Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

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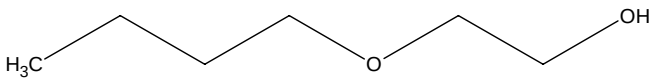
1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 111-76-2
EINECS Number: 203-905-0
IUPAC Name: 2-butoxyethanol
Molecular formula: $C_6H_{14}O_2$
Structural formula: CH_3-CH_2-CH_2-CH_2-O-CH_2-CH_2-OH



Molecular weight: 118.17 $g.mol^{-1}$
Synonyms: EGBE (this synonym will be used in the present study to refer to the chemical 2-butoxytehanol). Other synonyms: butoxyethanol ; 2-butoxy-1-ethanol ; n-butoxyethanol ; butyl ethoxol ; 3-oxa-1-heptanol ; o-butyl ethylene glycol ; butyl glycol ; butyl monoether glycol ; ethylene glycol butyl ether ; 2-BE ; ethylene glycol n-butyl ether ; ethylene glycol monobutyl ether ; glycol butyl ether
Commercial trade names : Dowanol EB ; Butyl Cellosolve ; Butyl Icinol ; Butyl Oxitol ; Eastman EB Solvent

1.2 PURITY/IMPURITIES, ADDITIVES

Purity :the purities quoted in IUCLID were all ≥ 99 % w/w

Impurities :

- as the synthesis reaction produces other glycol ethers, small concentrations of them can be found.
- 2-butoxyethoxyethanol (CAS 112-34-5) ≤ 0.3 % w/w
- 1,2-ethanediol (CAS 107-21-1) ≤ 0.3-0.5 % w/w
- 1-butanol (CAS 71-36-3) ≤ 0.1-0.2 % w/w
- water < 0.1-0.2 % w/w

Additives : 0.008-0.012 % w/w 2,6-bis(1,1-dimethylethyl)-4-methylphenol (CAS 128-37-0) added to prevent the formation of peroxides.

1.3 PHYSICO-CHEMICAL PROPERTIES

The physico-chemical properties are discussed below and summarised in Table 1.1.

Table 1.1.1: Summary of physico-chemical properties

Property	Value
Physical state	Liquid
Melting point	-73.4°C
Boiling point	170.5°C
Relative density	0.9 at 20°C
Vapour pressure	1 hPa at 20°C
Surface tension	26.6 mN/m at 20°C
Water solubility	Miscible (~1.10^6 mg/L)
Partition coefficient n-octanol/water (log value)	0.8
Granulometry	Not applicable
Flash point	63.2°C
Autoflammability	244.5°C
Flammability	Upper limit: 12.7 % (volume) Lower limit: 1.1 % (volume)
Explosive properties	Not explosive
Oxidising properties	No oxidising properties
Viscosity	3.28 mPa.s at 20°C
Henry's constant	0.08 Pa.m^3/mol at 25°C
Conversion factors (101 kPa, 20°C)	1 ppm = 4.9 mg/m^3 1 mg/m^3 = 0.204 ppm

1.3.1 Physical state

EGBE is a neutral, colourless liquid with a very weak pleasant odour (Ullmann, 2000). An absolute perceptible limit of 0.1 ppm (50 % recognition = 0.35 ppm and 100 % recognition = 0.48 ppm) was referred to EGBE (Verschueren, 2001).

1.3.2 Melting point

Several values are reported in literature. The value of -77°C appears in several studies (ECETOC, 1994 ; OECD SIAR, 1997 ; Dow, 2001) and handbooks show values ranging between -74.8 and -70°C (Lewis, 1999 ; Ullmann, 2000 ; Howard, 1989 ; Eastman, 2001 ; BASF, 2002 ; Verschueren, 2001).

The mean of all the values found is retained for this study : -73.4°C.

1.3.3 Boiling point

Most of the values found in literature are in the following range: 168-172°C (Ullmann, 2000). A boiling point of 170.8°C for EGBE can be found (ECETOC, 1994) which is close to those appearing in handbooks (170°C in Kirk-Othmer, 1983 and Verschueren, 2001). Two other handbooks show values between 171 and 172°C (Lewis, 1999 ; Howard, 1989). Boiling point of 171°C was found in technical data sheets or product information reports (BP, 2002b; Dow, 2001).

The mean of all the values above is 170.5°C. This value will be used for the risk evaluation.

1.3.4 Relative density

Several relative density values are available too. Most of them are about 0.9 (Verschueren, 2001 - value measured at 20°C ; Merck, 1996). Technical data sheets or product information reports give values of 0.9011 and 0.902, at 20°C (BP, 2002b; Dow, 2001).

A rounded value of 0.9 will be chosen for this study.

1.3.5 Vapour pressure

Vapour pressures ranging from 0.79 to 1.3 hPa, at 20°C have been reported. Data come from technical data sheets: 0.8 hPa (Shell Chemicals, 2001), 0.89 hPa (Dow, 2001), 1.3 hPa (BP, 2002b). The same range appears in Sax (1986) and other chemical handbooks give almost the same values: 0.89 hPa (Verschueren, 2001), 1 hPa (Ullmann, 2000).

The mean of these values gives a vapour pressure of 1 hPa, at 20°C. It will be used in the risk assessment.

1.3.6 Surface tension

Several values of surface tension appear in technical data sheets for commercial EGBE: Dow Chemical Company (2001) reports a value of 27.4 mN/m at 25°C, Eastman (2001) gives a value of 26.6 mN/m at 20°C and Shell Chemicals (2001) another of 28.9 mN/m. A value of 27.4 mN/m at 25°C is also reported in a handbook (Kirk-Othmer, 1983).

The value reported in Eastman (2001) will be retained for the study: 26.6 mN/m, at 20°C, the temperature recommended in the OECD guideline No 115.

1.3.7 Water solubility

EGBE is often reported as miscible with water in several data sheets and product information reports (Hoechst, 1992 and 1993 ; BP, 2002b). A solubility of about 50000 mg/L is reported by Eckel *et al.* (1996), 63500 mg/L by ASTER (1996). A value of 1.10^6 mg/L has been measured (Riddick *et al.*, 1985 cited in HSDB).

EGBE can be considered as highly miscible in water and the maximum value permitted by EUSES (EC, 1996), 1.10^5 mg/L, is retained for modelling purposes.

1.3.8 Partition coefficient n-octanol/water

Several QSAR calculations of partition coefficient n-octanol/water for EGBE have been performed. They are presented hereafter using an ascending order of logarithmic values: 0.57 calculated with KOWWIN v.1.66. – (US EPA and SRC, 2001) ; 0.81 (Verschueren, 2001), 0.84 (ASTER, 1996).

Partition coefficient measurements have been performed (Tanii and Hashimoto, 1982): 0.77 is the mean value of three determinations. Measurements have been carried out with gas chromatography.

Other measured log K_{ow} can be found in literature: 0.74 (Verschueren, 2001), 0.81 (Staples *et al.*, 1998), 0.83 (Korenman and Dobromyslova, 1975 ; Hansch and Leo, 1985 ; Staples *et al.*, 1998).

Mean of all measured data gives a log K_{ow} of 0.8 that is retained for this study.

1.3.9 Granulometry

Not applicable: the substance is a liquid.

1.3.10 Flash point

Values reported in literature range from 60 (INRS, 1996 and Merck, 1996) to 68°C (BP, 2002b). Both values are measured with closed cup method. Two other values were obtained using this method: 67°C (Shell Chemicals, 2001) and 61°C (Sax, 1986), while methods leading to values of 65°C (Dow Chemical, 2001) and 67°C (Ullmann, 2000) are not mentioned.

The mean of the values measured using a closed cup is retained: 63.2°C.

1.3.11 Autoflammability

Two temperatures of autoflammability have been found in technical data sheets: 244°C (Dow Chemical Company, 2001) and 245°C (BP, 2002b). The mean value of 244.5°C is retained.

1.3.12 Flammability

Limits of flammability are reported by BP (2002b) for EGBE. The upper limit is 12.7 % (volume) and the lower one is 1.1 % (volume).

1.3.13 Explosive properties

Not explosive.

1.3.14 Oxidising properties

No oxidising properties.

1.3.15 Viscosity

The range of viscosity values found in technical data sheets for EGBE goes from 2.9 (at 25°C, Dow, 2001) to 3.3 (Shell Chemicals, 2001) mPa.s. A value of 3.28 mPa.s is also reported by BP (2002b), at 20°C. BP (1998) also reports a range of viscosity values for several concentrations of EGBE in water (between 20 and 100 % volume): 2-6 mPa.s at 20°C. Several values have been found in handbooks: 3.26 mPa.s, at 20°C (Ullmann, 2000), 2.83 mPa.s at 25°C (Prager, 1995) and a catalogue of commercial substances gives a viscosity of 5.83 mPa.s, at 20°C (Merck, 1996).

The most common values are rounded and a viscosity of 3.3 mPa.s (at 20°C) is retained for this study.

1.3.16 Henry's constant

Different QSAR estimation methods calculated with HENRYWIN v.3.10. (US EPA and SRC, 2001) give values of Henry's law constant presented in Table 1.1.2.

Table 1.1.2: Henry's law constant values (at 25°C) in EPI suite (US EPA and SRC, 2001)

Method	Value ($Pa.m^3$ / mole)
Bond method	0.009 9
Group method	0.002 1
Experimental value quoted in EPI suite database	0.162 1
Calculated with VP.M / WSol ratio using EPI suite estimated values (QSAR)	0.116 1

Henry's law constant values have also been calculated using another program (MPBPVP), at 25°C. Depending on the method used, values of 0.01 and 0.002 $Pa.m^3/mol$ are reported (SRC, 1988).

Another study shows a calculated Henry's law constant of 0.551 $Pa.m^3/mol$ at 25°C (ASTER, 1996).

Henry's law constant can also be estimated from the ratio of the vapour pressure to the water solubility using selected values from this study: 100 Pa for vapour pressure and 1.10^6 mg/L for water solubility. Calculation gives a Henry's law constant of 0.012 $Pa.m^3/mol$.

Experimental measurements of Henry's law constant have also been reported (Kim *et al.*, 2000). Two different methods were used to determine Henry's law constant for the equilibrium partitioning of EGBE contained in an aqueous solution and the air. Results of measurements are presented in Table 1.1.3.

Table 1.1.3: Henry's law constant values as measured in Kim *et al.*, 2000

Bag method for equilibrium partitioning	Batch stripping method
T = 20°C ; H = 0.041 $Pa.m^3/mol$	T = 22°C ; H = 0.063 $Pa.m^3/mol$
T = 25°C ; H = 0.081 $Pa.m^3/mol$	T = 23°C ; H = 0.068 $Pa.m^3/mol$
T = 30°C ; H = 0.101 $Pa.m^3/mol$	T = 25°C ; H = 0.082 $Pa.m^3/mol$
	T = 30°C ; H = 0.135 $Pa.m^3/mol$

In the previous study, there is a good correlation between both method used, bag method for equilibrium partitioning and batch stripping method, and particularly at 25°C. Moreover, direct measurement of the Henry's law constant is recommended in TGD (EC, 2003) for water miscible compounds. So a Henry's law constant of 0.08 $Pa.m^3/mol$ will be used.

1.4 CLASSIFICATION

1.4.1 Current classification (28th ATP of directive 67/548/EEC)

Classification: Xn; R 20/21/22

Xi; R 36/38

Labelling: Xn; R 20/21/22, 36/38

S 2 – 36/37 - 46

1.4.2 Proposed classification

Unchanged.

2 GENERAL INFORMATION ON EXPOSURE

3 ENVIRONMENT

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

Humans may be exposed to EGBE at workplace, via consumer products and indirectly via the environment. The highest potential exposure is likely to occur during occupational exposure.

Workers and consumers are primarily exposed via inhalation and dermal routes. EGBE is readily absorbed through the skin including absorption from direct contact with liquid or aerosol form or contact with vapours. Because this compound has a relatively low vapour pressure (0.1 kPa at 20°C), dermal absorption after direct contact with the liquid may be predominant or may contribute significantly to overall exposure.

Environmental monitoring of breathing zone or air concentrations in the work area has been found to be inadequate to assess overall exposures: the total exposure to EGBE should take into account the respiratory uptake of vapours and aerosols and the dermal absorption of EGBE in liquid, vapour and aerosol form. Biological monitoring of the common toxic urinary metabolite, 2-butoxyacetic acid (BAA), is considered necessary for a complete assessment (Angerer *et al.*, 1990 ; Johanson *et al.*, 1988 ; Sohnlein *et al.*, 1993 ; Vincent *et al.*, 1993). Limited data on urinary BAA concentrations are available for occupationally exposed groups of the population. Biomonitoring data in the general population are not available.

Exposure may occur during manufacture and use as intermediate in the chemical and pharmaceutical industries, and during formulation and use of products. EGBE is a solvent used in many industrial activities or consumer applications. The main uses are in paints or surface coatings (solvent-based or water-based), followed by cleaners and printing inks.

In the Swedish product register (KEMI, 2002), 882 products containing EGBE have been identified, of which 58 % are paints or inks, 16 % cleaning agents, 8 % antirust and 8 % polishing agents.

In the Danish product register (Arbejdstilsynet, 2001), 1 204 products containing EGBE have been identified, of which 76 were private household products. The most common uses were paints and varnishes (37 %), cleaning/washing agents (20 %), surface treatment (8 %), corrosion inhibitors (6 %), surface active agents (3 %), adhesives/binding agents (3 %), solvents (2 %).

Other data extracted from the French product register SEPIA (INRS, 2003) showed that 368 products out of the 10 345 products registered between 1997 and 2003 contained EGBE. The main use categories were: paints, varnishes and inks (39 %), cleaning agents (37 %) and products for metallurgical and mechanical sectors, e.g. grease cleaners (14 %).

The distribution of concentration intervals in the main type of products is presented in the tables 4.1 and 4.2.

Table 4.1: Concentration of EGBE in the main use categories in the Danish product register (2001)

Content %	Total	Cleaning agents	Solvents	Paints	Corrosion inhibitors	Surface treatment	Surface active agents	Adhesives
[0-1]	526	51		278	23	46	4	21
]1-5]	276	74	5	72	36	14	4	
]5-10]	176	56		53	8	10	6	6
]10-20]	147	40	8	31	6	27	4	
]20-50]	51	13	6	9		4	8	11
]50-80]	10	4	12				6	
]80-100]	17							

Table 4.2: Concentration of EGBE in the main use categories in the French product register SEPIA (INRS, 2003)

Concentration (%)	Products for metallurgical and mechanical sectors	Paints, varnishes and inks	Cleaning agents
[0-1]	2	36	11
]1-5]	36	41	53
]5-10]	6	41	21
]10-20]	2	9	13
]20-50]		4	5
]50-80]			2
]80-100]			

Other minor uses are reported: adhesives, metal cleaning, electronic industry, cutting oils, agricultural products, fire fighting foams, leather treatment, oil field chemicals and cosmetics (hair dyes) (SIDS, 1996 and OSPA, 2002).

4.1.1.2 Occupational exposure

Definitions and sources

In this document, unless otherwise stated, the term exposure is used to denote external personal exposure as measured or otherwise assessed without taking into account the attenuating effect of any personal protective equipment (PPE) which might have been worn. This definition permits the effects of controls, other than PPE, to be assessed and avoids the considerable uncertainty associated with attempting to precisely quantify the attenuation of exposure brought about by the proper use of PPE. Furthermore, inappropriate use of gloves may even increase dermal uptake.

The worst-case estimates generated in this exposure assessment are considered to be reasonable worst-case estimates, as they describe high-end or maximum exposures in feasible but not unrealistic situations. They are not intended to account for extreme or unusual use scenarios. The majority of exposures are expected to be well below these estimates.

Available recent high-quality documentation prepared by national or international bodies has been largely used in this report to avoid duplication of efforts. The following documents deserve here a specific mention: ATSDR, 1998 ; NICNAS, 1996 ; SIDS, 1996.

Air sampling data are presented in this section from a number of sources and have been tabulated, where practicable. There is in general little or no information on the activities carried out while the sampling was running, the concentration of EGBE in the products, the control measures, and other important matters, such as sampling strategy and measurement methods, mean and 90^{th} or 95^{th} percentile of results; this is most often a serious difficulty for interpreting the data correctly.

Measured exposure data are compared with that predicted from the EASE (Estimation and Assessment of Substance Exposure) model version 2. EASE is a general purpose predictive model for workplace exposure assessments. It is an electronic, knowledge based, expert system which is used where measured exposure data is limited or not available. The model is in widespread use across the European Union for the occupational exposure assessment of new and existing substances.

A few measured dermal exposure data are available for EGBE or analogous substances. They will be considered together with modelling to predict occupational dermal exposure to EGBE. Many of the references stress the importance of dermal exposure, particularly during use of products. All sections on dermal exposure deal with liquid exposure.

All models are based upon assumptions. Their outputs are at best approximate and may be wrong. EASE is only intended to give generalised exposure data; it predicts inhalation exposure as ranges for concentrations for continuous exposure at the process under consideration. Dermal exposure estimates are provided by EASE as the quantity of a product adhering to the skin due to a task, they do not take into account evaporation of the product.

In the present assessment all inhalation exposures are expressed in parts per million (ppm), although the figures in the original publication are sometimes given as mg/m^3. All mg/m^3 have been converted to ppm using the following approximation:

ppm = mg EGBE/m^3 x 24.05/118.2 = mg EGBE/m^3 x 0.2

mg EGBE/m^3 = ppm x 118.2/24.05 = ppm x 4.9

A number of biological monitoring data are available for potentially exposed workers. Although the dataset is not extensive, they give useful indications about the total exposure and are presented in this section.

Routes of exposure and relevant scenarios

The major occupational routes of exposure to EGBE are inhalation and skin contact. Assuming proper hygiene measures are applied, oral exposure would normally not occur in the workplace.

Extremely low exposures have been reported for non-industrial office settings. Data from the National ambient VOCs database indicate that for 14 samples from non-industrial offices, the daily mean and median indoor atmospheric concentrations of EGBE were 0.214 ppb (1.03 µg/m^3) and 0.075 ppb (0.36 µg/m^3), respectively (Shah and Heyderdahl, 1988 ; Shah and Singh, 1988 in ATSDR, 1998). Due to the very low atmospheric exposure levels (comparable to the general population), non-industrial office settings will not be dealt with thereafter.

Workers may be significantly exposed during the production of EGBE, its processing as an intermediate or during the formulation and use of EGBE containing products.

Occupational exposure assessment will be carried out through three main categories of scenarios:

(a) the manufacture of EGBE and its use as an intermediate;

(b) the formulation of products containing EGBE;

(c) the use of products containing EGBE.

The third category will focus on particular sub-scenarios for exposure in the most frequent type of use, or particular pattern of use, when relevant.

Number of workers exposed

Due to the wide range of products containing EGBE, it is assumed that a large number of workers in many professional sectors may be exposed daily or occasionally.

Data from the National Occupational Exposure Survey (NOES) conducted by NIOSH from 1980 to 1983 indicate that an estimated 2,139,292 workers in 2,259 industry/occupation categories were potentially exposed to EGBE in the United States from 1981 to 1983. These numbers do not include workers potentially exposed to trade-name compounds that contain EGBE (ATSDR, 1998). In Denmark, a survey of chemical exposure in the workplace estimated that there were a total of 55,000 exposures to EGBE in 1989 (Brandorff *et al.*, 1995). Although these quantitative data are quite old, they confirm that EGBE is a widely used chemical with many workers potentially exposed.

Other data specific to some activities are reported further in this section.

Occupational exposure limits (OELs)

OELs apply to workplace air concentrations of chemicals. They are normally intended to protect workers against short-term adverse effects (irritation, acute CNS effects) or long-term effects (e.g. on liver, lungs, kidneys, or chronic CNS effects) after months or years of exposure. When applicable, a "short-term exposure limit" (STEL) may be proposed or imposed for the first ones, and/or a "time-weighted average" (TWA) for the second. The first value ordinarily refers to a 15 minutes or so duration, the second to a shift (generally considered as an 8-hour shift).

Table 4.3 details the OELs recommended for EGBE in various countries. They are provided for information and are not an indication of the level of control of exposure achieved in practice in workplaces. All of them add a “skin” notation.

Table 4.3: OEL values (Ariel Research, 2003)

	8-hour TWA		STEL, 15 min	
Country	mg/m^3	ppm	mg/m^3	ppm
EU*	98	20	246	50
Austria	100	20	200	40
Belgium	98	20	246	50
Denmark	98	20	-	-
Finland	98	20	250	50
France	9.8	2	147.6	30
Germany	98	20	392	80
Ireland	100	25		
Italy	97	20		
Netherlands	100	20	246	50
Norway	50	10	-	-
Spain	98	20	-	-
Sweden	50	10	100	20
Switzerland	100	20	200	40
United Kingdom		25	-	50
USA (ACGIH)	-	20	-	-
USA (NIOSH)	24	5		
USA (OSHA)	120	25	-	-

*Directive 2000/39/CE of 8 June 2000

Biological exposure levels (BELs)

Biological monitoring is recommended when applicable to measure both inhalation and skin exposure and can be carried out by determination of total urinary BAA (free and conjugated). Sensitive methods for the quantitative analysis of total urinary BAA were developed from the late 1980's and adapted by successive improvements in analytical chemistry. Sampling at the end of the shift would be the most appropriate (Johanson and Johnsson, 1991 ; Laitinen, 1998 ; Sakai *et al.*, 1993).

BELs proposed to characterize occupational exposure to EGBE are presented in table 4.4.

Table 4.4: Biological exposure levels (BEL)

Country	Determinant	Sampling time	BEL	Reference
Germany	BAA in urine	Post-shift at the end of the working week	100 mg/l	DFG, 2002
USA	BAA in urine	Post-shift	60 mg/g creatinine	NIOSH, 1990
France	BAA in urine		80 mg/g creatinine	INSERM, 1999
Finland	BAA in urine		70 mmol/mol creatinine	Laitenen, 1998

Nota :1 mmol/mol creatinine = 1.17 mg/g creatinine = 0.83 mg/l

The correlation between inhalation exposure to EGBE and urinary excretion of BAA was studied with persons mainly exposed through the respiratory system (Laitinen, 1998). The results indicate that an 8-hr inhalation exposure of 5 ppm in the absence of dermal exposure would correspond to a BAA excretion of about 75 mmol/mol creatinine (88 mg/g creatinine) in postshift urine samples.

4.1.1.2.1 Scenario 1 : Manufacture and use as intermediate

EGBE is manufactured continuously, either full-time during the year or within periodic campaigns of several weeks or months. Workers may be exposed approximately 8 hours for 5 days per week of campaign but typically there are no personnel working constantly on the plant, with only occasional visits from fitters, engineers and other technical staff. The process is enclosed as extensive precautions are taken to prevent and minimize exposure to workers in the production area, due to the toxicity of the ethylene oxide feedstock (SIDS, 1996).

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) (ECETOC, 1994).

Exposure during transfer to tankers or drums is generally minimized by the use of automated filling, where the operator is segregated from the area during transfer, and the use of local exhaust ventilation. Accidental exposure may occur when the process is breached or when spills occur. Exposure may also occur during maintenance and cleaning activities; however, the purging of plant and equipment is generally standard practice (SIDS, 1996 and NICNAS, 1996).

Inhalation exposure

Measured data

Airborne measurements provided by manufacturers of EGBE were collected for the OECD assessment in 1996. They are reported in table 4.5. No indication are available in the SIDS document whether they represent task measurements or 8 hour TWA exposures. Clarifications were found and they are given for the Australian plant in the NICNAS report (NICNAS, 1996).

Table 4.5: Measured inhalation exposure for EGBE manufacture (SIDS, 1996, slightly modified with NICNAS, 1996)

Manufacturer	Monitoring area	No of readings	Mean (ppm)	Maximum (ppm)
BASF (EU)	Production	97	0.09	1.2
	Filling	66	1.3	5.3*
	Technical unit	9	0.25	1.2
	Laboratory	14	1.3	11*
	Various	8	0.5	2.7
BP (EU)	All	311	<0.1	1.6
Eastman (US)	Production	16		<0.04
	Tanker loading	11	<0.25	1.8
Hüls (EU)	Production	30	<0.14	<0.31
	Filling	10	<0.14	0.22
	Laboratory sampling	20	<0.38	1.1
ICI (Australia)	All (STEL and TWA, personal) Maintenance (TWA, area)		0.1	1.8

*These values are reported as outliers by the department of Work Safety (Germany).

Older data are reported in open literature:

- measurements in a US plant showed that the highest results were obtained during drum filling, with a TWA of 1.7 ppm in area monitoring. The highest personal monitoring reading was 0.1 ppm (Clapp *et al.*, 1984 ; SIDS, 1996).
- TWA personal exposures in the manufacture of EGBE in Europe were in the range of 0.01-0.4 ppm (production), 0.03-0.3 ppm (drum filling), 0.5-2.7 ppm (blending), <1.6 ppm (roadcar filling) (ECETOC, 1985).
- personal exposures at a number of production sites during 1988-1993 gave results in the range 0.1-1.6 ppm and a mean of typically 0.13 ppm (data received from CEFIC, 1993 in ECETOC, 1994).

More recent measurements were received in the framework of this assessment from 4 EU producers of EGBE:

Producer 1: in 24 production and subsequent processing enterprises, analysis of 126 measurements (personal air sampling with exposure duration >1hour) carried out during the period 1995-2000 leads to a 90^{th} percentile of 0.2 ppm. The highest results are obtained in the laboratory with a 90^{th} percentile of 2.4 ppm. Details are presented in table 4.6.

Producer 2: 198 whole-shift personal measurements carried out in the production plant between 1995 and 2000 result in 8-hr TWA exposure <0.1 ppm in most operations. Details presented in table 4.7 show that personal exposure may be quite occasionally higher.

Producer 3: personal air measurements were carried out in 2002 in two departments of the production plant. Shift TWA exposures were all <0.1 ppm, task levels were slightly higher in the despatch department (up to 0.6 ppm). Frequency and duration of exposure of the main tasks were given. Details are presented in table 4.8.

Producer 4: 5 measurements made in the production plant in 2000 result in exposure <0.05 ppm (time span 420 to 690 minutes). No more details are available.

Table 4.6: Personal air measurements in Producer 1 enterprises (1995-2000)

Type of processing/workplace	No of results	No of enterprises	50 % value (ppm)	90 % value (ppm)	95 % value (ppm)
All	126	24	0.08	0.2	0.38
Production (closed system)	21	1	0.02	0.06	0.07
Subsequent users (closed system)	75	11	0.08	0.09	0.11
Laboratory (with LEV)	12	8	0.2	2.4	6.4
Filling/storage (with LEV)	18	4	0.08	0.6	0.68

Table 4.7: Personal air measurements in Producer 2 plant (1995-2000)

Job function	No of results	Average TWA (ppm)	Max TWA (ppm)	TWA (ppm) 5 to 95 % confidence range	
				Lower	Upper
Head of shift	106	0.2	9.0*	<0.1	0.4
Chemist	10	<0.1	<0.1	<0.1	<0.1
Engineer	6	<0.1	<0.1		
Fitter	2	<0.1	<0.1		
Operator	57	0.3	9.7*	<0.1	1.1
Physicist	4	<0.1	<0.1		
Technician	13	<0.1	<0.1	<0.1	<0.1

* Statistical analysis of the raw data shows that these two high measurements should be considered as outliers.

Table 4.8: Personal air measurements in Producer 3 plant (2002)

Job type	No of samples[1]	Sampling type	Range[2] (ppm)	Median (ppm)	90 % percentile (ppm)
Outside production operator	12 (3 <LOD)	Shift TWA	0.002-0.03	0.01	0.024
Mechanical maintenance operator	2 (1 <LOD)	Task	0.01-0.136		
Mechanical maintenance operator	1	Task	0.01		
Road gantry operator	10 (9 <LOD)	Shift TWA	0.002-0.008	0.002	0.004
Road gantry operator	8 (7 < LOD)	Task	0.006-0.052	0.022	0.042
Road gantry operator	1 (< LOD)	Task	0.003		
Road gantry operator	1 (<LOD)	Task	0.038		
Ship/jetty outside operator	2 (1 <LOD)	Shift TWA	0.02-0.366[3]		
Ship/jetty maintenance operator	1 (<LOD)	Task	0.064		
Ship/jetty maintenance operator	1	Task	0.628		
Road/jetty outside operator	2(2 < LOD)	Shift	Both 0.002		
Road/jetty outside operator	2(2 < LOD)	Task	Both 0.006		

Note 1: LOD = lower limit of detection (5µg which equates to 0.042 ppm for a 24 liter sample).

Note 2: for results <LOD, a value of 50 % of the LOD has been applied.

Note 3: one task ran over two shifts. Measurement was completed for the whole task and was therefore split between two operators, thus exposure approximates to 50 % of the level recorded.

Modelled data

The EASE model used to predict exposure during production in closed system with full containment provides an exposure estimation of 0 - 0.1 ppm. If the system is breached in some activities (like maintenance, sampling, cleaning, filling), concentrations could be in the range of 0.5 - 1 ppm (non dispersive use, low tendency to become airborne, presence of LEV).

Summary/statement of the exposure level

The results of monitoring data during production show very low workplace air levels. Due to automated processes for feeding reactors and for drum and tanker filling as well, typical inhalation exposure is < 0.5 ppm (using personal sampling) in most situations.

Higher exposure may occur during non-routine maintenance activities or during rare incidents such as spills or leaks. Such incidents are presented as exceedingly rare by industry, adding

that workers would wear PPE in such circumstances. Some measurement results show concentrations up to 2.7 ppm (SIDS, 1996) or 2.4 ppm (90 % of laboratory results of producer 1).

SIDS (1996) retained a maximum atmospheric concentration of 3 ppm. Recent data confirm that exposure is lower in nearly all situations. It is proposed to adopt the value of 2.4 ppm (12 mg/m^3) as a reasonable worst-case TWA atmospheric concentration in production activities.

Dermal exposure

Due to the enclosure of the process and control measures taken to minimize skin contact, for example, during transfer to tankers, dermal exposure at the plant is incidental and therefore likely to be low. The main source of potential exposure is during maintenance activities. Purging of plant and equipment is standard practice on site. Maintenance personnel are provided with butyl (or nitrile) rubber gloves and long-sleeved overalls, so exposure is not expected to be significant (NICNAS, 1996).

Questioned on the possibility of real skin exposure, industry answered that: "Operating controls are such that accidental spills and leaks are extremely rare. Campaign changes would not normally result in breaking of confinement. Equipment for transfer to drums/trucks use engineering controls of exposure; equipment is designed to virtually eliminate the possibility of exposure during such operations. The only real possibility for exposure would be during maintenance, but even in such a situation, the plant is normally purged to remove hazardous products before containment is broken and gloves would be recommended."

SIDS (1996) retained incidental contact time of 1 % of the working day with a 1000 cm^2 skin area exposed (a hand and a forearm). Incidental contact with the liquid seem appropriate for this scenario because exposure will be occasional (not daily) and intermittent contact would probably overestimate the exposure. The EASE model estimated a dermal exposure in the range of 0 - 0.1 mg/cm^2/day (non dispersive use with direct handling and incidental contact). Assuming exposed skin surface area is 420 cm^2 (palms of hands for consistency with other EU occupational risk assessments), maximum external dermal exposure would be 42 mg/day. This exposure will be mitigated by the use of suitable gloves.

4.1.1.2.2 Scenario 2 : Formulation of products containing EGBE

During the formulation of products containing EGBE, workers may be exposed during pre-weighing before mixing, during transfer to the mixing tank, during mixing and during the filling of containers with products. The whole operation is generally carried out at room temperature. Because of the similarity of scenarios, it will be assumed that exposure during formulation is the same whatever the final use of products is.

Quite a high number of workers are likely to be exposed during formulation of products. In Australia, during the assessment of EGBE only in cleaning products, 82 companies were identified, some producing cleaning products at more than one site, with at least 200 workers involved in formulation (NICNAS, 1996). An enquiry was recently conducted by CEPE (European council of the paint, printing and artists' colours industry) on the industrial uses of 4 glycol ethers in paints or inks, one of which is EGBE: 109 answers were received from all over Europe, 65 users and 44 non-users. They comprise both multinationals and small or medium size enterprises from most of the EU countries. The number of workers exposed was

indicated by 43 user companies out of the 65, the answers were in the range of 1 to 250 and represent a total number of 1760 workers (CEPE, 2002).

Exposure strongly depends on the process, which may be enclosed or relatively open. When the transfer of EGBE to the mixing vessel is carried out in a sealed system, potential exposure will be minimal, but when the operator adds the raw materials directly by drum to the mixing tank, exposure may be greater due to possible splashing and vapour and/or aerosol generation. Information obtained from the national assessment of EGBE in Australia indicated that a number of formulators of cleaning products containing EGBE use the latter procedure and that approximately 50 % of formulators carry out mixing in open top tanks, with greater potential for exposure (NICNAS, 1996). The situation is likely to be similar in the EU where many formulators are small or medium size enterprises.

Exposure will also strongly depend on the quantities handled, the concentration in the products and the duration and frequency of exposure.

While during preweighing and transfer to the mixing tank, workers are potentially exposed to pure EGBE, they are exposed to a more dilute form during filling. However the frequency and duration of exposure may be greater. As operators may be involved in both mixing and filling, assessment of exposure is for the formulation process as a whole.

From responses to a questionnaire sent to formulators in Australia (NICNAS, 1996), workers are potentially exposed to EGBE for an average of 3 hours/week (range 0.1-20). For most formulators, EGBE is an ingredient in only some of their products, so exposure is not continuous on a daily or weekly basis. From 74 responses to the questionnaire, the distribution for potential exposure duration was as follows:

Table 4.9: Distribution of potential exposure duration (NICNAS, 1996)

Duration/week	Number	%
< 1 hour	30	41
1 hour	11	15
2 hours	11	14
3-4 hours	8	11
5-8 hours	6	8
Greater than 8 hours	8	11

This suggests that 89% of formulators use EGBE for 8 hours or less per week.

Information about exposure frequency and duration has also been recently collected in the EU by CEPE (table 4.10) which suggests more frequent and/or longer exposures.

Table 4.10: Exposure frequency and duration in the EU paints and inks manufacturing industry (CEPE, 2002)

	Exposure in days/year (41 answers)	Exposure in hours/day (42 answers)
Arithmetic mean	173	5.1
Median	200	6.0
Range	2-250	0.5-8

Inhalation exposure

Measured data

There are a few measured data published for assessment of exposure during formulation. They are presented in table 4.11. Control measures are generally unknown.

Table 4.11: Atmospheric and biological monitoring during formulation of products (8 hr-TWA personal inhalation exposure and BAA urinary concentration)

Products	No of samples	Mean (range) (ppm)	No of post-shift urinary samples	Mean (range) (mg/g creatinine) [d]	Source
Printing inks	9	<1			Winchester, 1985
Varnishes	(12)[a]	1.1 (0.1-8.1)	(12)[a]	14.8 (0.8-42.7) (post-shift)	Angerer *et al.*, 1990
Varnishes	(12)[a]	0.5 (<0.1- 1.4) [b]	(12)[a]	0.30 (<0.03-1.83) [b] (post-shift)	Söhnlein *et al.*, 1993
	(12)[a]	0.6 (<0.1- 1) [b]	(12)[a]	23.1 (1.13-85.4) [c] (post-shift)	
Paints	4	1.26 (0.41-3.13)			Foo *et al.*, 1994
Unknown	15	0.7 (max 1.5)			SIDS, 1996 (industry)
Paints	328	0.4 (max 44.7)	300	3.9 (<2-59.6) (post-shift)	Vincent *et al.*, 1996
Paints	179	0.1 (0.0-1.4)			Wesolowski and Gromiec, 1997

[a] No of workers (No of samples not stated)

[b] Measurements on Monday

[c] Measurements on Tuesday

[d] BAA mg/l converted into BAA mg/g creatinine using the relation mentioned in nota of table 4.4

In the inquiry of CEPE (2002), 20 facilities out of the 65 EGBE users who answered the questionnaire said they performed workplace monitoring (11 replied negatively to the

question while the others did not answer at all). Twenty seven users indicated typical concentrations: 10 answers were <0.2 ppm, 5 in the range 0.2-2 ppm and 6 in the range 2-5 ppm (10-25 mg/m^3). No details are available.

Information from database

In the German MEGA database, 165 exposure measurements have been registered between 1996 and 2000, which were obtained in the paint formulation industry. The results (measurement values with an exposure duration ≥1 hour and a sampling duration ≥1 hour converted to 8-hour weighted averages) are presented in table 4.12. When possible, a distinction is made on the basis of whether or not control measures (LEV) were taken. In this regard, the results present an apparent paradox that the workplaces with LEV frequently do not exhibit lower exposures than those without LEV and the exposures may even be higher.

Technical measures are mostly taken in place where the situation may result in a higher release of vapours, for instance when large quantities of substance are handled or when process occurs at high temperature. By contrast, the release is comparatively low during use of small quantities or processing at ambient temperature. In most cases, control measures create a situation where the exposure level of workplaces with large release approximately reaches the level of workplaces with only low release but without control measures.

Table 4.12: Personal inhalation exposure in the MEGA database, 1996-2000 (BGAA, 2001)

Paint production activity	No of results	No of companies	50% value (ppm)	75 % value (ppm)	90 % value (ppm)	95 % value (ppm)
Raw EGBE handling, mixing and filling	165	39	a	a	1.4.	2.4
- without LEV	71	23	a	a	1.9	2.5
- with LEV	92	28	a	a	1.4	2.4
Cleaning of containers	74	36	a	a	2.7	7.6
- without LEV	23	15	a	a	3.2	6.8
- with LEV	49	23	a	a	2.2	6.6

a : measurement value < analytical determination limit

Modelled data

Using the EASE model (non dispersive use, low tendency to become airborne), the exposure estimate would be in the range of 0.5-1 ppm with LEV and 10-20 ppm in case of direct handling with dilution ventilation.

Summary/statement of the exposure level

SIDS (1996) and NICNAS (1996) estimated that exposure during formulation of products would be 2 ppm for formulations including up to 10 % EGBE, and 10 ppm for those including 30 % or more. As very few measured data were available for formulation, this estimate was based on monitoring data of Vincent *et al.* (1993) and Salisbury and Bennett (1987) during cleaning activities.

Although some of the published data are limited, results during formulation show that airborne concentrations are generally low. Recent data provided by industry indicate that exposure would not be higher than 5 ppm but very little information is available to the context of this measurements. Based on the 90th percentile of the MEGA database values, we propose in a first approach that worst case inhalation exposure during formulation of products containing EGBE would be 3.2 ppm (15.7 mg/m^3). Typical exposure levels are probably much lower (<1 ppm).

Information presented in tables 4.9 and 4.10 show that frequency and duration of exposure may considerably vary. A continuous exposure for full shift (8 hours per day) will be assumed, although the data available suggest that this is unlikely to be a daily exposure.

Dermal exposure

Measurements

As part of the "RISKOFDERM" project, a study was performed by the TNO (RISKOFDERM, 2002a ; Gijsbers *et al*., 2004) to directly assess dermal exposure to products containing 2-(2-butoxyethoxy)ethanol (DEGBE), a chemical of the same family as EGBE, but much less volatile (vapour pressure 2.7 Pa at 20 °C compared to 100 Pa at 20 °C for EGBE). Hand exposure was measured during loading (typically at the beginning of the formulation process, handled product is the substance, short task duration ranged between 1 and 15 minutes) and during filling (typically at the end of the formulation process, handled product is the formulation, task duration ranged between 22 and 125 minutes). The measurements were made using cotton sampling gloves which were worn over new protective gloves, where present. Exposure was mainly due to exposure on the hands. The most important source of variabilitywas due to between-company variability, rather than to either between-worker or within-worker variability. Results (given in DEGBE and product) are presented in table 4.13.

Table 4.13: Results of measurements of potential hand exposure to DEGBE in loading mixers and filling containers with products containing DEGBE (after RISKOFDERM, 2002a and Gijsbers *et al.*, 2004)

Exposure to DEGBE or product	*N*	*Range (mg)*	*AM (mg)*	*GM (mg)*	*GSD (mg)*	*AM (µg/cm²/min)*	*GM (µg/cm²/min)*	*GSD (µg/cm²/min)*
Loading (pure DEGBE)								
Hands DEGBE	28	0.28-28300.0	3313.6	218.9	19.9	727.4	52.9	17.2
Hands Product*	28	0.31-27745.1	3215.0	217.0	19.3	708.8	52.4	16.7
Filling (all data)								
Hands DEGBE	30	0.062 – 19000.0	1955.8	35.9	42.0	45.1	0.75	42.6
Hands Product*	30	4.1 – 18269.2	2726.6	555.4	9.4	58.5	11.5	9.6
Filling (only products < 10% DEGBE); data not published, but calculated from original data								
Hands Product*	21	4.1-11146	1216	249	8.5	15	4.1	7.6

N = number of measurements
AM = arithmetic average
GM = geometric average
GSD = geometric standard deviation)
* recalculated towards the full product by dividing the value measured for DEGBE by the fraction of DEGBE as analysed in the product

The 90th percentile from the measured data for loading was approximately 11,000 mg on 820 cm^2 (expressed as total product), approximately 11,100 mg on 820 cm^2 for filling (all products, including (almost pure) DEGBE) and approximately 3,300 mg for filling of products containing less than 10% of DEGBE (not published data, derived from original data). This value would lead to a level of 330 mg for DEGBE if the percentage of DEGBE would be 10%. It appears that the situations with handling (almost) pure products lead to higher exposure levels. That can be caused by the fact that products with small percentages of DEGBE, such as paints can be packaged in cans by highly automated equipment, while (almost) pure DEGBE is often packaged in larger containers with more handling of the container by the workers.

Although assessed in a direct way and in actual working situations, these results should not be given too much weight for the main following reasons:

- this is an isolated study, and it is known that there is an extreme variability in skin (and especially hand) exposure (Kromhout *et al*., 2004), depending of a number of most often qualitative factors that have been divided in six categories (Marquart *et al*., 2003);

- measurement durations are relatively short (range 1-139 min, arithmetic mean range 6-74 min, all tasks counfounded; Gijsbers *et al*., 2004) compared to a shift, so the results may represent rather task-associated exposure measurements than shift exposure assessments.

- although from the same chemical family as EGBE, DEGBE has very different physicochemical properties, among which volatility, viscosity and the octanol/water partition coefficient may play a significant role.

Biomonitoring studies

In this context, biomonitoring studies might be considered as an alternative means to assess skin exposure because: i) biomonitoring allows an assessment of the exposure on the whole shift, although it integrates dermal and inhalation routes; ii) several studies have been published on EGBE itself, so there is no need to extrapolate from other more or less related chemicals with the corresponding uncertainties. Technically, only studies where *total* (i.e. free *and* conjugated) urinary butoxyacetic acid (BAA) has been evaluated are sufficiently reliable (Jones and Cocker, 2003; Franks *et al*., 2006). In order to properly correct for EGBE absorption via the inhalation route, the latter must have been separately assessed on the same day and for the same worker and task as for dermal exposure. Difficulties may however arise, among which: i) too short sampling times when compared to a shift (e.g. more than 50% workers are exposed for 1 hour or less, according to table 4.9); ii) sampling conditions often unspecified (e.g. was it area sampling or personal sampling? were there incidents when sampling was running? how was the mean inhalation exposure concentration calculated?); iii) some skin absorption of vapours may occur (up to 39% of the total uptake in extreme conditions, after Jones *et al.*, 2003); (note: these difficulties may lead to an overestimation of inhalation exposure with the associated underestimation of skin exposure) iv) different values for the urinary excretion of total BAA per ppm EGBE inhalation exposure have been proposed (e.g. 12 mmol/mol creat per ppm, Laitinen *et al*., 1998; 15 mmol/mol creat per ppm, Laitinen, 1998; 10 mmol/mol creat per ppm, Franks *et al*., 2006).

Keeping these difficulties in mind, evaluations are tentatively proposed, based on biological monitoring with EGBE in real working conditions. If A is the result of biomonitoring (total BAA, mg/g creatinine) and B the corresponding inhalation exposure (ppm), a "dermal equivalent" (mg/day) may be evaluated using the formula:

$$[(A \times 5/88) - B] \times 88/5 /0.6 \quad [\text{which is equal to } (5A - 88B) / 3]$$

where 5 and 88 are the correspondence between inhalation exposure (5 ppm during a shift) and the excreted BAA (88 mg/g creatinine, post shift; after Laitinen, 1998), and 0.6 is the absorption efficiency by inhalation.

Among the studies from Angerer *et al.* (1990), Söhnlein *et al.* (1993) and Vincent *et al.* (1996), only peak measurements from Söhnlein show significant dermal absorption (85.4 mg/g creatinine with 1 ppm as a peak measured inhalation exposure), leading to a dermal equivalent absorption of 113 mg/day, that is to say a dermal load of 377 mg/day assuming 30% absorption. There is a high proportion of results entirely explained by the inhalation route or even leading to calculation of a negative dermal exposure, *i.e.* the biological monitoring results are (much) lower than would be expected due to the inhalation exposure data. This suggests a negative bias, especially for a chemical being known to be easily absorbed through the skin; this may originate from the many unknowns in sampling conditions, as already mentioned, and also from remaining uncertainties in the biological disposition of 2-BE (Franks *et al*., 2006).

If this bias is estimated as 30% (Franks *et al*., 2006, estimate a 31% discrepancy solely due to sampling time differences), the dermal load would be re-assessed as ca. 500 mg/day.

Modelling

Considering the process and the tasks where exposure may occur, SIDS (1996) retained intermittent contact time of 20 % of the working day with a 1000 cm^2 skin area exposed (a hand and a forearm).

Taking into account the same assumption, the EASE model estimates a dermal exposure in the range of 0.1-1 mg/cm^2/day (non dispersive use with direct handling and intermittent contact). Assuming exposed skin surface area is 420 cm^2 (for consistency with other EU occupational risk assessments and default assumptions recommended in table 3 of Appendix I of the Technical Guidance), maximum external exposure would be:

- 42-420 mg/day for loading (pure substance)
- 21-210 mg/day for filling (assuming 50% EGBE in the product as a worst case : in most situation, EGBE content will be much lower)

Assessment

Dermal exposure can occur during part of the working day during loading of mixers (short periods, up to approximately 20 minutes) and during packaging of products containing EGBE (up to 120 minutes per day). Measured data for this scenario for EGBE are not available. Data from DEGBE for similar processes may be relevant. The measured values, using cotton gloves as samplers, for DEGBE are generally high compared to the estimates by EASE. Geometric mean exposure levels to products, for situations where formulations were made, so excluding the filling of (almost pure) DEGBE, were in the order of 200-250 mg and the 90^{th} percentile was in the order of 3,300 mg for filling of products with less than 10% DEGBE and 11,000 mg for loading of DEGBE into mixers, both on 820 cm^2. The measured data for DEGBE may be an overestimate of potential dermal exposure for EGBE for two reasons. Firstly, the measurement method may have led to overestimation of dermal exposure, because cotton gloves are considered to retain more liquid than the skin would do. Secondly, EGBE is much more volatile than DEGBE and therefore, more of the substance may evaporate from the skin and not be available for uptake. The effect of both factors is difficult to estimate. The

estimate based on measured data is substantially higher than that based on EASE that is considered a weak model for dermal exposure. Although the measured data have a number of uncertainties, they should not be disregarded for risk characterisation. Therefore, a rounded value of 10,000 mg EGBE/day will be used for the loading step of the process. In the evaluation of the Margin of Safety (MOS) it will be considered that this may be a (substantial) overestimation.

For the purpose of determining the evaporation time, the following equation can be used (TGD Appendix I.E):

$T(s)= (mRT/M\beta pA)K$

This equation leads for EGBE to an estimate of evaporation time of 3 hours, with the following input values: m = 10 000 mg, R = 8.134 $J.K^{-1}.mol^{-1}$, T = 305 Kelvin, K = 3.6 * 10^4, M = 118.17, β = 8.7 $m.h^{-1}$ (default), p = 100 Pa, A = 820 cm^2. This indicates that in approximately 3 hours all EGBE would be evaporated from the skin.. For comparison, the same calculation with DEGBE (vapour pressure = 2.7 Pa) leads to an evaporation time of more than 100 hours.

The filling of packages with products leads to substantially lower exposure levels. Assuming a product with 50% EGBE and a 90^{th} percentile exposure level for the product of 3,300 mg leads to a reasonable worst case exposure level of approximately 1,600 for EGBE. The related evaporation time would be approximately half an hour.

Conservatively, it may be assumed that both tasks are done by the same workers, leading to a total exposure of approximately 11 600 mg that will take approximately 3,5 hours to evaporate from the skin. Due to the sampling method for the measured data and the higher vapour pressure of EGBE compared to DEGBE, these are considered to be overestimates of the real exposure levels, which should be taken into account in the evaluation of the MOS.

Biological monitoring data

On the one hand, exposure data have been obtained in real use conditions, which is a very strong argument to take them in consideration, but in an isolated work and with a chemical presenting physico-chemical properties very different from those of EGBE, which are strong arguments not to accept them at face value. On the other hand, limited biomonitoring data are available for EGBE itself, also in real conditions of use, with the advantage of not requiring any extrapolation of uncertain validity from another chemical substance. Its main weakness lies in the important penetration of EGBE through the inhalation route, which must then be assessed with sufficient reliability. Since neither of these sources can be ignored, a final assessment must be based on both. The difficulty to do so lies here in assessments that differ by a factor of 23 (11,600 mg/day for Risk of Derm, 500 mg/day for biomonitoring). This factor is in fact relatively small considering the extreme variability evidenced in assessments made by RISKOFDERM in a variety of situations. Moreover, there are good reasons to think that data obtained with DEGBE may be overestimates when transposed to EGBE. On the other hand, based on extensive data from this RISKOFDERM project, Marquart *et al*. (2006, table 5) consider that 550 mg is more a typical exposure value than a reasonable worst-case. According to them, “individual mean dermal exposure levels were on average within a 4-fold range”. In this context, it is proposed to re-evaluate the assessment of the limited data based on biomonitoring by a factor of 4. This eventually leads to propose a skin penetration of 2,000 mg/day for this scenario.

4.1.1.2.3 Scenario 3 : Use of products containing EGBE

EGBE is used in a wide variety of products. The following scenarios are considered as representative:

- use of paints and coatings

- use of printing inks

- use of cleaners

Cleaning related to painting and printing activities are included in the first and second scenarios.

Measured exposure levels in general

Exposures to EGBE were found in a 1983 survey of 336 Belgian businesses (Veulemans *et al.,* 1987a). In this study, EGBE was found in 25 of 94 air samples from sites using printing pastes; 10 of 81 samples where painting was done; 1 of 20 samples from automobile repair shops; and 17 of 67 samples from various other industrial sites where materials such as varnishes, sterilization agents and cleaning agents were used. The geometric mean atmospheric concentrations and ranges of EGBE at various sites were as follows: printing shops, 0.8 ppm, range 0.3-3.7 ppm; painting areas, 3.9 ppm, range 0.7-19.4 ppm; automobile repair shops, 1.2 ppm (one sample); various other industries, 1.8 ppm, range <0.1-367 ppm (this data are considered further when relevant to a specific scenario).

In a survey on industrial solvents conducted in 1994 to 1996 by Yasugi *et al.* (1998) in 95 different plants (196 unit work areas), EGBE was found 59 times (8 during printing and 51 during painting) with a median atmospheric concentration of 0.5 ppm and a maximum concentration of 1.3 ppm (10-l bag sample, analysed the day of sampling).

From 1987 to 1998, the French COLCHIC database collected 10,593 personal sampling results of glycol ethers for 602 facilities (Vincent, 1999). EGBE was found 1,195 times; the arithmetic atmospheric mean value of the 60 to 480 minutes samplings (622 results) was 2.3 ppm (median 0.6 ppm; range 0.02-110 ppm; 95th percentile 6.2 ppm). It should be noted that, globally, atmospheric concentrations have significantly decreased from 1987-92 (arithmetic mean of 147 personal samplings 1.1 ppm, median 0.6 ppm, range 0.02-10.7 ppm) to 1993-98 (arithmetic mean of 178 personal samplings 0.28 ppm, median 0.1 ppm, range 0.02-3.8 ppm) (Vincent and Jeandel, 1999).

For the years 1999 to 2002, the COLCHIC database collected 116 personal atmospheric sampling results of EGBE. The arithmetic mean value of 60 to 480 minutes samplings was found 0.52 ppm (median 0.14 ppm, range 0.02-14.84, 95th percentile 1.22 ppm) (Vincent, 2003).

- Scenario 3-1 Painting/Surface coatings

EGBE is used as a solvent in paints and surface coatings, particularly in water-based type. It is the main application of EGBE and due to the high volume use, a large number of workers are potentially exposed.

Twenty seven water-based paints and formulations used in the automotive industry have been analysed (Jargot *et al*., 1999). EGBE was found 18 times, with concentrations ranging from traces to 40.4 %; in thinners, its concentration ranged from 0 to 24.7 %.

SIDS (1996) reports that coatings may typically contain 1 % up to 8 % EGBE.

Analysis of the answers collected in the paint formulating industry by CEPE (2002) shows that the concentrations of EGBE range from traces to 39 % with an arithmetic mean up to 8 % (see table 4.14). Taking into account this data together with the information collected in European products registers (tables 4.1 and 4.2), a worst case EGBE content of 20 % in industrial paints and surface coatings and 3 % in decorative paints will be assumed in this assessment. Therefore the conclusions in this section refer to solvent-based paints. Exposure from use of water-based paints (lower EGBE content) would be much lower.

Table 4.14: Contents of EGBE in paints (CEPE, 2002)

	Industrial paints		Decorative paints	
	Water-based	Solvent-based	Water-based	Solvent-based
Number of answers	33	31	10	9
Arithmetic mean	7.8 %	7.1 %	1.4 %	1.6 %
Median	3.5 %	5 %	1.8 %	1 %
Range*	0.06-22.5 %	0.01-38.7 %	0.03-3.0 %	0.01-3.0 %

* minimum values probably correspond to incorporation of EGBE through additives.

Coatings and paints are applied by brushing, rolling, spraying or dipping in different industrial and skilled trade sectors, e.g. coating of metal and wood, vehicle production and repair, building trade. Application techniques inventoried in the CEPE enquiry are presented in table 4.15 (CEPE, 2002).

Table 4.15: Relative frequencies of application techniques in painting/surface coating (CEPE, 2002)

Application technique	No of mentions
Spray	40
Roll	22
Brush	21
Dipping	6
Roller coaters	3
Printing	3
Falling curtain	1

Inhalation exposure

Measured data

The data reported in the previous assessments (SIDS, 1996 ; NICNAS, 1996 ; ATSDR, 1998) completed by further references available in open literature (Collinot *et al.*, 1996 ; Delest and Desjeux, 1995) are presented in table 4.16. Details have been added when available.

Table 4.16: Atmospheric and biological monitoring during painting (8 hr-TWA inhalation exposure and BAA urinary concentration)

Activity	No/type of sampling	EGBE air concentration Mean (range)(ppm)	No of post-shift urinary samples	Post-shift BAA urinary excretion Mean (range) (mg/g creatinine)	Reference
Furniture production/finishing	64/p	1.5 (0.07-9.9)			Zaebst, 1984
Staining/varnishing	9	5 (≤71)			Denkhaus *et al.*, 1986
Car repair	1/a	1.2			Veulemans *et al.*, 1987a
General painting	10/a	3.9 (0.7-19.4)			Veulemans *et al.*, 1987a
House painting (water-based <1.4 % EGBE)	15/p	(0.4-12)			Hansen *et al.*, 1987
Cabinet finishing	6/p	Nd[1]-0.4			Newman and Klein, 1990
Spray painting	11/p	0.4 (0.2-0.6)			Winder and Turner, 1992
Painting (water-based paints <5 % EGBE)	54	0.1 (0.1-0.3)		(<2-13.2)	Delest and Desjeux, 1995
Indoors house painting (water-based paints)[3]	202/p	0.01 (<0.01-0.15)			Norbäck *et al.*, 1996
Cataphoresis	a	(≤7.5)			Collinot *et al.*, 1996

Can coating - external deco - internal decor	20/p 11/p	0.73 (0.35-1.23) 0.44 (0.15—0.67)		12.2 (0.3-51.4) 9.2 (0.6-20.4)	Haufroid *et al.*, 1997
Painting of plastic hup caps	79/p	<0.1 (<0.1-0.8)	19	n.d.	Vincent *et al.*, 1996
Cataphoresis	66/p	0.8 (<0 .1-6.2)	51	17.9 (<2-210)	
Can coating	168/p	0.2 (<0 .1-2.4)	79	5 (<2-33.9)	
New car painting	39/p	<0.1 (<0.1-0.5)	40	n.d.	
Coil coating	261/p	0.1 (<0.1-1.0)	213	2.3 (<2-28.4)	
Painting of metal frame	50/p	<0.1 (<0.1-0.3)	46	9.4 (<2-63)	
Painting of buildings	63/p	<0.1 (<0.1-0.2)	63	<2 (<2-13.2)	
Furniture varnishing		n.d.	50	2.9 (<2-31.2)	
Printed circuit boards varnishing	57/p	<0.2 (<0.1-2.8)	56	4.6 (<2-30.4)	

p: personal, a: area,
Note 1: Limit of detection not reported.
Note 2 : No of workers (No of samples not stated)
Note 3: EGBE found in 2 samples.

Information from database

In the HSE exposure database (Brown *et al.*, 1994), 12 results are available for painting and 20 for lacquering; the ranges (and arithmetic means) were 0 – 1.4 ppm (0.15 ppm) and 0.2 – 7 ppm (1.69 ppm) respectively. The single high exposure was taken during the spraying of lacquer onto wooden furniture and local exhaust ventilation was provided; this high level was considered as probably due to a failure in the LEV system.

Exposure measurements (sampling period 60-480 minutes) registered between 1987 and 1998 in the COLCHIC database were analysed by Vincent (1999). Results related to painting and coating are presented in table 4.17.

Table 4.17: Personal exposure in painting activities for measurements 60-480 minutes, years 1987-1998 (Vincent, 1999)

Type of work	No of results	Mean (ppm)	Range (ppm)	Median (ppm)	95th percentile (ppm)
Pneumatic spraying of paint or varnish	58	0.46	0.04-4.2	0.2	2.2
Varnishing (curtain)	19	0.14	0.02-0.6	0.1	0.6
Brush or roll coating of paint or varnish	21	0.18	0.04-0.6	0.12	0.42
Cataphoresis	3	0.2	0-0.6	-	-

In the German MEGA database, 441 exposure measurements have been registered between 1996 and 2000, which were obtained during application of paints or coatings, mainly in the plastic or metal processing industries, in electrical engineering or woodworking. The results (measurement values with an exposure duration ≥1 hour and a sampling duration ≥1 hour converted to 8-hour weighted averages) are presented in table 4.18. When possible, a distinction is made on the basis of whether or not control measures (LEV) were taken (see comments in scenario 2).

Table 4.18: Personal exposure measurements in the MEGA database, 1996-2000 (BGAA, 2001)

Type of company/working area	No of results	No of companies	50% value (ppm)	75 % value (ppm)	90 % value (ppm)	95 % value (ppm)
Painting,brush and roller application, filling work	40	24	a	a	a	2.4
- without LEV	26	16	a	a	a	2.2
- with LEV	10	6	a	a	a	a
Spraying (compressed air, airless, airmix)	224	112	a	a	a	3.8
- without LEV	23	14	a	2.4	11.6	24.6
- with LEV	189	98	a	a	a	2.6
Surface coating, mechanical	98	47	a	a	a	a
- without LEV	44	22	a	a	a	a
- with LEV	53	26	a	a	a	a
Surface coating, general	79	8	a	a	a	2.7
- without LEV	26	21	a	a	a	1.5
- with LEV	50	28	a	a	a	3.9

a : measurement value < analytical determination limit

Modelled data

Exposure to vapours during the use of paints or surface coatings is estimated by EASE to be in the range 100-140 ppm for wide dispersive, low tendency to become airborne, direct handling and dilution ventilation.

The model overestimates exposure levels, particularly because of non-consideration of the content of EGBE in the mixtures. The estimates cannot be corrected for the partial vapour pressure because the composition of the formulations is not known. A simple approach based on a reduction of the exposure by a factor equivalent to the EGBE concentration in the mixtures (up to 20 % for industrial paints and 3 % for decorative paints) would lead to exposure levels of 20-28 ppm for industrial paints and 3-4 ppm for decorative paints. However the validity of these extrapolations is rather questionable.

Summary/statement of the exposure level

Exposure to EGBE during painting may be extremely variable, due to differences in frequency and duration of use, concentration of EGBE in the paint, method of application and precautions taken during use. To some extent, this variation is reflected in the atmospheric monitoring data available for EGBE during painting and surface treatment.

SIDS proposed a maximum atmospheric concentration of 10 ppm for use of a paint/surface coating containing 10 % EGBE, the justification for this estimation is not clear. On the basis of the available data, results of air monitoring are generally much lower except during spraying.

For spraying, the available measured data are mainly extracted from the COLCHIC and the MEGA databases. The 90^{th} percentile of the MEGA (11.6 ppm) database values seems to be the most representative worst case inhalation exposure.

For other application techniques, inhalation exposure is likely to be lower. The 95^{th} percentile of the COLCHIC and MEGA databases results are lower than 4 ppm. The highest result from the largest study (Vincent *et al.*, 1996) is 6.2 ppm. This value will be used for risk characterisation to ensure that highly exposed workers are represented.

In conclusion, we propose in a first approach the following worst case inhalation exposures:

- 11.6 ppm (58.1 mg/m^3) for spray application of paint
- 6.2 ppm (30.4 mg/m^3) for other application techniques

Dermal exposure

Measurements

In a study performed by TNO (RISKOFDERM, 2002a and Gijsbers *et al.,* 2004), a part of the RISKOFDERM project, potential hand exposure to an analogous but less volatile glycol ether DEGBE (2-(2-butoxyethoxy)ethanol) was measured during indoors application of paint by brushing over of periods of 57-149 minutes. The sampling was ended when the painters (who usually painted for most of the working day) took a break for coffee or lunch, resulting in the measurement durations mentioned. The measurements were made using cotton sampling gloves which were worn over new protective gloves, where present. The amount of used product was between 0.5 and 2.5 litre (AM 1.2 litre) and the paint contained between 0.4 and

3.2 % DEGBE (AM 2 %). The treated area during measurements was between 2 and 15 m^2 (AM 6.4 m^2). Exposure was mainly due to exposure on the hands. Results (given in DEGBE and product) are presented in table 4.19.

Table 4.19: Dermal exposure rates during brushing (Gijsbers *et al*, 2004)

Hands exposure	N	W	Range (mg)	AM (mg)	GM (mg)	GSD (mg)	AM[c] (µg/cm²/min)	GM[c] (µg/cm²/min)	GSD[c] (µg/cm²/min)
- DEGBE	36	18	0.19-33.0	6.5	2.8	4	0.091	0.045	3.6
- product	24[a]	13	11.3-733.3	170.5	98.4	3	2.8	1.7	2.9

N, number of measurements; W, number of workers involved

AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation

Nota a : for 12 of the measurements, exposure to product could not be calculated due to contamination of samples by other sources of DEGBE.

Nota c : a surface area of 820 cm^2 was assumed for the hands exposure

The 90th percentile of the measured exposure levels was approximately 400 mg product (unpublished data, derived from original data).

No measurements for dermal exposure in spray painting of EGBE or other direct analogues (e.g. other glycol ethers) are available. A number of dermal exposure data sets is available for other spray applications. Exposure to a non-volatile pigment was measured in car painting. Dermal exposure to the hands, expressed as total formulation, was between 2 and 211 mg (n = 30), with a median of 41 mg. Amount of product used was up to 1.5 kg in an average duration of 16 minutes (RISKOFDERM, 2002b ; Delgado *et al.,* 2004). Exposure to another non-volatile pigment was measured in marine anti-fouling painting. Dermal exposure to the hands, expressed as total formulation was between 286 and 27.000 mg (n = 24) for sampling periods of 1 to 3 hours. In this period 40 to 140 litres of paint were sprayed. Exposure was not only to workers actually doing spray painting, but also to auxiliary workers (so-called linesmen) assisting the spray painters. There was no difference in exposure between the two jobs. (RISKOFDERM, 2002c ; Hughson and Aitken, 2004). Spray application of a cleaning agent containing DEGBE was measured 12 times in the food industry. Duration of measurements ranged between 6 and 18 minutes. The in-use concentration of DEGBE was between 0.007 and 1.1%. The total amount of diluted product used was between 16.5 and 97.2 L. Dermal exposure to the hands, expressed as total formulation, was between 37 and 1974 mg (RISKOFDERM, 2003a). The 90th percentiles of these three data sets were respectively approximately 100, 13,600 and 1000 mg per measurement period (unpublished data, derived from original data). Older data are reported in the TGD, where for spray painting on large surface areas a reasonable worst case value of 10 000 mg product per day (on 840 cm^2) is given (Appendix I.E).

Modelling

SIDS (1996) retained continuous skin contact over the work period (8 hours) and a 1000 cm^2 skin area exposed (a hand and a forearm). Intermittent contact seems more appropriate for tasks as brushing and rolling in this scenario. For spray application, extensive contact is assumed., For wide dispersive use, the EASE model estimates a dermal exposure in the range of 1-5 mg of product/cm^2/day for intermittent contact and 5-15 mg of product/cm^2/day for extensive contact. The estimation is made from a formulation containing up to 20 % of EGBE (industrial paint) and a formulation containing up to 3 % of EGBE (decorative paint) and an

exposed skin surface area of 840 cm^2 (two hands for consistency with other EU occupational risk assessments). This leads to estimated external dermal exposures of :

- 168-840 mg/day for industrial painting (excluding spray application)- 840-2520 mg/day for industrial spray painting
- 25-126 mg/day for decorative painting

Assessment

Skin contact due to manual transfer of liquids, spray application and brushing, rolling and cleaning is to be expected. In several of the available references, the importance of skin exposure is stressed.

In the TNO study, cotton sample gloves can retain more material than skin leads to overestimation of exposure. The size of this possible overestimation is difficult to estimate. It can be expected that this overestimation is smaller when more viscous paints are used than when less viscous pure product is used. The fact that workers (except for breaks) may almost continuously do the same tasks suggests that the task based exposures should be extrapolated to a full work shift. Whereas extrapolation is clearly not valid for high (product) exposures, due to saturation of the surface of the skin, this is not so much of a problem with lower task exposure levels. Given the fact that painters spend part of their working period in preparation, moving between rooms, cleaning up the material and other non-painting tasks, the exposure period for painting can be estimated to be up to 6 hours. Based on the 90^{th} percentile of the measured data (400 mg product in 2 hours), linear extrapolation to 6 hours of work and an assumed percentage of 20 % (industrial paint) or 3 % EGBE (decorative paint), the exposure value to be used in risk characterisation is estimated as 400x 3x 0.2 = 240 mg or 400x 3x 0.03 = 36 mg EGBE per day for brushing and rolling of industrial or decorative paints.

The exposure levels from industrial spray application apparently depend on the scale of application, as well as on control measures in use. Without further information, it is assumed that large scale application with limited exposure control can be done with paints containing up to 20% EGBE. The measured values over short periods cannot be extrapolated towards longer periods, because this would lead to oversaturation of the skin. Therefore, a reasonable worst case exposure level of 10,000 mg product per day is assumed, based on the levels mentioned in the TGD and the measurements by Hughson and Aitken (2004). This leads to an estimated exposure to EGBE of 2000 mg on 840 cm^2. Because EGBE is much more volatile than the measured substances, this may be an overestimation. Also, if less large scale tasks are done, the exposure levels may be substantially lower. These uncertainties should be taken into account in the evaluation of the MOS.

Dermal exposure may be lower if suitable gloves are worn.

Biomonitoring data

Three studies of biomonitoring have been reported for painting or coating activities. Results are presented in table 4.16.

In the study performed by INRS (Vincent *et al.,* 1996), the workers most exposed to EGBE were found in the area of cataphoresis. The authors note that cutaneous exposure was prevalent particularly in workers who cleaned car bodies with a rag dipped in EGBE before installing the electrode. Working conditions may have greatly improved since 1993.

In another French study (Delest and Desjeux, 1995), exposure of 54 house painters was also assessed by biological monitoring. Only 3 painters had BAA concentrations in urine higher than the detection limit (2 mg/g creatinine). The main reasons for such low figures were the application technique (brush or roller) and the low EGBE content in the products (<5 %).

An assessment of skin penetration may be made from these biomonitoring studies with the same reasons, techniques and limitations that have been presented in scenario 2. Highest results are also obtained with peak value measurements, especially in this case from metal painting (63 mg/g creatinine and 0.3 ppm inhalation concentration), which gives a dermal equivalent of 96.2 mg/day, corresponding to a dermal load of 320 mg/day, assuming 30% absorption efficiency. As in scenario 2, a negative bias of 30% is also considered; the dermal load would then be re-assessed as ca. 430 mg/day.

In the case of building painting ("decorative"; 13.2 mg/g creatinine and 0.2 ppm inhalation concentration), the calculated dermal equivalent is 16 mg/day, corresponding to a dermal load of 54 mg/day, assuming 30% absorption efficiency. If the same bias is also assumed, the dermal load would be assessed as ca. 70 mg/day.

- Scenario 3-2 Printing

EGBE is a solvent in a range of specialist inks particularly silk-screen inks used by professional trades. However there is a trend from solvent based inks to UV curing inks that contain no solvents.

SIDS (1996) reports that inks contain approximately up to 20 % EGBE. In the enquiry recently performed in the ink formulating industry by CEPE (2002), the information collected is very limited. Only 6 answers were obtained in relation with the EGBE content in printing inks. They indicated content from traces up to 31 %. Screen ink seem to contain the highest concentration. Other recent data provided by one of the main producer of screen printing show that typical percentages range from 2 to 35 % (BP, 2002a). Taking into account all this data, typical maximum contents of 35 % EGBE in silk-screen inks and 20 % in others will be assumed in this assessment.

Inhalation exposure

Measured data

Monitoring data are reported in previous reports (SIDS, 1996 ; NICNAS, 1996 ; ATSDR 1996), some of them are quite old and probably not representative of current working conditions. A compilation of these data is presented in table 4.20, completed with two further references (Auffarth *et al.*, 1998 and Laitinen, 1998). Details have been added when available.

Table 4.20: Atmospheric and biological monitoring during printing (8 hr-TWA inhalation exposure and BAA urinary concentration)

Activity	No/type of sampling	Mean (ppm)	Range (ppm)	Comments / Post-shift BAA urinary excretion (mg/g creatinine)	Reference
Printing press operators	3/p	<0.2	<0.04-0.49	2 %, cleaning printing press rollers	Lewis and Thoburn, 1981
Label production /plate makers and pressmen	7/p	2	1-2		Apol, 1981
Silk screening	6/p	3.4	1.1-5.3		Boiano, 1983
Silk screening Spray printing	p p		max 1.4 max 0.75		Clapp *et al.*, 1984
Silk screening Silk screeners Spray printers Controls	 16/p 5/p 6/p	 6.8 2.6 0.3		up to 45 %	Baker *et al.*, 1985
Silk screening	3/p	4	3-5		Apol, 1986
Silk screening	108/p	0.64	Max 1.14	Half-shift sampling	Veulemans *et al.*, 1987b
Printing (various)	25/a	0.8	0.3-3.5		Veulemans *et al.*, 1987a
Silk screening	14/p 8/a	25 63	13-36 23-169	100 %, open spray troughs and wash table areas	Kullman (NIOSH), 1988
Printing press operators	1/p		8.3		Lee, 1988
Silk-screening (clean-up and maintenance)	5/p	5.2	1.7-9.8	10-50 %; cleaning with a hydrocarbon-based solvent	Salisbury and Bennett, 1987
Printing press operators	2/p	<0.33	nd-0.53	2 %; cleaning printing press rollers	Kaiser, 1990
General printing	9/p	0.64	0.4 - 0.8	9 urinary samples	Sakai *et al.*,

				3.9 (1.3-9.9)	1993
Tampography	84/p	0.2	<0.1-0.7	48 urinary samples 2.2 (<2-7.1)	Vincent *et al.*, 1996
Silk screening	295/p	0.2	<0.1-1.6	154 urinary samples nd[c]	Vincent *et al.*, 1996
Offset printing	39	Nd[b]		11 urinary samples 2.2 (<2-3.8)	Vincent *et al.*, 1996
Printing press operation - semi automatic - 3/4 automatic process - fully automatic	 5/p 14/p 3/p	 0.5 0.5 0.5	 0.3-0.9 0.3-0.9 0.3-0.9		Auffarth *et al.*, 1998
Silk screening	5/p	0.5	0.2-0.7		Auffarth *et al.*, 1998

p: personal sampling; a: area monitoring

b : any concentration upper than 0.1 ppm was detected

c: any concentration upper than 2 mg/l was detected

More recent exposure measurements made in 2001 and provided by one of the main producer of screen printing inks in the EU have been presented by industry (BP, 2002a). Printers are exposed 1-8 hours per day but 2-3 hours is the typical length time when workers are exposed to solvents. Nearly all operations use LEV as well as general ventilation. EGBE was detected in 13 samples amongst 161. The results are presented in table 4.21. The figures represent TWA over 2-3 hours.

Table 4.21: Personal air measurements during screen printing (BP, 2002a)

Activity	No of results where EGBE detected	Mean (ppm)	Maximum (ppm)	5-95 % percentile (ppm)
Print shop	6	0.4	0.7	0.02-0.7
Reclaim	2	0.9	1.7	
Other	5	<0.1	0.2	

Information in database

In the HSE database (Brown *et al.*, 1994), 66 personal exposure (8-hour TWA) have been collected (1986-1991) for screen printing. The range was 0-9.2 ppm (arithmetic mean 1.54 ppm).

Referring to the already cited French COLCHIC database (Vincent, 1999), 45 atmospheric personal samplings have been made between 1987 and 1998 in the printing industry, resulting

in an arithmetic mean concentration of 0.6 ppm (range 0.02-5.2 ppm; median 0.04 ppm; 95th percentile 2.6 ppm). Results collected during the same period for printing activities in different industrial sectors are presented in table 4.22.

Table 4.22: Personal exposure in printing activities made for measurements 60-480 minutes, years 1987-1998 (Vincent, 1999).

Activity	No of results	Mean (ppm)	Range (ppm)	Median (ppm)	95th percentile (ppm)
Screen printing	92	1.1	0.02-6.5	0.4	4
Screen washing	9	0.4	0.04-1.6	-	-
Offset printing	13	0.3	0.02-1.2	0.04	1.2
Cleaning in general	2	2.5	1.2-3.8	-	-
Flexography	20	0.08	0.04-0.1	0.1	0.1
Rotogravure	7	0.04	0.02-0.1	-	

In the printing industry, it should be noted that exposure to EGBE has significantly decreased from 1987-92 to 1993-1998, with an arithmetic mean for personal samplings of 1.4 ppm (median 1.3 ppm; range 0.02-6.5 ppm; 68 samples) for the first period, and 0.3 ppm (median 0.1 ppm; range 0.02-2.5 ppm; 79 samples) for the second ($p<0.01$) (Vincent and Jeandel, 1999).

Modelled data

SIDS calculated the exposure during silk screening and general printing tasks using inks containing 50 % and 20 % of EGBE. Exposure estimates were 10 ppm and 2 ppm respectively. No information is available on the calculation method.

Exposure to vapours during printing is estimated by EASE to be in the range of 10-20 ppm for non dispersive use, direct handling with dilution ventilation.

The model overestimates exposure levels, particularly because of non-consideration of the content of EGBE in the products. The estimates cannot be corrected for the partial vapour pressure because the composition of the formulations is not known. A simple approach based on a reduction of the exposure by a factor equivalent to the EGBE concentration in the mixture (35 % for screen inks and 20 % for others) would lead to exposure levels of:

- 3.5-7 ppm for silk screening

- 2-4 ppm for general printing.

However the validity of these extrapolations is rather questionable.

Summary/statement of the exposure level

Considering the above data, it seems reasonable to make distinctions between the different situations and uses. Mainly based on the data from COLCHIC, the reasonable worst-case exposures proposed would be the following:

- Silk screening (including washing): 4 ppm (20 mg/m^3)

- General printing: 1 ppm (5 mg/m^3)

Dermal exposure

Measurements

In a study performed in Finland by Kuopio Regional Institute of Occupational Health (KRIOH)(RISKOFDERM, 2003b), a part of the EU RISKOFDERM project, potential hand exposure to EGBE was measured during silkscreening on 10 workers in three different enterprises. The measurements were made for 6 to 8 hours by giving protective gloves, which were collected after shift and analysed. Results (given in formulation) are presented in table 4.23a. In the same study, measurements of actual hand exposure indicate that the use of gloves does lower hand exposure significantly. Hands seemed to be the most dominant potential dermal route exposure because EGBE could be found only on a few parts of the body parts of printers.

Table 4.23a: Potential dermal exposure measurements during silkscreening (KRIOH, 2003)

	1st measurement day (µg/cm^2/h)	2nd measurement day (µg/cm^2/h)
No of samples	6	10
Average	5.328[a]	4.068[b]
90th %	15.684	12.937

a : 3 results were below the limit of detection

b : one result was below the limit of detection

The range of the measured exposure values for the formulation was <0.04-85 mg and the 90th percentile was 65 mg/day (unpublished data, from the original data).

Modelling

Dermal exposure may occur during mixing, application and cleaning activities. SIDS (1996) retained continuous skin contact over the work period (8 hours) and a 1000 cm^2 skin area exposed (a hand and a forearm). Intermittent contact seems more appropriate for this scenario (as for painting). Assuming non dispersive use, direct handling and intermittent contact, the EASE model estimates a dermal exposure in the range of 0.1-1 mg of product/cm^2/day. The estimation is made from a formulation containing up to 35 % (screen inks) or 20 % (others) of EGBE and an exposed skin surface area of 840 cm^2 (two hands for consistency with other EU occupational risk assessments). This leads to an estimated external dermal exposure of:

- 29-294 mg/day for silk screening
- 17-168 mg/day for general printing.

Assessment

For general printing, no relevant exposure data are available. The EASE estimate therefore has to be used for risk characterisation. For silk screen printing, a set of 16 potential full shift hand exposure data is available with a 90th percentile of 65 mg/day of product. The "product" in this case consisted of printing ink with up to 10% of EGBE and retarder (up to 100% EGBE) that was used by just two of the printers (as part of the total ink-system). Assuming that the concentration of EGBE in screen printing inks can be up to 35%, the reasonable worst case exposure level for EGBE in this process would be approximately 23 mg/day. Because the measured values are based on a more than 12 measurements and come from different workplaces, they can be considered sufficiently representative for use in risk characterisation. In conclusion, the following range are proposed for dermal exposure during printing:

- 23 mg/day for silk screening
- 168 mg/day for general printing.

Dermal exposure may be much lower if suitable gloves are worn.

Biomonitoring data

The results of two studies of biomonitoring performed in the printing industry are presented in table 4.20.

In the study performed by Vincent *et al*. (1996), EGBE was not the main solvent to which the workers were exposed to. BAA was not detected in the silk screening printing workshops while its concentration was up to 7.1 mg/g creatinine when pads were used.

In the study performed by Kuopio Regional Institute of Occupational Health (KRIOH)(RISKOFDERM, 2003b) and also described above in the paragraph "Dermal exposure – Measurements", biological monitoring data were reported. Workers internal exposure was evaluated by urinary excretion of 2-butoxyacetic acid (BAA) in the first measurements day and in the second measurements day; in the first measurements, the inhalation exposure was excluded by using a respirator and in the second measurements, the dermal exposure was excluded by using protective overalls and gloves. The results are reported in the table 4.23b.

Table 4.23b : Workers'internal exposures (Urany butoxyacetic acid (BAA) excretion, µmol/mol creatinine)

	Urinary butoxyacetic acid (BAA) excretion, µmol/mol creatinine			
	1st measurements day		2nd measurements day	
	Before work shift	After work shift	Before work shift	After work shift
Average	3.2	3.8	3.5	18.4
90th %	3.8	5.5	5.5	40.7

Because of many results below the limit of detection, the correlations based on the results between the actual exposure and BAA excretion are highly inaccurate as it is mentioned in the report KRIOH (**RISKOFDERM, 2003b**).

If a tentative assessment of skin penetration is made in the same conditions that have been presented in scenario 2, biomonitoring data may in this case be entirely explained by inhalation exposure alone. Supposing also a negative bias, it is proposed to come back to the low end of the EASE assessment.

- Scenario 3-3 - Cleaning

The NICNAS report (1996) focuses on the use of EGBE in cleaning products in Australia because of the specific concern raised in this context :

« Due to the large number of cleaning products containing EGBE, a large number of workers may be exposed to the chemical. The main groups of workers who handle cleaning products containing EGBE include:

. carpet cleaners;

. contract cleaners;

. food process workers;

. hospital and nursing home workers;

. hospitality industry workers;

. householders;

. laundry cleaners and workers;

. mechanics;

. metal workers;

. school and office cleaners; and

. window cleaners

Exposure during cleaning is extremely variable, due to differences in frequency and duration of use, strength of solution used, method of application and precautions taken during use…

The strength of solution used in the cleaning process is generally low as the product is usually diluted substantially before use; for example, most surface cleaners specify a dilution ratio in the range 1:3 to 1:100, depending on the application and the soil loading. A large proportion (68 %) of cleaning products contain less than 10 % EGBE (see table 4.24), so the final strength of solution is often less than 1%. A random survey of 20 general surface cleaning products containing <10 % EGBE indicated that the dilution ratio ranged from 1:1 to 1:250, with most ratios in the 1:5 to 1:100 range…

Some products are sold as high level concentrates (>50 % EGBE) which must be diluted with large volumes of water before use. In some cases, products are diluted with hot water (up to 80°C).

Exposure during dilution

Dilution is often carried out daily at the beginning of the shift. While the dilution procedure is usually of short duration, the potential exposure may be greater due to use of higher concentrations of EGBE and the possibility of splashing. Higher exposures may also occur if

the product is diluted with hot water as vapour concentrations may be higher and skin absorption facilitated. If dilution is carried out in a confined space or poorly ventilated area, exposure may be increased.

Exposure during application of cleaning solution

A number of different methods are used to apply the cleaning solution, for example, washing, wiping, mopping and spraying. Approximately half of the cleaning products were used in spray form, with a small number marketed in aerosol spray cans or trigger packs. This method of application will potentially increase both dermal and inhalation exposure as the atmospheric concentration of EGBE will be higher and dermal contact will be increased. The potential for exposure may also be increased where heat is applied during cleaning, for example, cleaning ovens and hot plates".

Table 4.24: Main uses of EGBE containing cleaners in Australia (NICNAS, 1996)

Use	Number	% of total	EGBE %	
			Min.	Max.
Surface cleaner	214	49	0.57	71
Floor stripper	49	11	<1	30.5
Glass/window cleaner	47	10	<1	40
Carpet cleaner	40	9	<1	10-30
Laundry detergent	15	4	<1.5	10-30
Rust remover	11	3	<10	30-60
Oven cleaner	11	3	<1	10-30
Ink/resin remover	9	2	1	10-93
Others	38	9	<10	94

AISE (BP, 2002b) sent recently a questionnaire to their 10 member companies most likely to be using EGBE. Six (belonging to the group of the biggest companies) responded to the request. According to the data collected, the main uses of EGBE for cleaning are:

- industrial and institutional cleaners such as professional laundry products

- institutional kitchen/floor/window cleaners

- strippers

- other industrial cleaners for the food and beverage industry

- industrial car/truck cleaners.

The concentration of EGBE in these professional products is always below 12 %, and typically not higher than 8 %.

Taking into account the NICNAS data together with the information collected in European products registers (table 4.1 and 4.2), a maximum content of 50 % EGBE in professional cleaners with dilution 1:1 and a task duration of 2 hours will be assumed in this assessment.

Inhalation exposure

Measured data

1) Workplace measurements

Monitoring data reported in NICNAS (1996) for cleaning activities are summarised in table 4.25.

Table 4.25: Measured atmospheric exposure (TWA) in cleaning activities.

Activity	Nb/type of sampling	Mean (ppm)	Range (ppm)	EGBE % and comments	Reference
Window cleaning in hospitals	(4 w)		<0.2	Cleaner applied in spray, gloves worn	Apol and Cone, 1983
Cleaning in food plant	(1 w)		1.6	0.3 %, mechanical floor scrubbing, 95 min sampling, gloves, overalls, boots worn	Apol and Johnson, 1979
School cleaning	(4 w)		<0.7	0.25 %, cleaner applied in liquid and spray form	Rhyder, 1992
Window cleaning Cleaning cars: garage A	10/p(2 w)	0.5	<0.1-1.2	14.4 %, 0.8-5 h exposure, no gloves worn	Vincent *et al.*, 1993
garage B	10/p(6 w)	0.84	<0.1-2.8	21.2 %, 0.3-4 h exposure, no gloves worn	
garage C	6/p (3 w)	<0.1		5.7 %, 0.7-2 h exposure, no gloves worn	
garage D	4/p (2 w)	4.9	2.9-7.3	21.2 %, 5.3 h exposure, short sleeves, gloves worn	
Office cleaners[1]: group A	32/p(8 w)	0.32	0.3-0.73	9.8 %, 15 min exposure, no gloves worn	
group B	8/p (2 w)	<0.3		0.9 %, 15 min exposure, no gloves worn	

p: personal sampling; a: area monitoring; w: workers.

Note 1: for office cleaners, results are for half-day shift air samples.

2) Experimental data

A series of experiments was conducted in a room-size, controlled environment chamber to evaluate a user's inhalation and dermal exposure to constituents of a household spray cleaner dispensed from either a hand-trigger pump bottle or an aerosol can (Furtaw *et al.*, 1997).

Airborne concentrations and the amounts transferred to gloves worn by the product user were measured for EGBE at 5 % weight product concentration. The measured air exposure concentrations of EGBE were on average 5.7 ppm; air exposure concentrations resulting from a typical 20-minute usage were well below the OSHA OEL of 25 ppm.

NICNAS estimation

The inhalation exposure during cleaning activities was estimated by NICNAS (1996) for various EGBE concentration in products and taken up by SIDS (1996). The reasoning and the results are presented below.

In the monitoring data available, TWA air concentrations up to 9.7 ppm were obtained for print machine operators using a cleaning solvent containing 10 – 50 % EGBE (Salisbury and Bennett, 1987) and up to 7.3 ppm for workers cleaning car windows with a 21.2 % solution applied as a spray (Vincent *et al.*, 1993). Based on this data, the EGBE air concentration selected for worst-case estimates for a 30 % cleaning solution was 10 ppm TWA.

For cleaning solutions containing <1% EGBE, the atmospheric concentrations were generally below the limit of detection; however, a personal monitoring reading of 1.6 ppm was obtained during floor scrubbing with a 0.3 % solution (Apol and Johnson, 1979). Consequently, an air concentration of 2 ppm was selected for 0.1 and 1 % cleaning solutions.

Table 4.26: Exposure estimates for use of cleaning products (SIDS, 1996)

% EGBE	Max. exposure (ppm)
0.1	2
1.0	2
10	4
30	10

Modelled data

Several parameters should be chosen to correspond to the real variability of conditions of use of cleaning products:

- wide dispersive use, low tendency to become airborne, direct handling would give 200-300 ppm,
- wide dispersive use, low tendency to become airborne, direct handling and dilution ventilation, would give an estimation of 100-140 ppm.

Assuming the exposure would occur 2 hours a day, this leads to exposure levels of 50-75 ppm or 25-35 ppm, averaged on a shift duration.

The model overestimates exposure levels, particularly because of non-consideration of the content of EGBE in the products. The estimates cannot be corrected for the partial vapour pressure because the composition of the formulations is not known. A simple approach based on a reduction of the exposure by a factor equivalent to the EGBE concentration in the mixture (25 % if the estimation is made for a formulation containing up to 50 % of EGBE

with dilution 1:1) would lead to exposure levels of 12.5-18.7 ppm or 1-8.7 ppm. However the validity of these estimates is rather questionable.

Summary/statement of the exposure level

Only limited measured exposure data are available and they are difficult to analyse because of the great variability of exposure conditions (in particular duration and content in the product).

The highest exposure of 7.3 ppm measured for workers cleaning cars with a 21.2 % solution may be considered as a worst case for this scenario. Therefore it seems cautious to keep the NICNAS estimation of 10 ppm (49 mg/m^3).

Dermal exposure

Measured data

A series of experiments was conducted in a room-size, controlled environment chamber to evaluate a user's inhalation and dermal exposure to constituents of a household spray cleaner dispensed from either a hand-trigger pump bottle or an aerosol can (Furtaw *et al.,* 1997). Approximately 10 % of the amount of product used was transferred to the user's gloves (simulating dermal exposure), with the great majority found on the palms rather than the backs of the hands (88 % according to data presented). Using results presented, skin contamination can be evaluated (for both hands) at 0.22 mg/cm²/day for a 5 % solution. Note also that the right hand is roughly 4 times more contaminated than the left one (right-handed worker).

In a study performed by TNO (RISKOFDERM, 2003a), a part of the RISKOFDERM project, potential hand exposure to an analogous but less volatile glycol ether DEGBE (2-(2-butoxyethoxy)ethanol) was measured during spraying of a diluted cleaning agent (sampling duration between 6 and 18 min) and during wiping (car washing, sampling duration between 5 and 12 min and cumulative duration of wiping per day 24-90 min). For spraying, the measurements were made using butyl rubber protective (recommended by the employers), the in-use concentration was between 0.0007 and 1.1 % of DEGBE. For wiping, hand exposure was evaluated by the hand wash method, the in-use concentration was between 0.04 and 0.82 %. Exposure was mainly due to exposure on the hands. Results (given in product as well as recalculated to in-use product) are presented table 4.27. They show a substantially higher exposure rate during wiping activities.

Table 4.27: Dermal exposure rates during spraying and wiping (RISKOFDERM, 2003b)

Hands exposure	Range (mg)	AM (mg)	GM (mg)	GSD (mg)	AM (µg/cm^2/min)*	GM (µg/cm^2/min)*
Spraying (n=12)						
- DEGBE	36-6302[a]	1000[a]	438[a]	3.73	0.15	0.04
- product	37-1974	719	522	2.72	68.4	48.1
Wiping (n=12)						
- DEGBE	1.8-29.8	10	6.8	2.50	1.8	1.1
- product	1508-4861	3161	2985	1.45	535	499

Nota a : these values are in µg

* Asurface area of 820 cm^2 was assumed for the hands

The 90th percentiles (expressed in mg of total diluted product) for the sampling durations were 1000 mg for spray application and 4000 mg for car cleaning (unpublished data, derived from original data).

Modelled data

Dermal exposure is clearly predominant during cleaning activities. SIDS (1996) retained continuous skin contact over the work period (8 hours) and a 1000 cm^2 skin surface area exposed (a hand and a forearm). Intermittent contact seems more appropriate for this scenario because the duration will generally be a part of the shift and continuous contact would probably overestimate the exposure.

Assuming wide dispersive use, direct handling and intermittent contact, the EASE model estimates a dermal exposure in the range of 1-5 mg of product/cm^2/day. The worst case estimation is made for a formulation containing up to 50 % of EGBE with dilution 1:1 and a 420 cm^2 skin surface area exposed (one hand). This leads to an estimated external dermal exposure of 105 - 525 mg/day for cleaning.

Assessment

The results of the TNO study during spraying and wiping indicate relatively high potential for dermal exposure. However interpretation is difficult due to the relative small data set of measurements and the fact that during spraying the use of sample gloves may lead to different exposure levels than direct contact to the skin. Furthermore, EGBE is much more volatile than DEGBE and this may lead to higher evaporation from the skin and lower effective exposure levels. Also, for tasks with cumulative exposure durations substantially longer than the sampling durations, extrapolation is difficult due to the effects of evaporation as well as the possibility of saturation of the skin with product, specifically for situations with direct contact and immersion, such as cleaning with a sponge or cloth. On the other hand, the kind of tasks performed with these products will often not be done for many hours per day.

Although the measured data are difficult to interpret, they provide much more real exposure information than the EASE model, whose dermal exposure part is not based on real dermal exposure measurements.

A rough estimator of reasonable worst case dermal exposure (to the hands) is therefore assumed to be the 90th percentile of the exposure levels during the measurement period. This appears to be reasonable, because the measurement periods were aimed at a covering one full "cycle" of the relevant activities and they were followed by periods without dermal exposure, when evaporation and other effects may lower the skin contamination levels before a new "cycle" will again contaminate the skin. Reasonable worst case exposure levels for hand exposure to diluted cleaning products containing up to 25% of EGBE are therefore expected to be:

- spray application: 250 mg/day;
- manual application including immersion of the hands into the solution: 1000 mg/day.

The uncertainty in these values is rather large, because of the aspects mentioned earlier. This should be taken into account in the evaluation of the MOS. The estimate for manual application is substantially higher than that of EASE, while the estimate for spray application

is similar to that of EASE. However, the measured data are preferred. Dermal exposure may be much lower if suitable gloves are worn.

Biological monitoring data

A study was conducted in France by INRS to evaluate the occupational exposure to EGBE of workers using window cleaning agents: 10 cleaning women working in a town hall and 13 automobile cleaners working in 4 garages (Vincent *et al.,* 1993). EGBE was detected in all the window cleaning agents (0.9 to 21.2 %). The results of exposures by job category are presented in table 4.28.

Table 4.28: Characterisation of exposure by job category (Vincent *et al.*, 1993)

Job category	Air concentration of EGBE (ppm)			Pre-shift urinary concentration of BAA (mg/g creatinine)			End-shift urinary concentration of BAA (mg/g creatinine)		
	No of samples	Mean	Range	No of samples	Mean	Range	No of samples	Mean	Range
Cleaner of new cars	15	2.33	<0.10-7.33	14	17.9	<2-98.6	12	111.3	12.7-371
Cleaner of used cars	15	0.36	<0.10-1.52	12	4.8	<2-33	11	6.3	<2-24.4
Office cleaner	32	0.32	<0.30-0.73	32	2.1	<2-4.6	32	2.1	2-3.3

The authors noted that the high concentrations of urinary BAA relative to the EGBE TWA concentrations, indicated that dermal absorption was the predominant route of worker exposure. They concluded that for workers in these occupations not wearing protective gloves, environmental sampling probably underestimates the exposure, and that biomonitoring of urinary BAA appears to be the best method for estimating overall exposures.

To indirectly assess skin exposure using these data as already proposed in the preceding scenarios, with the same limitations, it is seen that highest results are also obtained with peak value measurements in new car cleaning activity (272.4 mg/g creatinine and 7.3 ppm inhalation concentration), which gives a dermal equivalent of 240 mg/day, corresponding to a dermal load of 800 mg/day, assuming 30% absorption efficiency. For the same reasons already discussed in scenario 2, a negative bias is also assumed as 30%, which gives a dermal load re-assessed as ca. 1040 mg/day.

Rettenmeier *et al.* (1993) reported biological monitoring data on car cleaners. Total BAA (free BAA and BAA glutamine conjugate) in 6 post-shift samples averaged 243 (range 67 to 935) mg/g creatinine (assuming an average urinary creatinine concentration of 1.25g/l). No air monitoring was gathered in this study, which does not allow for an indirect evaluation of skin penetration.

- Data from other uses (miscellaneous)

Exposure of 13 workers using cutting oils containing EGBE (1 % to 5 %) in a mechanical workshop was measured by biological monitoring. No atmospheric measurements were made in this case. Concentrations of BAA in post-shift urine varied from <2 to 8.3 mg /g creatinine. This result reflects a low exposure (Vincent *et al.*, 1996).

Exposure of 53 hairdressers working in 10 shops was evaluated. The composition of 43 products was analysed, EGBE was detected in 8 products (0.5 to 5 %). No solvent was detected in the atmosphere and BAA in the post-shift urine was <2 mg/g creatinine (detection limit). This result was probably related to the very low quantities of products handled and the use of gloves (Vincent *et al.*, 1996).

Seven personal exposure measurements made during removal of asbestos-containing mastic result in EGBE concentrations in the range from 2 to 22 ppm with a mean of 12 ppm (Kelly, 1993 quoted in ATSDR, 1998).

4.1.1.2.4 Summary of occupational exposure

Differences in assessments of skin exposure are striking. One could a priori consider that the work undertaken within the "RISKOFDERM" project should be given priority, since it is recent and has explored a diversity of real working conditions. In fact, this project has objectified the great difficulties of this exercise, leading Kromhout *et al*. (2004) to conclude: "For the time being, actual dermal exposure measurements and a better understanding of actual determinants of dermal exposure seem to be a necessity in order to evaluate dermal exposure hazards properly." Important characteristics that may be retained are the extreme variability of skin exposure (e.g. "geometric mean levels of dermal exposure range over up to 4 orders of magnitude between dermal exposure operation units when exposure is expressed in milligrams.") and the predominance of hand exposure in very different tasks (e.g. Eriksson and Wiklund, 2004 ; Eriksson *et al*., 2004 ; Fransman *et al*., 2004 ; Gijsbers *et al*., 2004 ; Roff *et al*., 2004a and 2004b).
In the present situation, skin exposure may also be indirectly assessed in real situations with EGBE itself (and not a parent compound with very different physico-chemical properties), so these data should also be considered as reliable although somewhat different.
To take these 2 sources into account, it is proposed to take the available RISKOFDERM data as a basis and to adapt them to EGBE using biomonitoring data when applicable. This is done in the following table 4.29 (last column) where a factor of 4 has been applied to the biomonitoring data when already exposed reasons show that the RISKOFDERM results may not be directly used.

Table 4.29: Summary of proposed reasonable worst case exposures.

Scenario	8-hour TWA inhalation (mg/m^3)	Dermal worst case derived from biomonitoring data (mg/day)	External dermal (mg/day)	Dermal worst case, EASE & RISKOFDERM & biomonit. Retained values for risk characterisation (mg/day)
1 - Manufacture	12	No data	42[b]	42
2 - Formulation	15.7	500	11,600[c]	2,000[e]
3 - Use of products				
3.1.1.1 - Coating/Painting – industrial: spraying	58.1		2,000[c]	2,000
3.1.1.2 - Coating/Painting – industrial: other works	30.4	430 (metal painting)	240[c]	430
3.1.2 - Coating/Painting-decorative	30.4	70	36[c]	70
3.2.1 – Printing - silk screening	20	Negligible	23[b]	23
3.2.2 – Printing - general printing	5	Negligible[d]	168[b]	168
3.3.1 – Cleaning - spraying	49		250[c]	250
3.3.2 – Cleaning - wiping	49	1,040	1,000[c]	1,040

Nota b : modelled data from EASE
Nota c : data derived from measurements with a less volatile solvent (DEGBE)

Nota d: in this case, due to a probable negative bias (see text), it is proposed to apply the low end of the EASE assessment.

Nota e: see explanations in the § 4.1.1.2.2 – *Assessment*

4.1.1.3 Consumer exposure

4.1.1.3.1 Exposure from uses

EGBE is used as a solvent in many products available for consumers as, paints, paints thinners and cleaning products. EGBE is no more used in cosmetics.

Cleaning products which contain EGBE include general surface cleaners, floor strippers, window cleaners, carpet cleaners, spot cleaners, rust removers, oven and grease trap cleaners, laundry detergent, cars cleaners, bathrooms and toilets cleaners and disinfectants, ink and resin removers.

Paints that contain EGBE are both waterborne paints and solvent-borne paints (CEPE enquiry, 2002) including varnishes and products to preserve wood.

An investigation was made by the journal "Que choisir" in 2001 showing that two paints contained 1 % of EGBE and that in cleaners for cars, the concentrations of EGBE ranged from 0.3 to 4 %.

Another source of exposure is linked to the stoppers of bottles used for perfusion in medicine which are made with natural or artificial rubbers. Chemicals used in rubber formulations can leach from rubber stoppers during sterilisation and enter the bloodstream of patients when the perfusion is administered. The concentrations of leached stoppers components found in parenteral solutions concentrations were measured by GC/MS after extraction by methylene chloride. Two parenteral solutions were tested containing 5 % dextrose and one containing 5 % sodium bicarbonate. Results indicate a mean release of 68 µg/150 ml EGBE in dextrose solutions (respectively 70 and 65 µg/150 ml for each sample) and a release of 13 µg/500 ml EGBE in the sodium bicarbonate solution. The size of the sampling is too low to allow a good estimation of the real contamination of perfusion solutions by EGBE (Danielson, 1992). This scenario will not be used in the risk characterisation as it is of concern for a few people and it is not a frequent event to the single consumer. The consumer is exposed by this way only if two conditions are simultaneous: he needs a perfusion and the rubberstopper contains EGBE. By another way, the number of evaluations of the release of EGBE from rubberstoppers is too weak to allow a good estimation of the exposure.

Scenario 1: Household surface cleaners

Two routes of exposures have to be considered during the use of household surface cleaners: inhalation and dermal exposure.

Cleaning frequency and duration were assessed by a questionnaire applied to 342 subjects (75 % of women), 18 to 80 years old (Kovacs *et al.,* 1997). Subjects reported a longer duration for kitchen cleaning task that for the bathroom, with mean values of 37.7 and 30.7 mn respectively. ($t = 3.67$; $p<0.0005$). Depending on the frequency of cleaning in a week, the duration of exposure to household surface cleaners can be estimated to mean values of 98 and 59 mn/week spent cleaning kitchen and bathroom respectively. When broken down by gender, the mean values for the kitchen are respectively 105 and 78 mn/week for women and men and, for the bathroom cleaning, 64 and 44 mn/week respectively.

In the Technical Guidance Document, data from AISE (2002) are provided for the use of surface cleaners : the highest quantity of liquid surface cleaner used is 110 g/task when it is diluted in 5 l of wash water volume and the longest duration of exposure is 20 minutes. For sprays, the highest quantity of liquid used is 30 g/task during 10 minutes.

Five tests were conducted in a 24.5 m^3, controlled-environment chamber to evaluate a user's inhalation and dermal exposure to constituents of a household spray cleaner dispensed from either a hand-trigger pump bottle or an aerosol can (Furtaw *et al.,* 1997). The temperature of the room was 23 °C, the humidity was monitored but not controlled, outdoor air exchange rate was set at approximately 0.5 air changes per hour. The cleaning product contained 5 % EGBE. Subjects were asked to clean two "Formica" sheets (approximately 0.76 m x 1.83 m), one being placed horizontally in the room, the other being vertical on the wall. The cleaning process, which consisted on spraying the cleaning product on the "Formica" surface then wiping it with a cotton cloth continued approximately for 20 minutes. About 28 g of the product was used during the cleaning process. Five tests were performed. During the cleaning period, air from the breathing zone of the experimenter and air in a corner of the room were sampled on a charcoal tubes (type ORBO^{TM}-32) and analysed on an automated gas chromatograph (Microsensor systems Inc. Model MSI-301). During the cleaning, the experimenter wore cotton gloves over PVC gloves. The purpose of the cotton gloves was to absorb cleaning solution that would have been in contact with the hands of the person if he had not been wearing gloves. The amount of EGBE transferred to the gloves is about 6.3 % ±

3.8 % of the quantity used on the right hand and1.6% ± 3.0 % on the left one and a greater amount is found on the frontal area of the hand than on the back of the hand. The mass and size of particles emitted decay much faster than the air EGBE concentration in the room that suggests that EGBE is being reversibly absorbed and re-emitted from chamber surfaces. Median diameters of particles vary from 0.749 to 0.976 µm. The personal average ORBO^{TM}-tube result was 5.70 ppm.

The interactions between EGBE and indoor materials were studied by placing the indoor material as a membrane between two air compartments. Both compartments are constantly flushed with air, the airflow containing EGBE leading to the primary cell, the other airflow with clean air entering the secondary cell. A permeation factor was defined as: PF(%) = C_A x 100/C_B where C_A is the outlet concentration of the secondary cell and C_B the outlet concentration of the primary cell. Average inlet concentration of EGBE was 483.7 µg/m^3 with a standard deviation of 2.2 µg/m^3. PF values were 88.1 % and 9.4 % for wallpapers, 70.4 % for acrylic wall-covering, 61.6 % for carpets. The capacity of retention of each material was also calculated. It varies from 5.3 to 33.3 % (mean value = 19.2 %; SD = 9.8 %) according to the category of wall paper (n=6), 3.1 % for the textile wall-covering (n=1), 82.9 % for the carpet (n=1), 37.4 for the chipboard (n=1), 12.1 for the PVC flooring (n=1) and 80.4 % for the gypsum board (n=1). All those materials can release EGBE after the use of cleaning products (Meininghaus and Knoppel, 1999).

NICNAS (1996), using data available from monitoring of workers using window cleaners (n = 23) or surface cleaners (n = 19), estimates that a good worst-case estimate for concentrations in air during the use of cleaning solutions is:

- 10 ppm (49 mg/m^3) for a 30 % cleaning solution

- 2 ppm (9.8 mg/m^3) for a 0.1 or 1 % cleaning solution

- 4 ppm (19.6 mg/m^3) for a 10 % cleaning solution

OECD (1997) estimated the exposure of consumers by cleaning products to 0.58 mg/kg/event assuming a respiratory rate of 0.8 m^3/hour and a bodyweight of 60 kg for a 1 hour cleaning event. For dermal absorption, a skin absorption rate of 0.2 mg/cm^2/hour and a skin surface of 1,000 cm^2 were assumed.

Zhu *et al.* (2001) made the determination of EGBE emissions from selected consumer products and made assessment of the inhalation exposure associated with cleaning tasks. Two all-purposes cleaners were tested and two spray glass cleaners. Concentrations of the products ranged from 0.744 % to 1.28 % for general-purposes cleaners, from 0.50 to 0.87 for glass cleaners. A standard room of volume 17.4 m^3 was used for the test. An average air exchange of 0.5 h^{-1} was applied to the standard room. It was assumed that for all-purpose spray-cleaners a mass of 76 g of product was applied on a surface of 4.5 m^2 for each task whereas a mass of 17 g of spray glass cleaners was applied to a surface of 2.3 m^2 for each task. The test included four various tasks for general-purposes cleaners: clean outside of cabinets during 0.87 hours, clean counters during 0.42 hours, clean bathroom or other tiled or ceramic walls during 0.57 hours, clean outside of refrigerator and other appliances during 0.32 hours. Two tasks were assessed for glass cleaners: clean inside of windows during 2.12 hours, clean other glass surfaces like mirrors or tables during 0.40 hours. Samples of air in the chamber were collected every half hour or every hour or every 2 hours depending on the stage of the emission. An inhalation rate of 1.3 m^3/h and a mean bodyweight of 70.9 kg were used for exposure assessment. The daily exposure by task ranged from 0.013 to 0.063 mg/kg bw/d if only one of the tasks were performed in one day (mean value = 0.0325mg/kg bw/d; standard deviation =

0.017 mg/kg bw/d). If they were all performed on the same day, the sum of daily intake by inhalation is 0.186 mg/kg bw/d with the first product and 0.075 mg/kg bw/d with the second. In the case of glass cleaners, the daily exposure by task ranged from 0.001 to 0.004 mg/kg bw/d if only one of the tasks were performed in one day (mean value = 0.003 mg/kg bw/d; standard deviation = 0.001 mg/kg bw/d). If they were all performed on the same day, the sum of daily intake by inhalation is 0.006 mg/kg bw/d with the first product and 0.004 mg/kg bw/d with the second.

Fortmann *et al.* (1999) performed tests to determine gas-phase and particulate emissions during application of a water-based cleaner with a hand-pump sprayer. Tests were performed in the bathroom of a test house and in an 18m^3 controlled-environment test-room. The water-base cleaner which contained 50 mg/g of EGBE was applied to realistic surfaces (counter tops, glass, mirrors) with the manufacturer's hand-pump spray. During application, air samples were collected on Tenax for analysis of EGBE by gas chromatography. Concentrations of droplets were measured continuously with aerodynamic particle analysers. The average concentration of EGBE measured by collection on Tenax for a 10-minute period in a closed bathroom during and following application of 96 g of a 50/1 (v/v) dilution of the cleaner was 760 µg/m^3. The mean particle droplet size during the application period was in the range of 1 to 2 µm. Particle concentrations increased during application with the sprayer but dropped quickly.

Table 4.30: summary of studies about exposure by household cleaning products

Study	Route of exposure	Concentration of EGBE in product	Duration of exposure	number of tests	Concentration	Daily exposure
Furtaw *et al.*, 1997	Inhalation	5 %	20 min	n = 5	5.7 ppm (28 mg/m^3) in air	
Furtaw *et al.*, 1997	Skin	5 %	20 min	n = 5	6.3 ± 3.0 % (right hand) 1.6 ± 3.0 % (left hand)	
Meininghaus and Knoppel, 1999	Inhalation				0.48 mg/m^3 in air	
NICNAS, 1996	Inhalation	30 %		n = 42	10 ppm (49 mg/m^3) in air	
		10 %			4 ppm (19.6 mg/m^3) in air	
		0.1 to 1 %			2 ppm (9.8 mg/m^3) in air	
OECD, 1997	Inhalation + skin		1 hour	modelled data		0.58 mg/kg/event
Zhu, 2001	Inhalation	0.74 – 1.28 % 0.5 – 0.87 %	0.32-2.18 hr 0.4 – 2.52hr	n = 2 n = 2	2.8 – 62 mg/m^3	0.013-0.063mg/kg/day 0.001-0.004mg/kg/day
Fortmann *et al.*, 1999	Inhalation	0.1 %	10 min	n = 5	0.76 mg/m^3 in air	

The risk assessment will be based on data from NICNAS (1996) and AISE (2002) for inhalation exposure, assuming a cleaning product containing 10 % EGBE leading to an air concentration of 19.6 mg/m^3 and a duration of exposure of 20 minutes (1/3 hour)for each event. The concentration of 10% will be chosen as a reasonable worst case as according to the Danish product register (2001) (cf table 4.1), products containing 10 % EGBE or less represents 76 % of the whole market of products containing EGBE (93 % contain 20 % or less EGBE). According to the french register SEPIA from INRS (2003), in 81 % of French cleaning products containing EGBE, its concentration is equal or less than 10 % (93% contain 20 % or less EGBE). These data are furnished without any distinction between products sold to general consumers and these sold in the occupational field and generally, cleaning products sold in the occupational field are more concentrated in cleaning substances than those sold to consumers are. Data indicates that the great majority of cleaning products available to consumers contain less than 10% EGBE. It cannot be excluded that some products may be available with higher levels. However, anecdotal evidence suggests that such products are either used in small amounts (e.g. spot cleaners) or are diluted before use. Combined with other evidence to indicate a decline in use in this application, a figure of 10% is set as a reasonable worse case for the composition of EGBE in consumer cleaning products for the scenarios modelled. The worst case considered will be the use of undiluted product.

For inhalation, based on a 20 m^3 respiratory volume a day for an adult weighing 60 kg, the exposure will be:

$$\frac{20 \times 19.6 \times 1}{60 \times 24 \times 3} = 0.09 \text{ mg/kg/d}$$

For skin contact, measured data from Furtaw *et al.* (1997) will be used to characterise each event : use of 28 g of undiluted product for a 20 minutes event (AISE, 2002) is considered as the worst case, transfer of 6.3 + 3.8 % of the quantity used on the right hand and of 1.6 + 3.0 % to the left hand. For a 10 % EGBE product, the dermal exposure will be:

$$\frac{0.1 \times 28 \times 1000 \times (0.10 + 0.05)}{60} = 7 \text{ mg/kg/d}$$

Scenario 2: Measurements in indoor air

Measurements of EGBE concentrations were performed in the indoor air of flats (Kirchner, 2002). Preliminary results indicate that the 90th percentile is 3 µg/m^3 in the sleeping-room with a high value of 14 µg/m^3 in a flat. The 90th percentile is 3.5 µg/m^3 in the kitchen with a high value of 24 µg/m^3 in a flat. Releases from building materials were also evaluated. Simulations were made by installing new carpets or new floor coverings or by applying paints on the walls of reference rooms. After 24 hours, the concentrations of EGBE in air ranged from below the detection limit to 3.8 µg/m^3 (mean value = 1.7 µg/m^3; SD = 1.5 µg/m^3) when release from carpets were tested. After 28 days, it ranged from non detectable to 23.3 µg/m^3 (mean value = 6.1 µg/m^3; SD = 9.0 µg/m^3). With other categories of floor coverings, it ranged from non detectable to 5.6 µg/m^3 (mean value = 2.5 µg/m^3; SD = 2.7 µg/m^3) after 24 hours and from non detectable to 59.6 µg/m^3 (mean value = 27.5 µg/m^3; SD = 30.9 µg/m^3) after 28

days. Release by paints applied on the wall were at non detectable values after 24 hours and reached 24 and 298 µg/m^3 respectively for the two paints tested 28 days after application.

The same type of measurements was performed in apartments in Italy: in five of them, EGBE was not detected, in a sixth, it was found at a concentration of 8 µg/m^3 (De Bortoli *et al.,* 1986).

A study of the daily indoor concentrations for all indoor site types (n = 14) gave the following results: average air pollution by EGBE = 0.214 ppb (1 µg/m^3); median concentration = 0.075 ppb (0.4 µg/m^3); 25th percentile = 0.030 ppb (0.1 µg/m^3); 75th percentile = 0.360 ppb (1.7 µg/m^3) (Shah and Singh, 1988).

According to data furnished by the FIPEC and SIGMAKALOM (Conseil supérieur d'hygiène publique de France (CSHPF), 2001), consumers should inhale 1 g of EGBE during a period of 30 days.

Table 4.31: summary of studies about exposure by indoor air

Study	Concentrations in air	Number of measurements	Mean values	Reasonable Worst case exposure
Kirchner (2002)	Bedroom : 3 µg/m^{3*} (highest value : 14 µg/m^3 in 1 flat) Kitchen : 3.5 µg/m^3 (highest value : 24 µg/m^3 in 1 flat)	n = 57		24 µg/m^3 in 1 flat)
	Release from carpets, 0-28days : non detectable to 23.3 µg/m^3	n = 6	24 hours after : 1.7 µg/m^3 28 days after : 6.1 µg/m^3	23.3 µg/m^3
	Release from new floor covering, 0-28days : non detectable to 59.6 µg/m^3	n = 5	24 hours after : 2.5 µg/m^3 28 days after : 27.5 µg/m^3	59.6 µg/m^3
	Release from paints, 0-28days : non detectable to 298 µg/m^3	n = 2		298 µg/m^3
De Bortoli *et al.* (1986)	8 µg/m^3 (Found in only 1 out of 6 flats)	surveillance in 6 flats during 1983 et the beginning of 1984 n = ?		8 µg/m^3
Shah and Singh (1988)	1.7 µg/m^3 **	n = 14	1 µg/m^3	1.7 µg/m^3
CSHPF (2001)	1g/30 days	modelled data by WPEM***		1g/30 days

* 90th percentile
** 75th percentile
*** WPEM = Wall Paint Exposure assessment developed by US EPA

As a worst case approach, the scenario chosen will be: rooms with paints releasing 298 µg/m^3 of EGBE and with a new covering floor releasing 59.6 µg/m^3. For this scenario, the only exposure is by inhalation of indoor air. For inhalation; based on a 20 m^3 respiratory volume a day for an adult weighing 60 kg and a 20 hours duration of exposure, the exposure will be:

$$\frac{20 \text{ x } (59 + 298) \text{ x } 20}{60 \text{ x } 24} = \quad 0.1 \text{ mg/kg/d}$$

Scenario 3: Painting

Studies have been made to evaluate indoor concentrations of EGBE from paints.

Norbäck *et al* (1995 and 1996) measured painters exposure to EGBE by sampling air in their breathing zone for one hour as they were applying various water-based paints: wall paints, ceiling paints, wood paints and wet room paints (N = 20 samples). All paints were applied by roller except one case of wall paint that was sprayed. Most of the measurements (90 %) were performed during renovation of old buildings. The average room temperature was 19.3 °C (17.5-20.5 °C) and the relative air humidity ranged from 34 % to 83 % (arithmetic mean value = 58 %). EGBE was detected during painting of walls and wet room painting. The arithmetic mean for EGBE is a concentration of 59.6 µg/m^3, the geometric mean is 8 µg/m^3 (geometric standard deviation = 4.2 µg/m^3) with a highest value of 730 µg/m^3.

Hansen *et al.* (1987) measured volatiles emitted during the use of waterborne paints in one room during 3 days. 13 samplings were performed by adsorbing the organic vapours present in the ambient air.. The organic vapours were adsorbed on polymer in cartridges of stainless steel (Tenax AT, 60-80 mesh). The sampling time was 20 minutes corresponding to a sampling volume of 0.9 l. Samples were analysed by gas chromatography using a flame-ionisation detector. The content of EGBE in the paints ranged from 0 to 1.4 % w/w and the concentration in air of the workplaces ranged from 2 to 60 mg/m^3 .

OECD (1997) estimated the exposure of consumers by paints and surface coatings to 1.05 mg/kg/event assuming a respiratory rate of 0.8 m^3/hour and a bodyweight of 60 kg for a 6 hour painting period. For dermal absorption, a skin absorption rate of 0.2 mg/cm^2/hour and a skin surface of 1,000 m^2 were assumed.

EPA (2001) studied the emissions of EGBE from paints applied on two types of surfaces: stainless steel plate and gypsum board. The concentrations of EGBE in the paints ranged from 4.68 to 5.20 mg/g (mean value = 4.98, n = 4). Peak concentrations measured in the air of the environment chambers were higher when the surface is a stainless steel plate (11.6 mg/m^3) than on gypsum board (5.14 mg/m^3). 93 % of the EGBE is emitted during the 336-hour testing period when applied on stainless steel plate, 14 % when painting on gypsum board. The study was prolonged with gypsum board. After a rapid decrease in concentrations of EGBE in the environment chambers, levels of EGBE in the air remain at values near 0.01 mg/m^3 after 3 months.

CEPE (2002) considers that a 3% concentration in paints can be considered as a reasonable worst case for decorative paints based on data gathered from their member companies (see table 4.14).

Table 4.32: Summary of studies about exposure by paints

Study	Average exposure	Reasonable Worst case exposure	number of measurements	Comment
Norback, 1995-1996	59.6 µg/m^3	730 µg/m^3	N = 20	
Hansen *et al.*, 1987		2-60 mg/m^3	N = 13	EGBE content in paint 0-1.4 %
OECD, 1997	1.05 mg/kg/event		modelled data	6 hours painting period
EPA, 2001	5.14 mg/m^3 to 11.6 mg/m^3	11.6 mg/m^3	N = 4	2 weeks. Non adsorbent surface. Max EGBE content in paint 0.52 %
	0.01 mg/m^3	0.01 mg/m^3		value 3 months after painting
CEPE, 2002				EGBE content in paint 3 %

The CONSEXPO model can also be used to estimate exposure. Another alternative model is the USEPA Wallpaint model. The parameters used in both models are shown in Appendix A. The calculated inhalation exposure results are :

	CONSEXPO (worst case inhalation exposure)	WPEM inhalation exposure
Exposure per event (over 133 minutes)	377 mg/m^3	144 mg/m^3 instantaneous (8 hr TWA = 27 mg/m^3)
Calculated external dose per event (60 kg/person)	11.6 mg/kg/d	Based on TWA : 3mg/kg/d Model prediction : 19.8 mg/kg/d

To verify if this value can be considered as realistic, the same calculation was performed with a 1.5 % concentration of EGBE in paints to compare with the value obtained with measured data.
A reasonable worst case inhalation exposure can be based on data from Hansen *et al.* (1987) for a 1.5% concentration of EGBE as the data were collected in real situations (renovation of old apartments in a building) and frequent samplings were performed during a 3 days work. Assuming a respiratory rate of 20 m^3/day, a 6 hours event in one day and a mean bodyweight of 60 kg, the inhalation exposure by paints is:

$$\frac{20 \times 60 \times 6}{60 \times 24} = 5 \text{ mg/kg/d}$$

Modelling the exposure according to the Technical Guidance Document, the inhalation exposure linked to the use of a 1.5% EGBE paint should be: 64.2 mg/kg/d. This shows that in this case, the modelling strongly overestimates the inhalation exposure (factor = 12.84).

For CONSEXPO and WPEM models, calculations for a paint containing 1.5 % EGBE give the following results :

	CONSEXPO (worst case inhalation exposure)	WPEM inhalation exposure
Exposure per event (over 133 minutes)	188.5 mg/m^3	72 mg/m^3 instantaneous (8 hr TWA = 27 mg/m^3)
Calculated external dose per event (60 kg/person)	5.8 mg/kg/d	Based on TWA : 1.5 mg/kg/d Model prediction : 9.9 mg/kg/d
Comparison with measured data	116 % of measured data	198 % of measured data

As all models give an overestimation of consumer exposures, we have chosen to consider that a 3 % EGBE concentration in paint leads to a concentration of EGBE in air of 120 mg/m^3 (60 mg/m^3 x 2 – 20 min TWA) and an external body burden exposure by inhalation of : 10 mg/kg/d.

This value will be used as the worst case for inhalation exposure in this scenario.

For dermal exposure, the modelling proposed in the Technical Guidance document will be used. So the external exposure (E_{ed}) of skin to EGBE from paints will be:

$$E_{ed} = \frac{C_{prod} \times TH_{der} \times AREA_{der} \times n}{D \times BW}$$

where :
C_{prod} = concentration of EGBE in paint (kg/m^3)
TH_{der} = thickness of product layer on skin
$AREA_{der}$ = area of contact between paint and skin
n = number of events (n = 1)
D = dilution. There is no dilution so D = 1
BW = mean bodyweight for a consumer (60 kg)

Assuming the concentration of EGBE in the paint is 3 % i.e. 30 kg/m^3 (CEPE, 2002), the surface of the skin exposed is 840 cm^2 (840 x 10 $^{-4}$ m^2), the thickness of paint layer on the skin is 10^{-4} m, the external exposure of skin by paints will be:

$$E_e = \frac{30 \times 10^{-4} \times 840 \times 10^{-4} \times 1}{1 \times 60} = 4.2 \text{ mg/kg/d}$$

4.1.1.3.2 Summary of consumer exposure

Table 4.33: Summary of proposed "reasonable worst-case" exposures in the main scenarios

Scenario	Inhalation (mg/kg/d)	Skin (mg/kg/d)
1 – Household surface cleaners	0.09	7
2 – Indoor air	0.1	
3 – Painting	10	4.2

For risk characterisation, internal exposures will be estimated for each scenario.

As a consumer is also exposed to indoor air when he paints, or uses household cleaners, we will also include in the risk characterisation, the scenarios:

- household surface cleaners + indoor air

- paints + indoor air

Table 4.34: Summary of combined worst case scenarios

Scenario	Inhalation (mg/kg/d)	Skin (mg/kg/d)
Household surface cleaners + indoor air	0.19	7
Paintings + indoor air	10.1	4.2

4.1.1.4 Humans exposed via the environment

The information relating to the estimation of the indirect exposure of humans via the environment are presented in table 1. The concentrations calculated in intake media (drinking water, fish, plant roots and leaves, milk, meat, air) and the subsequent estimation of human intakes via different routes are shown hereafter with the corresponding total daily intakes. Both local and regional levels are taken into consideration and the estimation of local environmental exposures has been performed for all scenarios listed in chapter 3.1.2.2. Concerning the production step, only the worst case has been reported. Calculations have been performed using default parameters in EUSES except for the use of an absorption factor via inhalation of 60% and a body weight of 60kg.

Table 4.1: exposure assessment for human via the environment (concentrations in foodstuff, air and drinking water and connected daily doses)

	Conc. in drinking water ($mg.L^{-1}$) / Subsequent daily dose ($mg.kg^{-1}.d^{-1}$)	**Conc. in wet fish ($mg.kg^{-1}$) / Subsequent daily dose ($mg.kg^{-1}.d^{-1}$)**	**Conc. in plant roots ($mg.kg^{-1}$) / Subsequent daily dose ($mg.kg^{-1}.d^{-1}$)**	**Conc. in plant leaves ($mg.kg^{-1}$) / Subsequent daily dose ($mg.kg^{-1}.d^{-1}$)**	**Conc. in milk ($mg.kg^{-1}$ ww) / Subsequent daily dose ($mg.kg^{-1}.d^{-1}$)**	**Conc. in meat ($mg.kg^{-1}$ ww) / Subsequent daily dose ($mg.kg^{-1}.d^{-1}$)**	**Conc. in air ($mg.m^{-3}$) / Subsequent daily dose ($mg.kg^{-1}.d^{-1}$)**	**Total daily intake ($mg.kg^{-1}.d^{-1}$)**
Production (site-specific, worst case)	$1.20.10^{-1}$ / $4.01.10^{-3}$	$1.50.10^{-2}$ / $2.88.10^{-5}$	**$1.22.10^{-1}$ / $7.79.10^{-4}$**	$4.87.10^{-2}$ / $9.75.10^{-4}$	$7.89.10^{-5}$ / $7.37.10^{-7}$	$7.89.10^{-6}$ / $3.96.10^{-8}$	$1.49.10^{-3}$ / $1.78.10^{-4}$	$5.97.10^{-3}$
Paints I_F	$5.00.10^{-2}$ / $1.67.10^{-3}$	$7.06.10^{-2}$ / $1.35.10^{-4}$	$1.81.10^{-2}$ / $1.16.10^{-4}$	$9.75.10^{-2}$ / $1.95.10^{-3}$	$7.72.10^{-5}$ / $7.22.10^{-7}$	$7.72.10^{-6}$ / $3.87.10^{-8}$	$3.25.10^{-3}$ / $3.89.10^{-4}$	$4.26.10^{-3}$
Paints I_P	$1.25.10^{-1}$ / $4.16.10^{-3}$	$1.76.10^{-1}$ / $3.38.10^{-4}$	$5.34.10^{-2}$ / $3.41.10^{-4}$	1.24 / $2.48.10^{-2}$	$7.61.10^{-4}$ / $7.11.10^{-6}$	$7.61.10^{-5}$ / $3.82.10^{-7}$	$4.15.10^{-2}$ / $4.98.10^{-3}$	$3.46.10^{-2}$
Paints II_F and III_F	$5.12.10^{-2}$ / $1.71.10^{-3}$	$7.23.10^{-2}$ / $1.38.10^{-4}$	$1.85.10^{-2}$ / $1.19.10^{-4}$	0.10 / $2.00.10^{-3}$	$7.92.10^{-5}$ / $7.40.10^{-7}$	$7.92.10^{-6}$ / $3.97.10^{-8}$	$3.33.10^{-3}$ / $4.00.10^{-4}$	$4.36.10^{-3}$
Paints II_P and III_U	$1.82.10^{-2}$ / $6.07.10^{-4}$	$2.57.10^{-2}$ / $4.93.10^{-5}$	$7.45.10^{-3}$ / $4.77.10^{-5}$	$7.99.10^{-2}$ / $1.60.10^{-3}$	$5.34.10^{-5}$ / $4.99.10^{-7}$	$5.34.10^{-6}$ / $2.68.10^{-8}$	$2.67.10^{-3}$ / $3.20.10^{-4}$	$2.62.10^{-3}$
Paints IV_F	$7.35.10^{-3}$ / $2.45.10^{-4}$	$1.04.10^{-2}$ / $1.99.10^{-5}$	$6.40.10^{-3}$ / $4.09.10^{-5}$	$3.54.10^{-1}$ / $7.08.10^{-3}$	$2.05.10^{-4}$ / $1.92.10^{-6}$	$2.05.10^{-5}$ / $1.03.10^{-7}$	$1.19.10^{-2}$ / $1.42.10^{-3}$	$8.81.10^{-3}$
Paints IV_P	$1.70.10^{-2}$ / $5.67.10^{-4}$	$1.04.10^{-2}$ / $1.99.10^{-5}$	$1.72.10^{-2}$ / $1.10.10^{-4}$	**1.53 / $3.05.10^{-2}$**	**$8.76.10^{-4}$ / $8.19.10^{-6}$**	**$8.76.10^{-5}$ / $4.40.10^{-7}$**	**$5.11.10^{-2}$ / $6.13.10^{-3}$**	**$3.73.10^{-2}$**
Paints V_F	$7.35.10^{-3}$ / $2.45.10^{-4}$	$1.04.10^{-2}$ / $1.99.10^{-5}$	$4.05.10^{-3}$ / $2.59.10^{-5}$	0.10 / $2.00.10^{-3}$	$6.01.10^{-5}$ / $5.62.10^{-7}$	$6.01.10^{-6}$ / $3.02.10^{-8}$	$3.35.10^{-3}$ / $4.01.10^{-4}$	$2.69.10^{-3}$
Paints V_P	$1.64.10^{-2}$ / $5.47.10^{-4}$	$2.32.10^{-2}$ / $4.45.10^{-5}$	$6.27.10^{-3}$ / $4.01.10^{-5}$	$1.56.10^{-2}$ / $3.12.10^{-4}$	$1.60.10^{-5}$ / $1.50.10^{-7}$	$1.60.10^{-6}$ / $8.03.10^{-9}$	$5.14.10^{-4}$ / $6.17.10^{-5}$	$1.01.10^{-3}$
Paints VI_F	$1.54.10^{-1}$ / $5.13.10^{-3}$	$2.17.10^{-1}$ / $4.17.10^{-4}$	$5.20.10^{-2}$ / $3.33.10^{-4}$	$5.35.10^{-2}$ / $1.07.10^{-3}$	$9.71.10^{-5}$ / $9.08.10^{-7}$	$9.71.10^{-6}$ / $4.87.10^{-8}$	$1.73.10^{-3}$ / $2.08.10^{-4}$	$7.16.10^{-3}$
Paints VI_P	$9.67.10^{-2}$ / $3.22.10^{-3}$	$1.37.10^{-1}$ / $2.62.10^{-4}$	$3.28.10^{-2}$ / $2.10.10^{-4}$	$1.64.10^{-2}$ / $3.27.10^{-4}$	$5.12.10^{-5}$ / $4.79.10^{-7}$	$5.12.10^{-6}$ / $2.57.10^{-8}$	$5.08.10^{-4}$ / $6.10.10^{-5}$	$4.08.10^{-3}$
Paints VII_F	$5.86.10^{-2}$ / $1.95.10^{-3}$	$8.27.10^{-2}$ / $1.59.10^{-4}$	$2.02.10^{-2}$ / $1.30.10^{-4}$	$2.10.10^{-2}$ / $4.20.10^{-4}$	$3.73.10^{-5}$ / $3.49.10^{-7}$	$3.73.10^{-6}$ / $1.87.10^{-8}$	$6.79.10^{-4}$ / $8.15.10^{-5}$	$2.74.10^{-3}$
Paints VII_P	$1.45.10^{-2}$ / $4.83.10^{-4}$	$2.05.10^{-2}$ / $3.92.10^{-5}$	$9.42.10^{-3}$ / $6.03.10^{-5}$	$4.26.10^{-1}$ / $8.52.10^{-3}$	$2.49.10^{-4}$ / $2.33.10^{-6}$	$2.49.10^{-5}$ / $1.25.10^{-7}$	$1.43.10^{-2}$ / $1.71.10^{-3}$	$1.08.10^{-2}$
Paints $VIII_F$	$7.35.10^{-3}$ / $2.45.10^{-4}$	$1.04.10^{-2}$ / $1.99.10^{-5}$	$3.70.10^{-3}$ / $2.37.10^{-5}$	$6.13.10^{-2}$ / $1.23.10^{-3}$	$3.81.10^{-5}$ / $3.56.10^{-7}$	$3.81.10^{-6}$ / $1.91.10^{-8}$	$2.05.10^{-3}$ / $2.46.10^{-4}$	$1.76.10^{-3}$
Paints $VIII_P$	$7.35.10^{-3}$ / $2.45.10^{-4}$	$1.04.10^{-2}$ / $1.99.10^{-5}$	$3.18.10^{-3}$ / $2.04.10^{-5}$	$5.87.10^{-3}$ / $1.17.10^{-4}$	$6.56.10^{-6}$ / $6.13.10^{-8}$	$6.56.10^{-7}$ / $3.29.10^{-9}$	$1.93.10^{-4}$ / $2.32.10^{-5}$	$4.26.10^{-4}$
Paints IX_F	$2.20.10^{-2}$ / $7.33.10^{-4}$	$3.11.10^{-2}$ / $5.95.10^{-5}$	$8.35.10^{-3}$ / $5.35.10^{-5}$	$4.22.10^{-2}$ / $8.44.10^{-4}$	$3.36.10^{-5}$ / $3.14.10^{-7}$	$3.36.10^{-6}$ / $1.68.10^{-8}$	$1.40.10^{-3}$ / $1.69.10^{-4}$	$1.86.10^{-3}$
Paints IX_P	$9.12.10^{-3}$ / $3.04.10^{-4}$	$1.29.10^{-2}$ / $2.47.10^{-5}$	$5.82.10^{-3}$ / $3.72.10^{-5}$	$2.28.10^{-1}$ / $4.55.10^{-3}$	$1.34.10^{-4}$ / $1.25.10^{-6}$	$1.34.10^{-5}$ / $6.70.10^{-8}$	$7.62.10^{-3}$ / $9.15.10^{-4}$	$5.83.10^{-3}$
Detergents I_F	$1.76.10^{-2}$ / $5.87.10^{-4}$	$2.49.10^{-2}$ / $4.77.10^{-5}$	$6.58.10^{-3}$ / $4.21.10^{-5}$	$6.66.10^{-3}$ / $1.33.10^{-4}$	$1.15.10^{-5}$ / $1.07.10^{-7}$	$1.15.10^{-6}$ / $5.75.10^{-9}$	$2.15.10^{-4}$ / $2.58.10^{-5}$	$8.36.10^{-4}$
Detergents I_P	$3.28.10^{-2}$ / $1.09.10^{-3}$	$4.63.10^{-2}$ / $8.88.10^{-5}$	$1.58.10^{-2}$ / $1.01.10^{-4}$	$4.00.10^{-3}$ / $8.01.10^{-5}$	$1.64.10^{-5}$ / $1.54.10^{-7}$	$1.64.10^{-6}$ / $8.25.10^{-9}$	$1.15.10^{-4}$ / $1.38.10^{-5}$	$1.38.10^{-3}$
Detergents II_F	$1.16.10^{-2}$ / $3.87.10^{-4}$	$1.16.10^{-2}$ / $3.15.10^{-5}$	$4.59.10^{-3}$ / $2.94.10^{-5}$	$4.84.10^{-3}$ / $9.68.10^{-5}$	$7.82.10^{-6}$ / $7.31.10^{-8}$	$7.82.10^{-7}$ / $3.92.10^{-9}$	$1.57.10^{-4}$ / $1.88.10^{-5}$	$5.64.10^{-4}$

	Conc. in drinking water (mg.L^{-1}) / Subsequent daily dose (mg.kg^{-1}.d^{-1})	**Conc. in wet fish (mg.kg^{-1}) / Subsequent daily dose (mg.kg^{-1}.d^{-1})**	**Conc. in plant roots (mg.kg^{-1}) / Subsequent daily dose (mg.kg^{-1}.d^{-1})**	**Conc. in plant leaves (mg.kg^{-1}) / Subsequent daily dose (mg.kg^{-1}.d^{-1})**	**Conc. in milk (mg.kg^{-1} ww) / Subsequent daily dose (mg.kg^{-1}.d^{-1})**	**Conc. in meat (mg.kg^{-1} ww) / Subsequent daily dose (mg.kg^{-1}.d^{-1})**	**Conc. in air (mg.m^{-3}) / Subsequent daily dose (mg.kg^{-1}.d^{-1})**	**Total daily intake (mg.kg^{-1}.d^{-1})**
Detergents II_U	$1.67.10^{-2}$ / $5.56.10^{-4}$	$2.35.10^{-2}$ / $4.51.10^{-5}$	$5.69.10^{-3}$ / $3.64.10^{-5}$	$3.63.10^{-3}$ / $7.27.10^{-5}$	$9.32.10^{-6}$ / $8.72.10^{-8}$	$9.32.10^{-7}$ / $4.68.10^{-9}$	$1.15.10^{-4}$ / $1.38.10^{-5}$	$7.24.10^{-4}$
Intermediates I_P	$2.29.10^{-2}$ / $7.63.10^{-4}$	$3.17.10^{-2}$ / $6.08.10^{-5}$	$2.31.10^{-2}$ / $1.48.10^{-4}$	$4.83.10^{-3}$ / $9.66.10^{-5}$	$1.25.10^{-5}$ / $1.17.10^{-7}$	$1.25.10^{-6}$ / $6.27.10^{-9}$	$1.34.10^{-4}$ / $1.61.10^{-5}$	$1.09.10^{-3}$
Inks I_F	$9.36.10^{-2}$ / $3.12.10^{-3}$	$1.32.10^{-1}$ / $2.53.10^{-4}$	$3.19.10^{-2}$ / $2.04.10^{-4}$	$3.29.10^{-2}$ / $6.59.10^{-4}$	$5.93.10^{-5}$ / $5.54.10^{-7}$	$5.93.10^{-6}$ / $2.97.10^{-8}$	$1.07.10^{-3}$ / $1.28.10^{-4}$	$4.37.10^{-3}$
Inks I_P	$8.75.10^{-3}$ / $2.92.10^{-4}$	$1.24.10^{-2}$ / $2.37.10^{-5}$	$8.73.10^{-3}$ / $5.58.10^{-5}$	$5.56.10^{-1}$ / $1.11.10^{-2}$	$3.21.10^{-4}$ / $3.00.10^{-6}$	$3.21.10^{-5}$ / $1.61.10^{-7}$	$1.86.10^{-2}$ / $2.24.10^{-3}$	$1.37.10^{-2}$
Metal I_F	$6.27.10^{-2}$ / $2.09.10^{-3}$	$8.85.10^{-2}$ / $1.70.10^{-4}$	$2.18.10^{-2}$ / $1.39.10^{-4}$	$4.06.10^{-2}$ / $8.12.10^{-4}$	$5.03.10^{-5}$ / $4.70.10^{-7}$	$5.03.10^{-6}$ / $2.52.10^{-8}$	$1.33.10^{-3}$ / $1.60.10^{-4}$	$3.37.10^{-3}$
Metal I_P	$1.18.10^{-2}$ / $3.92.10^{-4}$	$1.62.10^{-2}$ / $3.11.10^{-5}$	$1.19.10^{-2}$ / $7.60.10^{-5}$	$7.99.10^{-1}$ / $1.60.10^{-2}$	$4.61.10^{-4}$ / $4.31.10^{-6}$	$4.61.10^{-5}$ / $2.31.10^{-7}$	$2.68.10^{-2}$ / $3.21.10^{-3}$	$1.97.10^{-2}$
Pharm I_P	$9.95.10^{-3}$ / $3.32.10^{-4}$	$1.12.10^{-2}$ / $2.14.10^{-5}$	$1.01.10^{-2}$ / $6.44.10^{-5}$	$4.52.10^{-3}$ / $9.04.10^{-5}$	$6.84.10^{-6}$ / $6.39.10^{-8}$	$6.84.10^{-7}$ / $3.43.10^{-9}$	$1.39.10^{-4}$ / $1.67.10^{-5}$	$5.25.10^{-4}$
Elec I_P	$2.44.10^{-2}$ / $8.13.10^{-4}$	$1.41.10^{-2}$ / $2.69.10^{-5}$	$2.46.10^{-2}$ / $1.58.10^{-4}$	$5.00.10^{-3}$ / $1.00.10^{-4}$	$1.32.10^{-5}$ / $1.24.10^{-7}$	$1.32.10^{-6}$ / $6.64.10^{-9}$	$1.38.10^{-4}$ / $1.65.10^{-5}$	$1.11.10^{-3}$
Leather I_P	**$1.60.10^{-1}$ / $5.33.10^{-3}$**	**$2.26.10^{-1}$ / $4.33.10^{-4}$**	$7.95.10^{-2}$ / $5.09.10^{-4}$	$6.36.10^{-3}$ / $1.27.10^{-4}$	$7.25.10^{-5}$ / $6.78.10^{-7}$	$7.25.10^{-6}$ / $3.64.10^{-8}$	$1.17.10^{-4}$ / $1.40.10^{-5}$	$6.41.10^{-3}$
Adhesives I_F	$2.10.10^{-2}$ / $7.01.10^{-4}$	$2.97.10^{-2}$ / $5.70.10^{-5}$	$7.73.10^{-3}$ / $4.95.10^{-5}$	$8.20.10^{-3}$ / $1.64.10^{-4}$	$1.38.10^{-5}$ / $1.29.10^{-7}$	$1.38.10^{-6}$ / $6.93.10^{-9}$	$2.66.10^{-4}$ / $3.19.10^{-5}$	$1.00.10^{-3}$
Adhesives I_P	$8.10.10^{-3}$ / $2.70.10^{-4}$	$1.14.10^{-2}$ / $2.19.10^{-5}$	$4.65.10^{-3}$ / $2.98.10^{-5}$	$3.59.10^{-3}$ / $7.18.10^{-5}$	$5.57.10^{-6}$ / $5.21.10^{-8}$	$5.57.10^{-7}$ / $2.79.10^{-9}$	$1.15.10^{-4}$ / $1.38.10^{-5}$	$4.07.10^{-4}$
Agri I_F	$1.46.10^{-2}$ / $4.87.10^{-4}$	$2.06.10^{-2}$ / $3.95.10^{-5}$	$6.48.10^{-3}$ / $4.14.10^{-5}$	$7.02.10^{-3}$ / $1.40.10^{-4}$	$1.03.10^{-5}$ / $9.67.10^{-8}$	$1.03.10^{-6}$ / $5.19.10^{-9}$	$2.27.10^{-4}$ / $2.73.10^{-5}$	$7.35.10^{-4}$
Agri I_P	$7.54.10^{-3}$ / $2.51.10^{-4}$	$1.07.10^{-2}$ / $2.04.10^{-5}$	$4.13.10^{-3}$ / $2.64.10^{-5}$	$5.07.10^{-3}$ / $1.01.10^{-4}$	$6.19.10^{-6}$ / $5.78.10^{-8}$	$6.19.10^{-7}$ / $3.10.10^{-9}$	$1.65.10^{-4}$ / $1.98.10^{-5}$	$4.19.10^{-4}$
Oilfield I_F	$9.36.10^{-2}$ / $3.12.10^{-3}$	$1.32.10^{-1}$ / $2.53.10^{-4}$	$3.19.10^{-2}$ / $2.04.10^{-4}$	$3.29.10^{-2}$ / $6.59.10^{-4}$	$5.93.10^{-5}$ / $5.54.10^{-7}$	$5.93.10^{-6}$ / $2.97.10^{-8}$	$1.07.10^{-3}$ / $1.28.10^{-4}$	$4.37.10^{-3}$
Oilfield I_P	$1.11.10^{-2}$ / $3.71.10^{-4}$	$1.57.10^{-2}$ / $3.01.10^{-5}$	$1.07.10^{-2}$ / $6.82.10^{-5}$	$3.81.10^{-3}$ / $7.62.10^{-5}$	$6.94.10^{-6}$ / $6.49.10^{-8}$	$6.94.10^{-7}$ / $3.48.10^{-9}$	$1.15.10^{-4}$ / $1.38.10^{-5}$	$5.60.10^{-4}$
Cosmet I_F and Fire I_F	$3.49.10^{-2}$ / $1.16.10^{-3}$	$4.93.10^{-2}$ / $9.44.10^{-5}$	$1.23.10^{-2}$ / $7.90.10^{-5}$	$1.29.10^{-2}$ / $2.58.10^{-4}$	$2.25.10^{-5}$ / $2.10.10^{-7}$	$2.25.10^{-6}$ / $1.13.10^{-8}$	$4.18.10^{-4}$ / $5.02.10^{-5}$	$1.64.10^{-3}$
Fire I_P	$7.78.10^{-3}$ / $2.59.10^{-4}$	$1.10.10^{-2}$ / $2.11.10^{-5}$	$4.02.10^{-3}$ / $2.57.10^{-5}$	$3.57.10^{-3}$ / $7.14.10^{-5}$	$5.42.10^{-6}$ / $5.07.10^{-8}$	$5.42.10^{-7}$ / $2.72.10^{-9}$	$1.15.10^{-4}$ / $1.38.10^{-5}$	$3.91.10^{-4}$
Cosmet I_U	$8.70.10^{-3}$ / $2.90.10^{-4}$	$1.23.10^{-2}$ / $2.35.10^{-5}$	$3.53.10^{-3}$ / $2.26.10^{-5}$	$3.55.10^{-3}$ / $7.10.10^{-5}$	$5.28.10^{-6}$ / $5.44.10^{-8}$	$5.28.10^{-7}$ / $2.92.10^{-9}$	$1.15.10^{-4}$ / $1.38.10^{-5}$	$4.21.10^{-4}$
Regional	$7.35.10^{-3}$ / $2.41.10^{-4}$	$1.04.10^{-2}$ / $1.96.10^{-5}$	$1.47.10^{-3}$ / $1.11.10^{-5}$	$3.48.10^{-3}$ / $4.05.10^{-5}$	$5.19.10^{-6}$ / $4.06.10^{-8}$	$5.19.10^{-7}$ / $2.18.10^{-9}$	$1.15.10^{-4}$ / $1.96.10^{-5}$	$3.32.10^{-4}$

The highest indirect exposure is estimated for the processing of can coating (Paints IV_P) : $3.73.10^{-2}$ $mg.kg^{-1}.day^{-1}$. It can also be noted that the highest exposures are to be expected through intake of drinking water and plants (leaves and roots). Moreover, based on the regional concentrations, the total daily intake for humans is $3.32.10^{-4}$ $mg.kg^{-1}.day^{-1}$. These two figures will be taken forward into the risk characterisation.

4.1.2 Effects assessment: Hazard identification and dose (concentration)-response (effect) assessment

4.1.2.1 Toxicokinetics, metabolism and distribution

4.1.2.1.1 Studies in animals

Inhalation

Rat

Sixteen male SD rats were exposed continuously (whole body) to EGBE (purity 99 %) by inhalation at doses of 20 ppm or 100 ppm for 1, 2, 3, 4, 6, 8, 10 or 12 days (Johanson, 1994). Urine was collected in 24-hr interval periods. After sacrifice, tissue samples of blood, muscle and liver were collected and analysed for EGBE and Butoxy Acetic Acid (BAA) content.

No clinical signs of toxicity were noted during the duration of the study. In all the tissues examined, the EGBE concentration rapidly increased during the first three days and continue to increase slower during the remaining days of exposure. The table 4.35 shows the tissue concentrations reached in the 20 ppm exposure group.

Table 4.35: Average tissues concentrations of EGBE and BAA following 20 ppm exposure

Tissue	Blood	Liver	Muscle	Testis
EGBE concentration	10 – 20 µmol/l	10 µmol/l	10 µmol/l	5 µmol/l
BAA concentration	30 – 40 µmol/l	15 – 20 µmol/l	10 µmol/l	10 µmol/l

Following a 100 ppm exposure the tissues concentrations were approximately 5 times higher.

(5 times higher in blood, 3.5 and 3.6 in muscle and testis respectively and 7.5 in the liver). The blood clearance of EGBE was estimated to 2.6 ± 1.3 l/h/kg bw whichever the dose administered. The kinetic profile of BAA was approximately the same as EGBE. The 100ppm/20ppm concentration ratio were: 4.4 for blood, 4.7 for muscle, 2.3 for testis and 5.3 for liver. The urinary excretion of BAA averaged 0.2 mmol/day in the 20 ppm group and 1.03 mmol/day in the 100 ppm group. This correspond to 64 % of the calculated respiratory uptake. The renal clearance was 0.53 l/h/kg.

[^{14}C]EGBE (purity 99 %) was administered to male F 344/N rats by inhalation (nose only – vapour form) for 6 hr at doses of 5, 50 or 450 ppm (Sabourin *et al*., 1992a). One group of 5 rats was used to determine the fractional uptake of inhaled EGBE and the body burden of the

compound at the end of the exposure. Respiratory measurements were performed on this group. After termination, the entire carcass was digested. The ^{14}C in these rats was used as a measure of the body burden. A second group of 4 rats was used to determine the paths of excretion of EGBE equivalents and the major metabolites excreted. Urine, faeces and exhaled breath were collected for 66 hr following the end of the 6 hr exposure. The rats were terminated and the carcass was digested and analysed for ^{14}C. A third group of 30 rats was used for analysis of blood ^{14}C and blood metabolite concentration at the 5 ppm exposure concentration only. At various time points and up to 24 hr following the start of exposure, 3 rats were euthanized and bled by cardiac puncture. Blood samples were analysed for haematocrit as well as ^{14}C associated with whole blood and plasma where

^{14}C associated with red blood cells = ^{14}C associated with whole blood - ^{14}C associated with plasma

Plasma metabolites were also analysed.

The respiratory rate and tidal volume were not significantly different form those of unexposed rats for any of the exposure. The amount of EGBE inhaled was proportioned to the exposure concentration in the 5 and 50 ppm exposures. A less than proportional amount of EGBE was inhaled at the high exposure concentration due to a lower minute volume.

At all exposure concentrations, the majority of ^{14}C was eliminated in the urine, less than 7 % of the parent compound was exhaled following the exposure. 10-20 % of the (^{14}C)EGBE equivalent remained in the carcass up to 66 hr post exposure at all concentrations. 7 % of the excreted ^{14}C was in the form of $^{14}CO_2$ (see table 4.36).

Table 4.36: Excretion of [^{14}C]EGBE

[^{14}C]EGBE equivalents as percentage of amount metabolised Exposure concentration in ppm (values in μmol)			
Route of excretion	5	50	450
$^{14}CO_2$ during exposure	0.2 (0.008)	1.3 (0.6)	0.9 (3.5)
$^{14}CO_2$ post exposure	7.6 (0.25)	7.2 (3.3)	5.9 (22.7)
Urine	67.3 (2.3)	64 (31.4)	75.9 (296.8)
Faeces	1.6 (0.06)	2.3 (1.1)	1.2 (4.6)
Exhaled EGBE post exposure	3.3 (0.09)	5.1 (2.5)	2.3 (9)
Carcass	19.8 (.068)	19.1 (9.4)	12.9 (50.6)
Total	100	100	100

Urinary ^{14}C excreted during the first 41 hr represented more than 88 % of the total ^{14}C excreted in urine. BAA was the major metabolite in urine at all exposure concentrations. EG and BEG were also found in lesser amounts (see table 4.37). As the exposure concentration increased, the proportion of 2 unidentified minor metabolites increased. At 5 ppm about 60 %

of the urinary ^{14}C was excreted during the 6 hr exposure. Whereas at 450 ppm only 10 % was excreted during the exposure. The urinary metabolites profile varied with time following exposure : metabolism to Glucuronide conjugate of EGBE (BEG) was favoured during the exposure whereas metabolism to BAA and Ethylene Glycol (EG) was favoured at later time post exposure. This observation was more apparent at the high exposure concentration. Metabolism of EGBE leading to EG and BAA appear to be easily saturable.

Table 4.37: Urinary metabolites of EGBE

Percentage of retained dose excreted in urine by 41 hr Exposure concentration in ppm (values in μmol)			
Metabolite	5	50	450
EG	16.1 (0.55)	13.4 (6.3)	7.9 (34)
BAA	43.2 (1.47)	41.9 (19.9)	36.6 (143)
BE glucuronide	3.4 (0.20)	6.7 (3.2)	10.4 (40)
4 min unknown	ND	10.5 (0.5)	5.3 (22)
10 min unknown	ND	ND	1.5 (6)
Others	0.5 (0.03)	0.7 (0.4)	2.2 (10)

Analysis of whole blood as well as plasma indicated that the majority of the EGBE equivalent were in the plasma.

During the first 2 hr of exposure about 20 % of the blood ^{14}C was associated with the red blood cell fraction. However at the later time points the proportion of ^{14}C in the cellular fraction declined and was undetectable following exposure. Bound ^{14}C metabolites was greater after the exposure than during the exposure.

In an inhalation study, F344 rats were exposed whole body for 6h per day, 5 days per week for 104 weeks, to EGBE (purity > 99 %) at concentrations of 31.2, 62.5 and 125 ppm (Dill *et al.,* 1998). Post exposure blood samples were collected after 1 day, 2 weeks and 3, 6, 12 and 18 months for EGBE and BAA determination. The post exposure time points depends of the day of collection. Post exposure urine samples were collected from animals after 2 weeks and 3, 6, 12 and 18 months of exposure. This study was also performed on mice.

The EGBE blood concentrations showed a rapid drop after the cessation of exposure. The elimination $t_{1/2}$ for EGBE after 1 day of exposure was < 10 min, this $t_{1/2}$ does not depend on the dose level. Increases in the initial blood concentration of EGBE and Area Under the Curve (AUC) were proportional to exposure concentration, supporting that elimination of EGBE from the blood follows linear kinetics. At the common concentration levels (62.5 and 125 ppm), mice eliminated EGBE faster than rats, values of $t_{1/2}$ and AUC_{EGBE} were significantly lower in mice than in rats (see table 4.38). The rate of EGBE elimination from blood slowed with increasing days of exposure, this change was more obvious in rats than mice as their $t_{1/2}$ and AUC dramatically increased after 3 months of exposure. Male rats generally exhibited larger AUC than females, indicating a lower blood clearance of EGBE. k was not significantly different between sexes, implying that the volume of distribution, rather than the rate of elimination might be responsible for the sex-related difference in EGBE clearance.

Unlike EGBE, BAA was not rapidly cleared from the systemic circulation. The BAA concentrations in the blood did not start to decline until 20 to 80 min post exposure. It seems that BAA was eliminated from blood following saturable, nonlinear kinetics. The k was not constant, decreasing as EGBE exposure concentrations increased. As the rate of BAA production reflects the EGBE elimination, it seems that the nonlinear characteristics of BAA kinetics likely result from BAA distribution or elimination processes. When the number of exposure days increased, the rate of 2BAA elimination tended to decrease. Females eliminated BAA more slowly than males (smaller k, longer $t_{1/2}$ and larger AUC). The maximum blood concentration was also higher in females at each exposure concentration. In mice, elimination of BAA was faster than rats when compared at common exposure concentrations. There was a clear difference in the urinary excretion of BAA between rats and mice (see table 4.39). Female rats excreted less BAA than males regardless of exposure concentration. Excretion of BAA tended to increase with time.

Table 4.38: Comparison of EGBE elimination between rats and mice at the same dose level tested.

	C_0 (µg/g)				k				T1/2 (min)				AUC (µg/min/g)			
62.5 ppm																
	Rats		Mice		Rats		Mice		Rats		Mice		Rats		Mice	
1d	1.86	1.59	1.29	1.35	0.0793	0.0809	0.229	0.185	8.74	8.56	3.03	3.75	23.5	19.6	5.6	7.3
2w	3.06	2.24	1.14	1.54	0.0828	0.0839	0.161	0.199	8.37	8.26	4.31	3.49	36.9	26.8	7.1	7.7
3m	0.54	0.47	1.30	1.3	0.0166	0.0248	0.126	0.199	41.66	27.9	5.51	3.49	32.3	18.9	10.3	6.5
6m	1.31	0.69	1.48	0.9	0.0308	0.0230	0.128	0.098	22.51	30.20	5.41	7.08	42.5	29.9	11.6	9.2
12m	1.08	0.97	1.17	1.54	0.0354	0.0439	0.091	0.129	19.6	15.79	7.63	5.38	30.5	22.0	12.9	12.0
125 ppm																
1d	4.27	3.11	3.78	3.18	0.0735	0.0736	0.171	0.139	9.43	9.42	4.06	4.99	58.1	42.3	22.1	22.9
2w	5.36	4.10	2.13	2.31	0.082	0.0852	0.126	0.147	8.45	8.13	5.5	4.72	65.3	48.1	16.9	15.8
3m	3.17	2.75	4.36	6.53	0.0379	0.0411	0.153	0.188	18.30	16.87	4.53	3.69	83.6	67	28.5	34.7
6m	3.58	3.58	2.43	2.39	0.0514	0.0589	0.084	0.082	13.47	11.76	8.29	8.44	69.6	60.7	29	29.1
12m	2.23	1.33	2.19	2.31	0.0347	0.0342	0.094	0.094	19.96	20.28	7.41	7.41	64.3	38.8	23.4	24.7

Table 4.39: Comparison of BAA urinary excretion between rats and mice.

	k				T1/2 (min)				AUC (µg/min/g)			
	Rats		Mice		Rats		Mice		Rats		Mice	
62.5 ppm												
1d	0.0174	0.0109	0.0221	0.0224	39.9	63.7	31.3	31	5541	8228	1330	2808
2w	0.0168	0.0088	0.0326	0.0304	41.2	78.4	21.3	22.8	4849	10605	797	1872
3m	0.0168	0.0094	0.0217	0.0178	41.3	73.7	31.9	39	-	-	3059	3872
6m	0.0099	0.0090	0.0222	0.0225	70.3	76.8	31.2	30.8	-	-	4592	7138
12m	0.0139	0.0087	0.0134	0.0108	49.8	79.6	51.6	64.1	7279	8445	9026	13941
18m	0.0067	0.0122	0.0144	0.0208	103.3	56.7	48.2	33.3	9351	8976	8158	7521
125 ppm												
1d	0.011	0.0031	0.0241	0.0285	63.2	224.1	28.7	24.3	19010	33403	4475	8145
2w	0.0116	0.0053	0.0263	0.0237	59.5	130.2	26.4	29.3	13597	37943	4640	10912
3m	0.0059	0.0039	0.0248	0.0227	117.9	178.8	28	30.5	13554	29094	8295	11772
6m	0.0035	0.0018	0.0124	0.0167	195.9	395.8	55.8	41.6	14142	26485	30151	18022
12m	0.0037	0.0024	0.0124	0.0054	187	283.4	55.7	127.8	16649	25897	21094	40782
18m	0.0023	0.0058	0.0143	0.0166	297.5	119.3	48.5	41.7	30768	25745	34787	21126

Mouse

In an inhalation study, B6C3F1 mice were exposed whole body for 6h per day, 5 days per week for 104 weeks, to EGBE (purity > 99 %) at concentrations of 62.5, 125 and 250 ppm (Dill *et al.,* 1998). Post exposure blood samples were collected after 1 day, 2 weeks and 3, 6, 12 and 18 months for EGBE and BAA determination. The post exposure time points depends of the day of collection. Post exposure urine samples were collected from animals after 2 weeks and 3, 6, 12 and 18 months of exposure. Before the core study started, a separate set of mice was moved into the control chamber and designated as the "aged (naïve)" mice. At 18 months into the chronic study, these mice (about 19 months old) were moved to the 125 ppm exposure chamber and exposed for 3 weeks. Blood was collected after 1 day and 3 weeks of exposure at post exposure time points of 10, 20, 40, 80, 180, 360, 720 and 1440 min. Post exposure urine samples were collected from the aged mice for 16 h after 2 weeks of exposure. This study was also performed in rats.

The EGBE blood concentrations showed a rapid drop after the cessation of exposure. The elimination $t_{1/2}$ for EGBE after 1 day of exposure was < 5 min, this $t_{1/2}$ does not depend on the dose level. Increases in the initial blood concentration of EGBE and AUC were proportional to exposure concentration, supporting that elimination of EGBE from the blood follows linear kinetics. At the same concentration levels mice eliminated EGBE faster than rats. Values of $t_{1/2}$ and AUC were significantly lower in mice at both exposure concentrations. The rate of EGBE elimination from blood slowed with increasing days of exposure , this change was more obvious in rats than mice as their $t_{1/2}$ and AUC dramatically increased after 3 months of exposure. The kinetic parameters were not significantly different between male and female

mice. Unlike EGBE, BAA was not rapidly cleared from the systemic circulation. The BAA concentrations in the blood did not start to decline until about 40 min post exposure. It seems that BAA was eliminated from blood following saturable, non-linear kinetics. The k was not constant, decreasing as EGBE exposure concentrations increased. As the rate of BAA production reflects the EGBE elimination, it seems that the non-linear characteristics of BAA kinetics likely result from BAA distribution or elimination process. When the number of exposure days increased, the rate of 2BAA elimination tended to decrease. Elimination of BAA was faster than rats when compared at common exposure concentrations. No differences were seen between males and females. There was a clear difference in the urinary excretion of BAA between rats and mice. No sex differences in BAA excretion were seen regarding exposure concentration. Time-dependent changes in urinary excretion of BAA were not comparable between males and females as the trend appeared to change over time in the opposite direction (decreasing in males mice and increasing in females).

Elimination of EGBE and BAA in aged mice : EGBE was rapidly cleared from systemic circulation and kinetic parameters were not significantly different from those of young mice. A slower terminal elimination phase was observed in aged mice suggesting that time-dependent reduction in the rate of EGBE elimination in chronically exposed mice could be, in part, due to the age of animals. As with young mice, there was no sex difference in elimination kinetic. There was a great difference with young animals in blood concentration of BAA after 1 day of exposure. Aged mice eliminated BAA from blood more than 10 times slower than young mice. Consequently both $t_{1/2}$ and AUC were greater in old mice compared to young animals after 1 day of exposure. This age-related difference mostly disappeared after about 3 weeks of exposure.

In an inhalation study, female B6C3F1 mice were exposed whole body to 2-butoxy[^{14}C]ethanol (purity 97.6 %) at dose of 250 ppm for 6 hours (Green *et al.*, 2000a). The mice (4 per time point) were terminated at 5 minutes, 24 and 48 hours post exposure. One animal for each time point was submitted to a whole body autoradiography, the remaining 3 animals were submitted to an analysis of the free and bound radioactivity of the stomach and contents.

The mice killed 5 min after the end of exposure contained high level of radioactivity without showing preferential labelling in any tissue or organ. The highest concentrations were found in liver, blood and nasal passages with lower concentrations in the gastro-intestinal. Similar concentration was observed in the glandular mucosa of the stomach but no radiolabelling were present in the forestomach. Very high levels of radiolabelling were present on the skin and fur near the hindquarters.

At 24 hours post exposure, the highest concentrations of radioactivity were observed in the liver and buccal cavity. Similar high amount of radiolabelling was seen in the mucosa of the caecum, in the forestomach mucosa, the lower gastro-intestinal tract mucosa and the oesophagus. The glandular stomach was conspicuously different from the forestomach in having low, background levels of labelling. High levels of radiolabel were seen in the skin and fur particularly on the back and near the hind quarters. A lower level of labelling was observed in the salivary glands, thymus, kidney medulla, adrenal and spleen. Background labelling was seen in the rest of the internal organs.

48 hours after exposure, high levels of radiolabelling was seen in the buccal cavity, the oesophagus, the forestomach and in the liver and the mucosa of the lower gastro-intestinal tract. The skin and fur, particularly near the hind quarters, were also heavily radiolabeled.

Low levels of labelling were observed in the duodenum, glandular stomach and the remainder of the internal organs.

The analysis of stomach and contents showed that immediately after exposure, a greater level of radioactivity was present in the stomach and its contents than the two later time point and that most of this radioactivity was due to EGBE. At the 24 and 48-hour time point, more of 80 % of the radioactivity present in the stomach tissues was covalently bound to protein. There was no significant difference between the glandular and forestomach.

High concentrations of radioactivity were seen on the fur and skin of the animals in this study and according to the authors, this observation together with the presence of radioactivity in the buccal cavity, oesophagus and stomach contents suggests that EGBE is ingested during grooming (during exposure and post exposure) and mucous removal (muco-ciliary clearance) through the nasopharynx (during exposure). Although the whole body autoradiograms very clearly demonstrated highly selective labelling of the mucosa of the forestomach at the two later time points, the analysis of the stomachs failed to detect this, probably due to the high levels of background labelling in the remainder of the stomach tissues.

In conclusion, the retention of radioactivity in the forestomach mucosa clearly indicates that the forestomach is a target organ following an inhalation exposure to EGBE. The high concentrations of radioactivity on the skin and fur and the presence of radioactivity in the buccal cavity and the oesophagus suggest that the radioactivity enters the forestomach as a result muco-ciliary clearance from the nasopharynx and grooming and ingestion of the material present on the skin and fur.

A complementary study from Poet *et al*., 2003 identified both BAA and EGBE in the saliva of mice administered EGBE by both oral gavage and intraperitoneal routes. The author concluded that EGBE can distribute to the forestomach by multiple mechanisms: grooming of the fur, mucociliary clearance, saliva and from systemic blood circulation and that BAA can also distribute to forestomach tissues from saliva and blood circulation as well as being formed locally from EGBE.

In this series of studies, Poet *et al*., 2003 also assessed the deposition of EGBE on fur after inhalation exposure: female mice were exposed either by whole-body or nose-only inhalation to a single 6-hour EGBE exposure of 250 ppm (vapour). At the end of each exposure, 5 mice were killed and immersed in hot water to collect the EGBE deposited on the fur. Additional groups of 5 mice were killed immediately after inhalation exposure for blood analysis. Two other groups of 5 mice were subjected to an 18-hour urine collection after the inhalation exposures.
An average of 205 µg of EGBE was detected on the fur of mice exposed whole-body. For mice exposed nose-only an average of 170 µg was found. After corrections, the estimation of the authors was that whole body exposures averaged 25 % more EGBE on the fur than the corresponding nose-only exposure.

At the end of each exposure, the mean concentrations of EGBE in blood were 3.0 and 3.9 mg/l for whole-body exposure and nose-only exposure respectively. As the same time, the concentration of BAA in blood were 235 and 390 mg/l for whole-body exposure and nose-only exposure respectively. Low levels of EGBE were found in the 18-hour post exposure urine collection (about 68 µg) to either route. These concentration are supposed to come from the fur because EGBE is not expected to be excreted in the urine unconjugated. Concentrations of free BAA in the urine were about 2020 µg or 1780 µg for whole-body exposure and nose-only exposure respectively.

Summary inhalation route :

Three studies performed with rats (two with F344 and one with SD rats) and 3 studies performed with B6C3F1 mice were reported. In one study, the distribution of EGBE and its metabolites was studied in relation with time. This study clearly showed that the liver and the forestomach were the main target organs. The great amount of radiolabelling found in the gastro-intestinal tract demonstrated that EGBE was ingested during grooming and by muco-cilliary clearance from nasopharynx and not directly via inhalation route. However this mechanism cannot explain the great amounts found in the forestomach. EGBE metabolism leaded to the formation of a majority of BAA, EG and BEG and 2 metabolites unidentified in small quantities. BAA and EG formation followed a saturable mechanism and increased doses of EGBE leaded to a greater and greater formation of BEG compared to BAA and EG. Like the other routes of administration, elimination via urinary route was rapid and predominant. A small amount of the administrated dose was eliminated via inhalation route in the form of CO_2 (less than 10 %).

The blood half-life of EGBE was about 10 minutes in rats and 5 minutes in mice whatever the level of exposure. Unlike BAA which seemed to be eliminated by a saturable mechanism, elimination of EGBE followed a linear kinetic. When repeated doses of EGBE were administered, the rate of BAA elimination tended to decrease with increasing doses of EGBE.

In rats, elimination of BAA varied with the sex of animals: females tended to eliminate BAA slower. This difference between the sex could be due to a difference in the renal excretion between males and females. This sex difference was not found in mice. A species difference was also reported, mice eliminating 2 fold faster than rats. An age difference was also described in mice, the older mice eliminating BAA 10 fold slower than the younger mice for an unique administration of EGBE. When exposures were repeated , the difference between aged and young mice tended the disappear. After 3 months of daily exposure, no difference was seen for the elimination of BAA when age is considered.

Dermal

In vivo studies

Rat

EGBE (99 % purity) was tested in a series of studies in rats for assessing its kinetic parameters via the dermal route (Bartnik *et al*., 1987).

Cutaneous absorption *in vivo*: EGBE was applied to 6 males and 6 females Wistar rats (12 cm^2 on shaved skin) at a dose of 200 mg/kg. To avoid oral uptake, the site of application was kept covered with a perforated glass capsule. Urine was collected for 48 hours. After termination, treated skin area was dissected for the determination of the radioactivity.

Percutaneous absorption was assessed by using the measurements of urinary excretion of ^{14}C from 0 to 48 hr following cutaneous and subcutaneous administration of EGBE and expressing the results as total radioactivity (urine) following topical application / total radioactivity (urine) following subcutaneous application.

In a similar experiment, blood levels were determined following cutaneous application of EGBE. EGBE was applied to twenty four female rats at a dose of 200 mg/kg (12 cm^2 on shaved skin). Animals were killed 0.5, 1, 2, 4, 6, 8, 16 and 24 hours after application of EGBE. EGBE and BAA were measured in blood.

In vitro absorption of EGBE was also studied (see *in vitro* section)

Within 48 hr following topical application of EGBE, approximately 20 to 23 % of the applied radioactivity was found in the urine (95 % eliminated during the first 24 hr). A percutaneous absorption of approximately 25-29 % of the applied topical dose was determined.

The highest radioactivity in blood and plasma occurs at 2 hr following application. Measurement of BAA in the plasma presented the same qualitative course. Based on BAA levels in the plasma, it appears that the major part of the absorbed EGBE is metabolized to BAA.

Male F344/N rats were exposed dermally (non occlusively) to EGBE (99.9 % purity) (Sabourin *et al*., 1992b) at dose levels of 122, 367 and 650 μmol/rat). Parent compound, CO_2, urine and faeces were collected for 72 hr. After 72 hr, rats were killed and the skin around the site of dosing was removed from the carcass. Digested tissues, urine and faeces were assayed for radioactivity.

Analysis of urine from [^{14}C]EGBE dosed animals indicated the presence of the parent compound. Some experiments demonstrated that 7 % of the volatilized [^{14}C]EGBE was trapped in the water in the urine receptacle. It demonstrate that urine dripping down the sides of the metabolic cage can absorb significant quantities of the volatilized EGBE.

At the mid dose, 3 rats per time point were killed at 0.5, 1, 2, 4, 8 and 16 hr after exposure. Blood was collected and haematocrit and radioactivity measurement made. Urinary and plasma metabolites were analyzed.

The total recovery of the ^{14}C was 74 to 90 %. 43 to 64 % of the dermally applied dose was trapped as volatile ^{14}C. 20 to 25 % of the dermally applied dose were absorbed and metabolized. A small amount of the applied ^{14}C (0.3-2 %) was still present at the site of application 72 hr following dosing. The majority of radioactivity was excreted in urine. Small amounts of ^{14}C were found in faeces and exhaled CO_2 on air. There was no significant effect of the dose on the excretion of ^{14}C except for a slight increase in the proportion of $^{14}CO_2$.

At the end of the collection period 7-16 % of the absorbed radiolabel remained in the carcass. Urinary metabolites were analyzed up to the time at which < 10 % of the ^{14}C excreted within 72 hr remained. For EGBE this included urine collected up to 23 hr after exposure. BAA was the major urinary metabolite. Amounts of glucuronide conjugates were also detected. The metabolite profile was not significantly affected by the dose. Analysis of ^{14}C in whole blood indicated that over 80 % of radioactivity was associated with the plasma. The maximum concentration of total plasma metabolites was reached at about 1 hr following dermal application of the mid-dose of radiolabelled EGBE, after which the concentration decreased with a half-life of about 4 hr. Plasma BAA, the major metabolite, was measured at 1-2 and 4 hr post exposure and represented 53 to 75 % of plasma ^{14}C.

Guinea pig

The percutaneous uptake rate of EGBE was studied in Guinea Pigs (Johanson and Fernström, 1986d). Female Guinea-Pig (GP) were kept under anesthesia throughout the experiment. After *Intra Venous* (IV) bolus of 42 or 92 μmol/kg EGBE, blood was sampled after 5, 10, 20, 30, 40, 50, 60, 75, 90, 105 and 120 min. At 150 min after the IV dosing, animals were exposed to

1 ml of undiluted EGBE percutaneously (3.14 cm^2 of skin exposed) with occlusive conditions. Blood samples were collected at the same time intervals as the intravenous dose.

The concentration of EGBE in blood after IV administration declined rapidly. Estimates of the total clearance (Cl) was 128 ml/min/kg bw corresponding to 2.7 ml/min/g of liver (0.8 ml/min/g of liver in human and 2 ml/min/g of liver in perfused rat liver). After dermal administration, the blood concentration of EGBE rose rapidly and reach a plateau level during the later half of the exposure period (average 21 µmol/l). The average uptake rate was estimated to be 0.25 µmol/min/cm^2.

The influence of water on the percutaneous absorption of EGBE was studied in Guinea Pigs (Johanson and Fernström, 1988). Animals were kept under anesthesia for 6 hr and killed at the end of the experiment. GP were exposed for 2 hours to pure or aqueous EGBE concentrations of 80 %, 40 %, 20 %, 10 % and 5 %. After a recovery period of 2 hours a second 2-hour exposure to pure EGBE was performed. The skin uptake of diluted EGBE was compared with the skin uptake of undiluted EGBE, each animal serving as its own control. Arterial blood were collected at intervals of 10 to 30 min throughout the experiment and assayed for EGBE.

The concentration of EGBE in blood increased with time after administration. After removal of the exposure source, concentration started to decreased immediately. During the 2nd hour of exposure to pure EGBE, the blood level appeared to approach a plateau level. The average concentration during the last hour was 4.6 µmol/l and the calculated skin uptake rate was 132 nmol/min/cm^2. The absolute skin uptake rate and the average blood concentration of EGBE during the first exposure period increased as the percentage of EGBE in the applied solution increased up to a maximum of about 40 % and then decrease again. The relative skin uptake rate showed a similar pattern. The average relative uptake rate of EGBE was approximately the same from the 5, 10 and 20 % solution as from the undiluted EGBE, while it was twice as high from the 40 and 80 % solutions. In contrast, the concentration of EGBE in blood declined approximately twice as rapidly after the end of exposure to the 40 and 80 % solution (average $t_{1/2}$: 28 min compared to 55 min for the other solutions).

In vitro studies:

Cutaneous absorption of EGBE *in vitro* was assessed by a modified method of Zesch and Schaefer (1973) (Bartnik *et al*., 1987). These studies also included an evaluation of cutaneous absorption of EGBE under semi occlusive and non-occlusive conditions. Skin of rats, pigs and humans was used. Before and at the end of each study, the skin was checked visually for integrity of the stratum corneum. For application of test materials, an area of 5 cm^2 (animal skin) or 3 cm^2 (human skin) was demarcated on the skin samples. 30 microliters of each of the following test solutions was applied : EGBE (100 %), EGBE (3.5 %) in water, EGBE (10 %) in water. All test solutions were evaluated for absorption in rat skin (1, 6 and 16 hr semi-occlusive, 1hr exposure non-occlusive) and pig skin (6 hr exposure semi-occlusive). Absorption of EGBE (3.5 % in water) were also assessed on both rat and pig skin under non-occlusive conditions (10, 30 and 60 min exposure). A comparative study involving samples of rat, pig and human skin was performed with EGBE (10 % in water) using an exposure period of 1hr under both semi-occlusive and non-occlusive conditions. The studies with 3.5 and 10 % EGBE were repeated with 5 % 2-propanol (IPA) or 5 % alkyl sulfonate (LAS) added to the test solution. IPA nor LAS had any measurable effect on the dermal uptake of EGBE.

Under semi-occlusion *in vitro*, EGBE is readily absorbed and completely absorbed after 16 hr of exposure. Penetration depends on time as well as concentration. The penetration rate of pure EGBE is slower than from aqueous solutions, but more complete after 16 hr. A comparison of EGBE penetration at 6 hr under semi-occlusive conditions showed that for all the tested formulations, the penetration through the pig skin was 2 or 3 times less rapid than through rat skin. Application of EGBE on rat skin under non-occlusive conditions results in apparently great reduction in the absorption due to the volatility of the compound. Within 10 min following application, a major proportion of the absorbed material had penetrated. In comparison with the pig skin, rat skin showed an approximate 2 fold higher absorption. Under semi-occlusive condition, the penetration rate of EGBE through human skin was comparable with that through pig skin (much slower than through rat skin). Under non-occlusive conditions, human skin exhibits the lowest percutaneous absorption (6.9 % of the applied dose).

Percutaneous absorption of EGEE and EGBE was studied in humans and rats (Lockley *et al*., 1999 – Abstract). 2 butoxy [1-^{14}C] ethanol (1.41 mg/cm^2) was applied to rat dorsal skin and human breast skin *in vitro*. The results were compared with an *in vivo* study in which EGBE (9.22 mg/cm^2) was applied to the skin of male Wistar rats. Skin surface swab (unabsorbed material), tape strip (material in the stratum corneum), skin (material in the epidermis and remaining dermis) and absorbed dose (receptor fluids *in vitro* and mass balance cumulative dose *in vivo*) were analyzed up to 24 hr after application. Evaporated EGBE was trapped with carbon filters (> 80% of the dose within 1 hr of application).

Absorption of EGBE *in vitro* through rat skin most closely reflected penetration *in vivo*. Absorption through human skin *in vitro* was less than rat skin but reflected dermal absorption described in a previous study (Johanson *et al.*, 1988). This study demonstrates that for EGBE *in vitro* studies are able to reflect *in vivo* conditions.

Summary dermal route :

Five studies were reported on EGBE kinetic properties via dermal route. Two performed with Wistar rat, one performed with F344 rats and two performed with Guinea pigs. A series of *in vitro* studies was also available, studying percutaneous uptake of EGBE in various species, at different concentrations in different solvents and with various patterns of application (occlusive or not). Under semi-occlusive conditions, dermal uptake of pure EGBE was between 20 and 30 % of the administrated dose. Dermal uptake of aqueous dilutions of 5, 10 and 20 % EGBE was similar to that of the pure substance. Uptake was increased and was maximal for the 40 and 80 % aqueous solutions of EGBE. The rate of penetration was less for pure substance than for concentrated aqueous solutions. Some *in vitro* studies demonstrated that dermal uptake for pig skin was 2 or 3 times slower than rat skin. The results found with human skin were more or less equivalent to those seen with pig skin.

If EGBE was administrated under non-occlusive conditions, the dermal uptake decreased dramatically (uptake < 10 %), mainly because of the volatility of EGBE.

A plasma peak was obtained in about 2 hours after the beginning of the application and relatively constant until the cessation of exposure.

The metabolism mainly lead to the formation of BAA and also to smaller quantities of BEG (Sabourin *et al*., 1992b). The half-life of metabolites in plasma was about 4 hours. The majority of metabolites was eliminated via urinary excretion and only a very small amount

were found in faeces. One part of administered EGBE was metabolised in CO2 and eliminated via respiration, this amount increasing with the increasing dose.

Oral

Rats

The metabolism and disposition of EGBE was studied in F344 rats (Ghanayem *et al*., 1987a). Male rats were given a single doses of radiolabeled EGBE by gavage of either 125 or 500 mg/kg bw. Collection of urine, faeces and expired air was made during 48 hours after the administration. Biliary excretion of EGBE was also monitored. Determination of the radioactivity in each organ and in blood was performed at the end of the study.

The amount of $^{14}CO_2$ exhaled in the first 48 hours after dosing was approximately 18 and 10 % of the administered doses of 125 and 500 mg/kg bw respectively. Exhaled Volatiles accounted for no more than 2 % of the administered doses. Faecal excretion of EGBE ranged between 2 and 3 % of the administrated doses. Urine was the major pathway of excretion and most of the radioactivity was excreted during the first 24 hours after dosing. A higher percentage (70 %) was found for the 125 mg/kg dose as compared to the 500 mg/kg dose (40 %). Two major metabolites were detected by chromatography in the urine (Butoxy Acetic Acid (BAA) and the Glucuronide conjugate of EGBE (BEG). Three minor peaks corresponding to the Sulfate conjugate of EGBE (BES), EGBE and an unknown substance were also measured in urine. All 5 metabolites were observed in urine collected in the first 8 hours after treatment with 125 mg/kg bw; EGBE and BES were not seen in later time periods, and EGBE and BES were not seen at any time in the urine of rats treated with 500 mg/kg bw. Analysis of urine collected between 8 and 24 hr after dosing revealed that approximately 90 % of the EGBE derived radioactivity was BAA at both doses. While BAA was the only metabolite observed in the 24-48 hr urine of animals receiving 125 mg/kg bw, the ratio of BAA to BEG was approximately 3 to 1 in urine of rats receiving 500 mg/kg bw (see table 4.40).

Table 4.40: Effect of time and dose on the excretion of BE metabolites in urine

Dose						
	125 mg/kg			500 mg/kg		
	8 hr	24 hr	48 hr	8 hr	24 hr	48 hr
Metabolite						
X	3.0	<2 %	ND	<1 %	<1 %	<1 %
BAA	74.4	93.5	100	76.4	89.9	75.3
BEG	21.3	5.3	ND	23	9.2	23.8
BES	2.7	ND	ND	ND	ND	ND
BE	<2 %	ND	ND	ND	ND	ND

Significant increases in bile flow were seen as early as 0.5 hr after EGBE administration (for the 500 mg/kg dose) that returned to normal after 4 hr. Biliary excretion of radioactivity

continued to increase in a manner parallel with the increase in bile flow. The cumulative excretion reached 8 % of the 500 mg/kg dose in 8 hr. BAA, BEG and EGBE were excreted in bile. EGBE was detected only during the first 2 hr after treatment. BEG was the major metabolite excreted in bile.

Organs which had the most radiolabelling were forestomach, liver and kidneys.

The modulation of EGBE metabolism after pyrazole (alcohol dehydrogenase inhibitor) or cyanamide (aldehyde dehydrogenase inhibitor) administration was studied in F344 rats (Ghanayem *et al*., 1987b). A single dose of 500 mg/kg of [^{14}C]EGBE was administered to rats by gavage and another group received the same dose after a pre-treatment with 250 mg/kg Intra Peritoneal (IP) one hour before. Rats were placed immediately in metabolism cages for urine and faeces collections (8, 24 and 48 hr after dosing for urine and 24 and 48 hr after dosing for faeces). Expired volatiles and $^{14}CO_2$ were collected over the 48 hr housing period. At the end of the 48 hr rats were sacrificed and tissues were collected. Biliary excretion and radioactivity after treatment or rats with radiolabeled EGBE of radiolabeled EGBE + pyrazole was determined. Qualitative and quantitative determination of metabolites in urine and in bile was performed.

Approximately 11 % of the 500 mg/kg dose was exhaled as $^{14}CO_2$ in 24 hr after dosing. However pre-treatment with pyrazole resulted in a significant decline in the percent of EGBE dose exhaled as $^{14}CO_2$. In contrast pyrazole significantly increased the urinary excretion of EGBE derived radioactivity. BAA was the major metabolite collected whatever the collection period (75 – 90 % of the total urine radioactivity). Most of the remaining was the glucuronide conjugate of EGBE. In contrast the major metabolite of pyrazole pre-treated rats was BEG (75 – 85 % of the total radioactivity). Furthermore, 8 – 19 % of the radioactivity was the sulfate conjugate which was not detected in rats treated with EGBE only. The increase of biliary excretion in general was more important for pyrazole pre-treated than EGBE only animals. Moreover, the biliary excretion of EGBE derived radioactivity was higher in pre-treated rats (16 % vs 8 % for animals treated with only EGBE. A similar metabolic profile was obtained in bile compared to urine (BAA constituted 10, 21 and 46 % of the total radioactivity excreted in the bile fractions at 0 to 1, 2 to 4 and 6 to 8 hr after treatment. In contrast, no BAA was detected in any of the bile fractions collected from pyrazole pre-treated rats. In these rats approximately 12 % of the radioactivity was excreted in the first hr as unchanged EGBE with the remaining portion being BEG. In addition no metabolite other than BEG were detected in bile fractions collected at 2 to 4 and 6 to 8 hr after dosing with EGBE. With cyanamide pre-treatment, the same results were obtained than with pyrazole. This study clearly demonstrates that metabolism of EGBE to BAA is mediated by alcohol and aldehyde dehydrogenases via the formation of BAL.

The metabolism and disposition of orally administered EGBE (500 mg/kg) in young (4-5 weeks old) or adult F344 rats (9-13 weeks old) was compared (Ghanayem *et al*., 1987c).

There was a significant higher percentage of the EGBE exhaled as CO_2, 48 hr after dosing, by young rats (approximately 10 and 22 % of the administered dose in adult and young rats respectively). Similarly, young rats excreted a significantly higher percentage of the administered dose in the urine than adult rats. BAA, BEG and BES were identified in the urine of EGBE treated rats. Young rats excreted significantly less BAA than adult rats. Further, young rats excreted more BEG in urine than adults. No BES was detected in the urine of either age group. An unknown metabolite was detected in the urine of both age groups. It constituted less than 1 % of the total radioactivity excreted in the urine of adult rats compared

to young rats. Lower levels of EGBE derived radioactivity were seen in all tissues of young animals compared to tissues of old animals.

In a study performed by Medinsky *et al*., in 1990, EGBE was administrated via drinking water to male F344/N rats. In parallel 2 others rat groups were dosed with Ethylene Glycol Methyl Ether (EGME) and Ethylene Glycol Ethyl Ether (EGEE) for comparison with EGBE. Rat were allowed access for 24 hours to 2-butoxy[U-^{14}C]ethanol (50 mCi/mmol) (purity 99.9 %) at three doses: 290 ppm (237 μmol/kg bw), 860 ppm (401 μmol/kg bw) and 2590 ppm (1190 μmol/kg bw). Exhaled CO_2, urine and faeces were collected during 72 hours from the beginning of the exposure. At the end of the 72-hour collection period, cages were washed to determine the amount of radioactivity remaining, the total amount of water consumed by each rat in the drinking water was measured and recorded. Rats were killed and carcasses analysed for total ^{14}C. Urine was analysed for parent compound and metabolites.

80 % of the radioactivity excreted appeared during the first 24-hour period. For metabolites, the major compound identified was BAA (50-60 % relatively constant for all doses), others compound identified were ethylene glycol, the glucuronide conjugate of EGBE and EGBE. No N-acetylglycine conjugate of BAA was identified in this study.

Effects of multiple dosing of male Fischer 344 rats with EGBE on its own disposition and metabolism were studied (Ghanayem *et al*., 1992). This study was performed together with mechanistic studies on haematotoxicity (see § 4.1.2.2.3). Rats were treated with 125 mg/kg EGBE for 3 or 7 days by gavage followed by a single dose of 125 mg/kg dose of ^{14}C EGBE on day 4 and 8 respectively. Rats were placed in metabolism cages and EGBE metabolism analysis were performed.

No quantitative or qualitative alteration of EGBE metabolism and disposition were caused by repeat exposure compared to those observed in rats treated with a single dose. The ratio of BAA, the glucuronide and sulfate conjugates and parent EGBE excreted in the urine of rats treated for 4 or 8 days were relatively similar to those from rats treated with a single EGBE dose. Therefore, it is unlikely that tolerance development to the hemolytic effects of EGBE (seen in many studies) is caused by increased detoxification of EGBE or inhibition of EGBE metabolism to BAA.

[^{14}C]EGBE (purity 96.3 %) was administered by gavage to male F344 rats (3 rats/dose) at doses of 10 and 125 mg/kg bw as a solution in water. An additional group of 3 rats was dosed with [^{14}C]EGBE at 10 mg/kg in a corn oil vehicle (Dow, 1993). Samples of blood were taken from the rats administered EGBE in water at 1, 3, 6, 12 and 24 hr after dosing for determination of total ^{14}C (plasma) and for analysis of EGBE and BAA (whole blood). Urine from each animal was collected at intervals of 0-12 and 12-24 hr after dosing and the metabolic profile assessed. Faeces were collected for 24 hr. Expired $^{14}CO_2$ was collected throughout the study. Each animal was killed 24 hr after dosing.

Haematological effects were seen in 2 rats of the 125 mg/kg bw dose group (the third rat was eliminated because of mis-dosing). 59 % of the dose was excreted in the urine during the first 24 hr following administration of 10 mg/kg EGBE in either corn oil or in water. 10 % was excreted as $^{14}CO_2$ in 24 hr. At 125 mg/kg bw the 2 rats eliminated 37.7 and 70.3 % of the dose in urine during the first 24 hr and 7.6 and 8.5 % of the dose as $^{14}CO_2$. The maximum radioactivity plasma concentration was reached apparently just before the first blood sample was taken. Metabolite profiles in urine were similar to than of the Medinsky *et al.* (1990) study and the Ghanayem *et al*., 1987a study. Nearly 40 % of the ^{14}C in urine was present as BAA during the first 12 hr following 10 mg/kg bw and 65 % after 125 mg/kg bw EGBE

dosing. An additional 15 % of the ^{14}C in urine was present as the glucoronidase-labile conjugate of EGBE at 10 mg/kg bw and 10 % at 125 mg/kg bw. Ethylene Glycol (EG) was confirmed in this study as a metabolite of EGBE, but was present in small quantities.

The potential of EGBE to form fatty acids conjugates was studied in F344 rats and *in vitro* (Kaphalia *et al.*, 1996). [Ethyl-1, 2-^{14}C] BE (purity 99 %) was administered as a solution of water via oral route to 3 rats at a dose of 500 mg/kg bw. Three other rats served as controls and were given the same amount of water. The animals were killed 2 hr after exposure. The liver was excised and analyzed for radiolabeled lipids.

Approximately 16 % of the total radioactivity detected in the liver was associated with lipids, mostly in the phospholipid fraction (about 85 % of the total lipids). 3 % radioactivity of the total lipids was detected in the ester fraction.

The effects of co-administration of EGBE (99 % purity) with alcohols (Ethanol (EtOH), n-Propanol (n-PrOH) and n-Butanol (n-BuOH)) was studied (Morel *et al.*, 1996). Three sets of four groups of 10 male SD rats were used for the experiment in which all substances were administered orally by gavage (each set of groups differed from the others by the alcohol administered). In each set, the first group received distilled water and served as control. The second group was treated with only alcohol (10 or 30 mmol/kg) in distilled water, except for n-butanol (no vehicle), this group served as alcohol control group. The third group was dosed with EGBE at dose of 5 or 1 mmol/kg. The fourth group was given simultaneously alcohol and EGBE at the same doses than group 2 and 3 in distilled water (except for n-butanol: no vehicle). The haemolytic effect of EGBE (5 mmol/kg bw) was evaluated 4 hr after treatment by Red Blood Cell (RBC)-counting. Urinary concentration of BAA was determined on urine collected during 24 hr.

Administration of EGBE alone (5 mmol/kg) resulted in a significant decrease on average RBC (26 %) accompanied by a large increase in the concentration of free plasma Haemoglobin (Hb). The co-administration of n-BuOH or n-PrOH (10 mmol/kg bw) partially reduced the haemolytic effect of EGBE. No effect was seen with co-administration of 10 mmol/kg bw EtOH. At the dose of 30 mmol/kg bw, each of the three alcohols co-administrated with EGBE produced a complete protection against haemolytic effects. The alcohol control treatment did not have any effect on the haematological parameters.

Urinary excretion of BAA in rats treated only with 1 mmol/kg bw EGBE was 0.083 mmol/24 hr, which represents about 30 % of the ingested dose. The simultaneous administration of each of the three alcohols at a dose of 10 mmol/kg with EGBE did not modify the 24 hr urinary excretion of BAA, whereas at dose of 30 mmol/kg, decreases of 43, 33 and 31 % were observed for EtOH, PrOH and BuOH respectively.

Mice

In a series of studies, the mechanism of EGBE (purity 99 %) accumulation in forestomach was assessed (Poet *et al*., 2003). Five experiment were conducted to examine various end-points:

- target tissue histology / forestomach irritation

- tissue dosimetry and pharmacokinetics

- fur deposition

- salivary excretion

- retention in stomach content

For tissue dosimetry and pharmacokinetics, groups of 30 female mice were administered EGBE (in saline solution) at doses of 50 or 250 mg/kg by IP injection or 250 mg/kg by oral gavage. Blood samples were taken and then the mice were killed 0.5, 1, 3, 6, 9, 12 and 24 hours after dosing. Kidney, liver and stomach tissues were rapidly collected at each time point. The AUC and kinetic parameters for both EGBE and BAA were calculated.

EGBE concentration in tissues paralleled the levels in blood regardless of dose or exposure route. For the 250 mg/kg dose to either route, concentration of EGBE were higher and persisted longer in forestomach than in blood or in other tissues. Regardless of the route, T1/2 and AUC were higher in forestomach than in other tissues (Blood T1/2 could not be calculated because it was shorter than the earliest time point collected. The T1/2 for BAA was 2.1 hours after gavage administration.

By 24h, about 50 % of the total dose was eliminated in the urine (48 % for oral route) as EGBE, BAA or a conjugate. Following gavage administration of EGBE, BAA was the major urinary metabolite (38 % of the dose); there was also a small quantity (less than 0.2 %) of free EGBE, a conjugate of EGBE (up to 3 % - probably glucuronide).and a conjugate of BAA (between 0 and 7 %).

Male and females B6C3F1 mice were administered EGBE (in distilled water) at dose of 600 mg/kg (Deisinger and Boatman, 2004). Control animals were treated with distilled water. At 5, 15, 45 and 90 min following the dose (5 min only for controls) animals were sacrificed and about 100 mg of blood, liver and forestomach were collected. Samples were analysed for EGBE, BAL and BAA by GC-MS.

Five minutes after the administration, forestomach contained 50 fold more EGBE than blood or liver. Elimination from blood and liver samples was rapid (but still measurable at 90 min post dosing) whereas EGBE concentration in the forestomach decreased gradually and represent still 62 % and 31 % of the dose measured 5 minutes after administration in females and males respectively. Low concentrations of BAL were observed in all organs at maximum levels five minutes after administration. Forestomach levels were 10 fold higher than blood or liver concentrations. BAA concentrations increased till 45 and 90 min sample time. At all time points, BAA concentrations were lower in the forestomach than in blood or in the liver. No sex differences were detected for organ concentrations of EGBE and BAA but females seems to have higher BAL organ concentrations than males (at all time points).

Summary oral route :

EGBE kinetic properties via oral route were reported in up to 10 studies, 7 performed with F344 rats and one performed with SD rats. Two series of studies were also performed on mice These studies demonstrated the following results.

Absorption of EGBE orally administered was rapid and essentially complete (assumed to be 100 %). Target organs in which the most radiolabeled compound were found were: the forestomach, the liver and the kidneys. The major metabolite of EGBE is BAA which formed through a mechanism involving alcohol and aldehyde dehydrogenases which seems to be saturable. Simultaneous administration of EGBE and a primary alcohol (ethanol, n-propanol or n-butanol) in sufficient quantity inhibited BAA formation. The other metabolites were, in

order of magnitude: the glucuronide conjugate of EGBE (whose percentage increases relative to the dose at the expense of the BAA formation) and two minor metabolites, the sulphate-conjugate of EGBE and ethylene glycol (which was not observed in all studies). Elimination is rapid and occurs mainly by urinary excretion. A small amount of metabolised EGBE is eliminated as CO_2 in expired air (0 to 20 % for a high and low dose respectively). A small amount of unchanged EGBE (approximately 1 %) is also eliminated in expired air. In two studies, BAA, BEG and EGBE were found in bile. EGBE did not accumulate in tissues. The metabolic profile was not changed after repeated exposures compared to acute exposure. Age-related difference was observed: young rats eliminating EGBE via CO2 and urine (with significantly less BAA and more BEG) to a greater extent than adult rats.

Other routes

Rat

Female SD rats (4 rats) were administered EGBE (purity unknown) via IP route at dose of 2.5 mmol/kg EGBE concurrently with 20 mmol/kg EtOH (Romer *et al*., 1985).

The degradation of EGBE was almost completely inhibited in combination with simultaneous EtOH treatment.

Radiolabeled EGBE (purity 99 %) was administered subcutaneously to 3 male Wistar rats at the dose of 118 mg/kg. Faeces, urine and expired air were collected during the 72-hour period following EGBE administration. After termination the following organs and tissues were collected: liver, kidneys, spleen, fat, testes, thymus, sternum (including bone marrow) and blood (Bartnik *et al*., 1987).

EGBE was metabolised and eliminated primarily in urine. Within 72 hr over 78 % of the radioactivity was found in the urine. A small amount (less than 1 %) was found in the faeces and 10 % was eliminated in the exhaled air as CO_2. 1.6 % of the dose was found absorbed on activated charcoal indicating that the exhaled air contained only a small amount of the test compound or volatile metabolites. At the end of the experiment, 4.8 % of the radioactivity was found in the carcass. Spleen and thymus followed by the liver were the 3 major organs where the concentrations of radioactivity were highest.

In a series of studies, EGBE was administered via intravenous route in order to study the effect of dose, age and inhibition of metabolism on the kinetic parameters of EGBE (Ghanayem *et al*., 1990a). Male Fisher 344 rats (young: 3-4 months old and old: 12-13 months old), three to four animals per treatment group were given IP doses of either pyrasole (250 mg/2.5 ml of 0.9 % saline/kg – alcohol dehydrogenase inhibition), cyanamide (50 mg/2.5 ml of 0.9 % saline/kg - aldehyde dehydrogenase inhibition) or probenecid (50 mg/2.5 ml of 0.9 % saline alkalinised with $NaHCO_3$/kg – renal tubular transport inhibition). A second dose of probenecid was administered 4 hr later. Twenty to 30 minutes after initial treatment, all groups received a single bolus dose of radiolabeled EGBE (31.25, 62.5 or 125 mg/kg) delivered IV. Blood samples were collected at 5, 15 and 30 min and at 1, 2, 4, 6 and 8 hours for analysis of radioactivity. EGBE and metabolites were determined in whole blood and in plasma.

- Effect of dose and age: EGBE in blood is proportional to the administrated dose. At the same dose level, concentrations obtained in old animals were higher than in the young ones. The mean concentration of radioactivity in plasma was higher than in whole blood.

Only EGBE and BAA were found in the plasma. There was no significant effect of dose on $T_{1/2}$ or Vd (Volume of distribution) of EGBE. C_{max}, AUC and Cl of EGBE were affected by the level of administrated dose (increase of C_{max} and AUC and decrease of Cl with increasing doses of EGBE). $t_{1/2}$, Vd and Cl were not affected by the age of rats whereas C_{max} and AUC were found to be age-dependent (increased in the older rats). For BAA, there was a significant increase in $t_{1/2}$ in the older rats at the low and high dose but not in the middle. There is also a significant effect in AUC and C_{max}, which tend to be higher in older animals than in young, this effect is more pronounced with high doses.

- Effect of alcohol dehydrogenase inhibition by pyrazole: the radioactivity was higher in plasma than in whole blood. The total radioactivity was lower at all time point and at both dose levels than in rats treated with EGBE only. BE-glucuronide conjugate (BEG) was detected in the plasma (not in rats treated without pyrazole). Pyrazole resulted in significant increases in $t_{1/2}$, AUC and Cl of EGBE at both dose levels with no effect on Vd. For BAA, there was a significant decrease in the values of AUC, C_{max} and $t_{1/2}$ in rats treated with pyrazole.

- Effect of aldehyde dehydrogenase inhibition by cyanamide: the mean concentration of radioactivity was higher in whole blood than in plasma at all time points beyond the first 30 minutes after EGBE administration. Furthermore, the mean concentration of total plasma radioactivity time profiles was higher at all time points in EGBE treated rats compared with that in rats treated with cyanamide + EGBE. Increases of $t_{1/2}$, AUC, Vd and Cl of EGBE were seen in the cyanamide treatment group. No effect was seen on C_{max}. The mean $t_{1/2}$ of BAA was more than doubled but the difference was not statistically significant. A significant decrease of the C_{max} and AUC of BAA was seen.

- Effect of inhibition of renal tubular anion transport by probenecid : a consistent increase in the mean whole blood and plasma concentration of total radioactivity was seen. There were no significant changes in C_{max}, AUC, $t_{1/2}$, Vd or Cl of EGBE. In contrast, a significant increase in $t_{1/2}$ of BAA (the increase being greater at the lower dose). Probenecid also significantly increased the AUC of BAA (also greater at the lower dose). C_{max} of BAA was not significantly affected.

In summary, this study has confirmed that BAA is the proximate hemolytic agent and that it is formed from EGBE by a metabolic pathway involving alcohol and aldehyde dehydrogenase. The renal organic acid transport may play a role in the clearance of BAA. The higher sensitivity of older rats compared to younger rats concerning haemotoxicity was due to a combination of factors: compromised renal clearance of BAA by the renal anion transport system in older rats, increased EGBE metabolism to BAA and diminished degradation of BAA to CO_2 and greater sensitivity of erythrocytes in the older rats.

Mouse

Radiolabeled EGBE (purity 97.6 %) was injected intravenously to female B6C3F1 mice at dose of 10 mg/kg (Green, 2000b). Animals were terminated at 4, 24 and 48 hours after dosing (4 animals per sacrifice time). One animal for each sacrifice time was submitted to whole body autoradiography. The remaining three animals were submitted to an analysis of stomach and contents, the determination of radioactivity was made for forestomach, glandular stomach and stomach contents (free and bound radioactivity).

Four hours after dosing, the highest concentrations of radiolabel were found in the liver, Harderian glands, salivary glands, nasal passages, oesophagus, buccal cavity and on the

surface of the feet. A lower concentration of radiolabel was seen in the stomach, Gastro Intestinal (GI) tract contents and mucosa, in the kidney cortex and associated with bones. Background levels of labelling were seen in the lung and the remaining internal organs.

After 24 hours, the highest concentrations of radiolabel were observed in the liver, bone, Harderian glands, surfaces of the feet and buccal cavity. Slightly lower amounts were seen in the mucosa of the stomach, GI tract and the oesophagus. A lower level of labelling was observed in the salivary glands and kidney cortex. A much lower level of labelling was seen in the spleen, GI content and the kidney medulla. Background labelling was seen in the lung and the rest of internal organs.

48 hours after dosing, radiolabel was observed in the liver, bone, buccal cavity and oesophagus. The mucosa of the forestomach and glandular stomach were also radiolabeled although there was no significant difference between them. Lower levels were observed in the salivary glands and GI tract mucosa. The lung and the remainder of the internal organs had background levels of labelling.

The total radioactivity in the forestomach and glandular stomachs was similar over the duration of the study with the bulk of the radioactivity being present in the stomach walls: 80-95 % of the radioactivity was bound to protein at the two later time points.

In this study, the radioactivity in the stomach tissue appears to be derived from the systemic circulation and by ingestion from the buccal cavity. The origin of the latter is unknown, but according to the author, it may be derived from the salivary and Harderian glands which contained significant amounts of radioactivity.

In a series of studies, the mechanism of EGBE (purity 99 %) accumulation in forestomach was assessed (Poet *et al*., 2003). Five experiment were conducted to examine various end-points:

- target tissue histology / forestomach irritation
- tissue dosimetry and pharmacokinetics
- fur deposition
- salivary excretion
- retention in stomach content
- For Tissue dosimetry and pharmacokinetics, groups of 30 female mice were administered EGBE (in saline solution) at doses of 50 or 250 mg/kg by IP injection or 250 mg/kg by oral gavage. Animals were sacrificed 0.5, 1, 3, 6, 9, 12 and 24 hours after dosing. The AUC and kinetic parameters for both EGBE and BAA were calculated.

  EGBE concentration in tissues paralleled the levels in blood regardless of dose or exposure route. For the 250 mg/kg dose to either route, concentration of EGBE were higher and persisted longer in forestomach than in blood or in other tissues. For BAA, during the first three hours after IP administration the maximum concentration were found in blood, kidneys and liver. After 3 hours, forestomach concentration of BAA were higher than for other tissues. Unlike in the forestomach, BAA concentrations in the glandular stomach tissue were similar to other tissues.

  Regarding kinetic parameters, for EGBE both T1/2 and AUC were higher for gavage dosing than IP dosing. Regardless of the route, T1/2 and AUC were also higher in

forestomach than in other tissues (Blood T1/2 could not be calculated because it was shorter than the earliest time point collected. The T1/2 for BAA were 1h for IP injection.

By 24h, about 50 % of the total dose was eliminated in the urine (54 % for IP) as EGBE, BAA or a conjugate for the 250 mg/kg administrations. A majority of BAA was found (50 for the IP), a small quantity (less than 0.2 %) of free EGBE and of a conjugate of EGBE (up to 3 % - probably glucuronide) was detected. The last substance detected was a conjugate of BAA (between 0 and 7 %).

- For saliva excretion assessment, groups of mice were administered EGBE at doses of 250 mg/kg by IP injection or oral gavage. Salivation was induced by injection of pilocarpine a few minutes before saliva collection. Saliva was collected under anesthesia at various times after administration (up to 2.5 hr) by periods of 15 to 30 min (to obtain aliquots of saliva of 100-200 µl). Blood was collected at the midpoint of each saliva collection interval and at the end of saliva collection. Kinetic parameter of EGBE and BAA for blood and saliva were calculated.

  For EGBE, peak blood and saliva concentration were detected at 15 min and 7.5 min for blood and saliva respectively, regardless of the route. Concentration in blood and saliva were nearly identical at all time points and were below the level of detection after 1.3h. AUC and T1/2 in blood and saliva were similar.

  For BAA, the time curve was identical for blood and saliva, the values of saliva concentrations being 4 fold lower than the blood levels. The T1/2 was 2 fold higher in blood than in saliva (1.4 and 1.6 hr for IP and gavage respectively in blood).

- For retention in stomach contents, a group of animal was treated with 250 mg/kg EGBE by IP route. After sacrifice (3, 6 and 9 hours after exposure) EGBA and BAA were quantified in stomach tissue and stomach contents.

  The concentration of EGBE was higher in the stomach content than that of the forestomach tissue. No EGBE was detected in either blood or glandular stomach tissue at any time point. The estimation of the T1/2 for EGBE in the content of the stomach was 4.8h following IP injections, longest of all the tissues.

Summary other routes :

Two studies were performed via IV route (one in rats, the other in mice), two studies were performed via IP route and one via SC route in rats. Target organs evidenced in the previous described studies were confirmed in these studies: spleen, liver, thymus and stomach. However, a slight difference was seen in the distribution of the substance between the forestomach and the glandular stomach after an IV injection. The distribution to stomach comes from systemic circulation and also from ingestion of EGBE (which could come from salivary glands).

In these studies, which are generally mechanistic studies, it was demonstrated that the BAA formation was caused by Alcohol Dehydrogenase and Aldehyde Dehydrogenase in the liver. This finding is validated by competitive inhibition studies performed with Ethanol. Inhibition of renal tubular anion transport caused a decrease in the renal excretion of BAA and therefore an increase of the EGBE toxicity.

4.1.2.1.2 Studies in humans

Inhalation

Seven human male volunteers were exposed to 20 ppm EGBE (0.85 mmol/m^3) via inhalation route for a period of 2 hours during light physical exercise (50W) (Johanson *et al*., 1986a). Blood samples were collected during the exposure period and 3 hr afterwards and analysed for the determination of EGBE blood concentration. Urine was collected for a period of 24 hours, the first sample was collected immediately before the volunteer entered the exposure chamber and thereafter sampling was made at 2-hour intervals for 6 hours. The samples were assayed for EGBE and BAA.

No signs of adverse effects could be related to the exposure of EGBE and no effects were seen on electrocardiograms. The concentration of EGBE in blood rose rapidly reaching an apparent plateau level within 1-2 hours. The decay after exposure was rapid, with a biphasic slope in a semi-logarithmic plot (data are too limited to permit the calculation of more than one slope constant). EGBE could no longer be detected in the blood 2-4 hours after the end of exposure. The average elimination $t_{1/2}$ was 40 min, the average plateau level in blood was 7.4 µmol/l, the average blood clearance was 1.2 l/min and the steady-state volume of distribution was 54l. The total amount of EGBE excreted in urine was less than 0.03 % of the total uptake and the $t_{1/2}$ of EGBE in urine was 1.36 hr. In urine, BAA reached a maximum concentration between 5 and 12 hours after the beginning of exposure. Elimination also reached a maximum between 2 and 10 hours after the beginning of the exposure. Great variations are observed between subjects. $T_{1/2}$ for BAA in urine was calculated at 5.77 hours.

According to the authors and on the basis of the very high *in vitro* blood/air partition ratio (above 1000, Johanson and Dynesius, 1988), one would expect the respiratory retention of EGBE to be limited by the alveolar ventilation and approximate 80 % at 50 W. However, the retention was about 57 % in the present study. The explanation may be that EGBE is partly adsorbed on the surface of the respiratory airways during inhalation and desorbed during exhalation.

Five human volunteers were exposed to EGBE at 20 ppm for 2 hours during light physical exercise (50 W) (Johanson and Johnsson, 1991). Venous blood samples were collected and analysed for BAA before and immediately after exposure and also at 4 and 6hr after the beginning of exposure. Urine was collected every 2 hours and assessed for BAA content.

After 2 hours of exposure BAA was found in blood. An average maximum concentration of 45 µM BAA was reached after 2-4 hr. The decrease in BAA blood levels from 4 to 6 hr corresponds to an average half-time of 4.3 hr. The time profile in blood seems to be similar to that for urinary excretion, where the maximum occurred at about 5 hr and the half-time was estimated to 4 hr. The average clearance of BAA was estimated to be 23-39 ml/min and is only about 1/3 of the glomerular filtration rate of about 125 ml/min. The low renal clearance may be due to binding of BAA to blood proteins and absence or low efficiency in tubular secretion. The low pKa of BAA (3.5) indicates that more of 99 % of BAA present in urine is present in the ionised form and is not available for tubular re-absorption at normal urine pH. The average Vd of BAA was calculated to be 15 l. This value is approximately equal to the volume of extracellular water.

Respiratory uptake of EGBE was investigated (concurrently with 9 other solvents) (Kumagai *et al*., 1999). The uptake of a chemical with high blood/air partition coefficient is predicted to

be high. But when a chemical is very water soluble, it can be partially absorbed in the mucus layer during inhalation and release during exhalation, leading to a low respiratory uptake.

Four human volunteers were submitted to 25 ppm of EGBE (whole body in an inhalation chamber) for 10 minutes. The same people were also submitted to inhalation of 9 other substances in the same test conditions. Exhaled air was collected from 1 min before exposure to the end of the exposure period.

The mean respiratory rate for each solvent was from 12.1 to 14 min^{-1}. The mean tidal volume was from 470 to 530 ml, no differences were found among the solvents. The results obtained with the different solvents suggests that the wash in/wash out behaviour cannot completely explain the actual respiratory behaviour of these solvents.

Summary human inhalation route:

Three studies were reported in which human volunteers were exposed to EGBE by inhalation route. Kinetic parameters found in these studies were equivalent to those described in human studies earlier in the report for others routes of administration. Theoretical absorption (calculated) of EGBE via inhalation route was found to be 80 %. However, measurements performed showed a real absorption of 55 to 60 %. This difference is explained by a "wash in / wash out" mechanism: due to its hydrophilic properties, EGBE is adsorbed to the surface of the respiratory tract during inspiration and it desorbed during exhalation leading to a decrease in the real uptake of substance. In the risk characterisation section, a 60 % of absorption for EGBE inhalation is used .

Dermal

In vitro

In an *in vitro* skin penetration test, the mean absorption rate of undiluted liquid EGBE (purity > 99 %) through human epidermis assessed (ICI, 1982a and 1982b). In this test, the penetration rate of EGBE was compared with 3 other glycol ethers (EGEE, EGME and Propylene Glycol Methyl Ether (PGME)) and 1 acetate (EGEEA). Disks of human abdominal skin were placed in diffusion chambers, the membrane integrity was assessed before the test by measurement of their permeability to tritiated water. The glycol ether absorption rate was measured for a period of 8 hours. After the test, the potential of the tested substance for impairing the epidermal diffusion barrier function was assessed by measuring the permeability to tritiated water again. A calculation of the damage ratio was made: Permeability constant after glycol ether contact / permeability constant before glycol ether contact.

For EGBE the mean rate of penetration calculated in this study was 0.20 $mg/cm^2/hr$ (±0.03 SEM, n=8). EGBE did not produce large alteration in permeability in this study: damage ratio = 2.07. Human skin *in vitro* will always deteriorate over time and a ratio greater than 1 would be expected. The measured ratio indicates a marginal effect with little damage to the skin following prolonged exposure.

Another skin penetration study *in vitro* was performed with liquid EGBE (Eastman Kodak, 1991). Human abdominal skin (stratum corneum) was exposed to EGBE in Franz-type diffusion cells. The integrity of each skin sample was determined before and after the test by measuring the permeability to tritiated water.

The skin absorption results for EGBE were measured twice due to high variability observed in the first study. The mean absorption rates were: 0.857 mg/cm^2/hour ± 0.282 in the first experiment and 1.52 mg/cm^2/hour ± 0.37 in the second experiment. The damage ratio was 3.25 ± 3.33 in the first experiment and 5.14 ± 4.99 in the second experiment. Due to the high variability in each experiment, mean absorption rates were calculated separately for the undamaged skin (n=8) and the damaged ones (n=4), the results from the damaged skin specimens were about 3 times higher than the undamaged ones (3.39 mg/cm^2/hour vs 1.19 mg/cm^2/hour). When the results from the four cells showing the high damage ratio are excluded from the calculation of the overall mean result, the mean damage ratio is 1.66 ± 1.31.

In a study performed by PMIC in 2001, *in vitro* percutaneous absorption of EGBE through human skin was assessed. Two different EGBE concentrations 10 and 5 % in an oxidative hair dye formulation was applied on human skin during a period of 30 minutes. For each experiment 33 mg of the different formulations (corresponding to 20 mg/cm^2) were applied on the skin surface (1.76 cm^2). At the end of the 30 minutes of exposure, the surface of the skin was washed to eliminate the residual mixture. The diffusion of EGBE was monitored during the 24 hours following the applications. The receptor fluid was collected 2, 4, 6, 10, 21 and 24 hours after the beginning of exposure. At the end of the 24 hr observation period, tissues (horny layer, epidermis and dermis) were analysed for EGBE remaining. This study was performed in compliance with GLP and following the recommendations of FDA, AAPS, COLIPA, SCCNFP and OECD for this kind of test.

Results are expressed in µg equivalent of EGBE (µg/cm^2) and in % of the applied dose for all the compartment analysed (see table 41).

Table 4.41: Quantities of EGBE analysed in the different system compartments for the 2 tested concentrations (5 and 10 %)

	EGBE 5 %		EGBE 10 %	
	µg/cm^2	% of the applied dose	µg/cm^2	% of the applied dose
Filter (F)	29 ± 3	3.13 ± 0.34	53 ± 8	2.82 ± 0.38
Washing (W) (30 min)	639 ± 66	68.6 ± 7.5	1223 ± 178	65.2 ± 9.5
Skin excess (F+W)	668 ± 68	71.7 ± 7.8	1275 ± 182	68.1 ± 9.8
Epidermis (E) Including stratum corneum (SC)	0.112 ± 0.065	0.012 ± 0.007	0.283 ± 0.128	0.015 ± 0.006
Dermis (D)	0.023 ± 0.01	0.002 ± 0.001	0.101 ± 0.051	0.005 ± 0.003
Receptor fluid (RF)	113 ± 54	12.1 ± 5.9	235 ± 138	12.5 ± 7.3
Total skin +RF (SC+E+D+RF)	113 ± 54	12.1 ± 5.9	235 ± 138	12.5 ± 7.3
Total recovery (%)	84 ± 4		81 ± 4	

These results showed that no accumulation of EGBE in skin occured. For these two concentrations, the percentage of absorption were similar (12.1 and 12.5 % for the 5 % and 10 % concentrations).

In vitro percutaneous absorption of liquid glycol ethers (including EGBE purity 98 %) was studied (Wilkinson and Williams, 2002). Human breast skin, full thickness or dermatomed (stratum corneum + upper dermis) was exposed to EGBE in aqueous solution at different concentrations (3 mg/ml or 6 mg/ml, 100 or 200 µl in the skin) or undiluted (10.5 µl). Percutaneous absorption was measured for 24 hours using flow through diffusion cells. Tissue culture medium was used as a receptor fluid with 2 % (w/v) Bovine Serum Albumin (BSA) or 2-6 % (w/v) PolyEthylene Glycol 20 (PEG 20) added for some studies.

In aqueous solution, a steady state flux of 544 ± 64 nmol.cm^{-2}.h^{-1} (0.064 mg/cm^2/hr) was found with dermatomed human skin. Reducing the dose to 100 µl decreased the steady state flux of EGBE by about 55 %. Using full thickness skin increased the time to steady state (tau) and reduced the steady state flux. Absorption rates of undiluted EGBE in finite dose exceeded those measured with aqueous solutions, though the apparent permeability coefficient was higher with the aqueous doses.

Modelling

An alternative for dermal permeation estimation is the model SKINPERM (version 3.0), which provides an estimation of the amount of EGBE that penetrates the skin either from the vapour phase, the neat liquid or an aqueous solution. SKINPERM describes dermal exposure and absorption using a quantitative structure activity relationship (QSAR) which is based solely on the biological structure of the skin and the physico-chemical properties of the substance under investigation. SKINPERM 3.0 was validated with an extensive database of experimentally measured permeation coefficients and is considered valid for substances with log Kow values in the range from 0 to 4. The model estimates the permeation rate for EGBE to be 0.63 mg/cm^2/hr, which is consistent with the measured *in vitro* data. This model is used for EGBE only for comparative reasons as a lot of measured data is available.

In vivo

Five men, who have been involved in another study 2 years ago (Johanson *et al.*, 1986a), were exposed to neat EGBE for 2 hr by placing four fingers of the left hand into a vessel filled with pure liquid solvent (Johanson *et al.*, 1988). At regular intervals, thickness of the skin and the finger volume were measured. Blood and urine analysis were performed during a 24-hour period after the beginning of exposure.

The skin of the exposed fingers was not irritated but appeared somewhat more rigid and less elastic after exposure and a wrinkled appearance. This effect reaches a maximum 2-4 hours after exposure and gradually disappeared. Concurrently, the volume of the fingers and the thickness of the skin decreased and then return to the normal. EGBE was detected in the blood of all subjects after exposure. Estimates of the skin uptake showed great variations between individuals (more than ten-fold variation): 7 to 96 nmol/min/cm^2 (0.05 - 0.63mg/cm^2/hr) with a geometric mean of 20 nmol/min/cm^2 (0.14mg/cm^2/hr). The elimination $t_{1/2}$ for EGBE in blood ranged from 0.6 to 4.8 hr (mean 1.3 hr). The excretion rate of BAA in urine increased during the first hours of exposure with a maximum 5 hours after the beginning of exposure. The cumulative excretion of BAA ranged from 2.5 to 39 % of the uptake of EGBE (with a mean of 17 %).

The percutaneous absorption of EGBE vapour was assessed in four male volunteers (Johanson and Boman, 1991). Subjects were exposed to EGBE by inhalation during 2 hours at concentration of 50 ppm (2 mmol/m^3). The subjects were breathing chamber air while sitting outside the exposure chamber, mouth breathing was assured by the use of a nose clip. After the two hours, one hour of pause was made then a second period of exposure was performed. For this second exposure, subjects were sitting inside the exposure chamber (subjects were naked apart from a pair of shorts – the estimated surface area exposer being 16000 cm^2) but breathing air was supplied from a tank of clean air. Heart rate was monitored during the entire day. Each volunteer participated in two experiments, one at normal (23° C, 29 % relative humidity) and one at raised temperature and humidity (33° C, 71 %). The two experiments were separated by two weeks or more. Capillary blood samples were collected during the experiments for the determination of EGBE. Based on earlier observations (Johanson *et al.*, 1986a), the relative respiratory uptake of EGBE was assumed to be 60 %.

The EGBE blood concentration during the mouth exposure increased during the first hour and seemed to reach a steady state at about 3μM (1.8-4μM) during the 2nd hour. The mean respiratory uptake was 1.3 mmol or 11 μmol/min and the apparent blood clearance 3.8 l/min. For the percutaneous exposure, the concentration of EGBE in blood increased to about 9 μM during the second hour of exposure. In seven of the experiments the EGBE blood concentration was 2.4-5.5 times higher after skin exposure than after mouth exposure, in one it was lower. This difference was also reflected in the high percutaneous absorption of 31 (8.6-48) μmol/min, or 2.5-5.9 times higher than the respiratory uptake. The half life of EGBE in blood after the skin exposure averaged 34 min (19-53 min). Only small and inconsistent differences in heart rate were found between the 2 routes of exposure, thus the observed differences in blood concentration and uptake rates of EGBE cannot be explained by differences in pulmonary ventilation. The concentration of EGBE in blood appeared to be slightly raised during mouth exposure to hot and humid, compared with normal chamber air. The increase may be partly explained by a raised pulmonary ventilation. The tendency towards higher blood concentrations in hot and humid conditions was more pronounced during the skin exposure. However, the differences between the two exposure conditions were not statistically significant.

Six human volunteers were exposed arm only to 50 ppm ($^{13}C_2$)EGBE (purity 99.54 %) for 2 hr (Corley *et al.,* 1997). Blood samples were collected into the antecubital vein of the unexposed arm for the analysis of both EGBE and its major metabolite BAA. Finger prickblood samples were taken from the exposed arm only at the end of the 2 hr exposure to compare with the blood obtained in the antecubital vein. Blood samples were obtained before exposure and at 10, 20, 30, 40, 60 and 1.5, 2, 2.25, 2.5, 3, 3.5, 4, 8, 12, 16 and 24 hr after the initiation of the 2 hr exposure. Urine samples were collected before exposure and at 0.12 and 12-24 hr intervals following the initiation of exposure for metabolite analysis.

EGBE was not detected in blood samples from the unexposed arm until 30 minutes. By 1.5 hr EGBE was detectable in all 6 subjects. An apparent steady state was reached for EGBE between 1.5 and 2 hr. EGBE was rapidly cleared from the blood with an apparent elimination $t_{1/2}$ of 0.66 hr (in 2 of the 6 subjects).

As EGBE, BAA was not detected in blood samples from the unexposed arm until 30 minutes. BAA was detectable in all six subjects by 1 hr. Peak blood concentrations were reached 3-4 hr after the start of the 2 hr exposure. BAA was less rapidly cleared from the blood than EGBE, with an apparent elimination $t_{1/2}$ of 3.27 hr.

The ratio of the finger prick blood sample taken at the end of the 2 hr exposure to the corresponding blood sample taken from the unexposed arm averaged nearly 1500/1 indicating a considerable influence by local absorption.

No free EGBE was detected in any urine samples. Following hydrolysis to cleave acid-labile conjugates, EGBE was detectable in only 2 urine samples (0-12 hr). No EG nor glycolic acid were detected in urine.

BAA was eliminated in urine during the first 12hr collection interval.2/3 of the total amount of BAA excreted in the urine was in the form of an acid-labile conjugate. In this study the skin permeability coefficient was estimated to be 3 cm/hr.

Four volunteers (2 males and 2 females, aged 28-33) were exposed on 9 separate occasion, each occasion separated by at least 3 weeks (Jones *et al.*, 2003). All exposure were 50 ppm EGBE for 2h. Two exposure were performed whole body and two skin only at 25° C, 40 % relative humidity and volunteers wearing shorts and T-shirts (baseline conditions). Other exposures were skin only and were performed with one parameter changed: high humidity (60 % and 65 %), low and high temperature (20 and 30° C), minimal clothing (shorts (and bra) only and overalls (all-in-one boiler suit) and an industrial scenario (overalls and high humidity and temperature (30° C and 60 % relative humidity). Total BAA in urine was measured to assess the absorbed EGBE. Urine samples were collected before and after each exposure (0, 4, 6, 8, 10, 12, 22, 26, 30 and 34h). To record any physiological changes in the volunteers under the different conditions, physiological monitoring equipment was used. The results obtained for skin only exposure are expressed as a percentage of the whole body measurement.

The mean dermal absorption for baseline conditions contributes for 11 % of the total body burden. Low temperature did not affect significantly the dermal absorption whereas high temperature increased significantly the dermal absorption (14 %). Increasing humidity increased the dermal absorption but not significantly. Clothing has also a little effect on dermal absorption. In the industrial scenario skin absorption is significantly increased compared to baseline conditions. The maximum dermal absorption contributes for 42 % of the total body burden (mean 39 %). No significant differences in any of the physiological parameters were seen.

This study shows that temperature is an important factor to take into account for assessing the percutaneous absorption of EGBE and also that the use of protective equipement under high temperature and relative humidity can lead to a high dermal absorption (higher than without protective equipment).

Six male volunteers were dermally exposed to EGBE at various concentration: neat or 50%, 90 % in water for 4 hours on the forearm (about 40 cm^2) (Jakasa *et al.*, 2004). An inhalation exposure of EGBE vapour was also performed on each volunteer (93 ± 6.8 mg/m^3 for 30 minutes) and served as a reference dosage. Dermal absorption parameters were calculated during 24 hours after the beginning of exposures by measuring excretion of total BAA (free + conjugated) in urine and EGBE in blood. Blood samples were collected for 8 hours (16 samples per experiment). Urine samples were collected every 4 hours during the 24 hour period. Each volunteer was exposed twice to a 50 % EGBE concentration (on each arm), once to the 90 % concentration and once to the pure EGBE. The period between two dermal exposures of the same site was at least 4 weeks.

EGBE in blood was below the detection limits after exposure to pure EGBE. The average dermal flux and the apparent permeability coefficients were greater for the 50 % dilution than for the 90 % dilution (see table 4.42). The same results were obtained with urinary excretion

of BAA. The permeation rate reach a plateau between 1 and 2 hours after the beginning of exposure, indicating a steady state permeation. The maximum permeation rates are presented in the table 4.43 (apparent permeability coefficients). The uptake after dermal exposure was also compared with the uptake after inhalation exposure (see table 4.42) showing that significant amounts of EGBE are due to dermal exposure. Half life of BAA was calculated to be 3.4 hr (range 1.3 to 3.8 hr for inhalation experiment). From the inhalation study, it was calculated than 57 % of the inhaled EGBE was excreted as BAA in urine.

Table 4.42: Kinetic parameters measured in the Jakasa study

	50 % EGBE	90 % EGBE	Neat EGBE
Average dermal flux (mg/cm^2/h)			
Based on blood data	0.92 +/- 0.34	0.74 +/- 0.25	ND
Based on urine data	1.34 +/- 0.49	0.92 +/- 0.60	0.26+/- 0.17
Apparent permeability coefficients (x10^{-3} cm/h)	1.75 +/- 0.53	0.88 +/- 0.42	ND
Estimated dermal uptake of EGBE(mg)	1,340	926	263
Pulmonary uptake	346		

Intra and inter-individual variation of dermal fluxes were also studied with 50 % dilution. The results are reported in the table 4.43.

Table 4.43: Intra and inter-individual variation of dermal fluxes after dermal exposure to 50 % EGBE in water.

	Average dermal flux (mg/cm^2/h)	90 % confidence interval	Intra individual CV (%)	Inter individual CV (%)
Blood data	0.92 +/- 0.34	0.63 – 1.19	16	35
Urine data	1.34 +/- 0.49	0.94 – 1.74	20	34

The results obtained in this study are in good agreement with animal data and *in vitro* data on human skin. It also demonstrate that demal absoption of EGBE is important when compared with inhalation exposure.

Summary human dermal route:

Five *in vivo* studies on volunteers and four *in vitro* studies are available for this end point. The four *in vitro* studies, measuring the rate of absorption of liquid EGBE through human skin, gave results that varied by a factor of 25 (0.064 mg/cm^2/hr for the lowest and 1.66 mg/cm^2/hr for the highest). *In vitro*, rate of absorption is highly dependent on the concentration of the aqueous solution of EGBE used. *In vivo*, in one study, an estimation of the skin penetration gave results in the following range: 7 to 96 nmol/min/cm^2 (0.008 mg/cm^2/hr to 0.0114 mg/cm^2/hr) for pure liquid EGBE. Another study performed with liquid EGBE has showed that greater absorption was found with EGBE 50 % in water than neat EGBE (dermal flux 1.34 mg/cm^2/h and 0.26 mg/cm^2/h respectively). This is consistent with data available *in vitro* and in animals. This is also critical for risk characterization because percentage of absorption

taken into account in each scenario can be different if exposure is exposure is to EGBE pure or EGBE in formulations.

EGBE half life was approximately 1 hour in all studies whereas BAA half life was about 5 hours. If temperature and humidity conditions increased, the percutaneous uptake increased also (in contrast with the inhalation uptake which remained constant).

In one *in vivo* study, percutaneous absorption of vapour EGBE was assessed. Depending on the external conditions during exposure, the internal dose of EGBE due to percutaneous absorption varies between 11 % and 39 %. The percentage of 11 % was found for "normal" conditions of use (temperature, humidity) and 39 % for the worst case of industrial use (high temperature, high humidity and overalls wearing). This worst case percentage is use in the risk assessment section to estimated the internal dose of EGBE due to dermal absorption of vapour EGBE.

Based on the PbPk modelling it is estimated that for a worst-case exposure to EGBE vapour (100 % of the body exposed and no cloths), percutaneous absorption would account for 15-27 % of the internal dose of EGBE (Corley *et al.*, 1997).

Oral

In a suicide attempt, a 50-year woman ingested 250-500 ml of a window cleaner containing 12 % of EGBE, corresponding to about 0.5 to 1 g/kg bw (Rambourg-Schepens *et al.*, 1988). On admission to the intensive care unit, the patient was comatose. Biologically she presented with metabolic acidosis, hypokalaemia, a rise in serum creatinine level and a markedly increased urinary excretion of oxalate crystals.

In another suicide attempt, quantitative analysis of blood BAA was performed (Gualtieri *et al.*, 2003). A high maximum blood concentration was found (4.86 mmol/L). Symptoms recorded were metabolic acidosis and hepatic biochemical disorders. Si sign of haemolytic anaemia were described.

4.1.2.1.3 Other data

In vitro studies have been conducted to determine the relative degree to which some glycol ethers act as substrate for alcohol dehydrogenase (ADH) (Dow Chemicals, 1983). Four concentrations of EGBE (purity 98.55 %) were used to define the kinetic constants. Each concentration was run in duplicate. Experiments were conducted at both pH 8.8 and 7.4 in order to determine if pH was a critical factor.

Assays conducted at pH 7.4 resulted in data sets which were not suitable for plotting. For EGBE, Vmax was 4.06 μM/min, Km was 1.18×10^{-3} M with a correlation coefficient of 0.98.

Kinetic of EGBE was studied in a perfused rat liver system with and without ethanol (Johanson *et al.*, 1986b). The liver of a Sprague-Dawley rat was isolated and perfused with EGBE (99 % purity). Concentration ranging from 0.057 to 2.7 mM of EGBE were achieved and submitted to the perfusion system for periods of 10 min. Two samples from the perfusion medium and from the perfusate were collected during the last 4 minutes and were analysed for EGBE concentration determination. Preliminary studies indicated that the half life of the adaptation of the liver to new concentrations of EGBE was approximately 1 min. Hence, 6 min was regarded sufficient to reach a plateau level. At the end of 3 experiments, the effect of

EtOH was studied while maintaining the concentration of EGBE at 0.45 mM. The liver was then perfused with a medium containing 17.1 mM EtOH during 20 min, followed by an equal period of time of perfusion with EtOH free medium.

The elimination data fit well to the Michaelis-Menten equation. The estimates of the apparent maximum elimination rate of EGBE range between 0.59 and 1.3 µmol/min/g. The estimate of the Km vary from 0.19 to 0.4 mM. Maximum clearance ranged from 2.7 to 3.1 ml/min/g. In the presence of EtOH, the extraction ratio of EGBE decreased from 0.44 to 0.11. When EtOH is withdraw, the liver returned nearly to its previous elimination capacity in approximately 10 min. This finding supports the hypothesis that EGBE is mainly metabolised by ADH in the rat liver.

Testicular and hepatic capacities to metabolise EGBE by alcohol dehydrogenase (ALD) was assessed (Moslen *et al.*, 1995). These comparisons were done with SD rats (highest testicular capacity to metabolise EGME) and with Syrian Golden Hamster (no detectable testicular capacity to metabolise EGME).

Testicular and hepatic activities of ALD for EGBE was greater in hamster than in rat. Comparing with other glycol ethers (GE), testicular and hepatic activities of ALD for EGBE were the less important in rats whereas testicular ALD in hamster was greater for EGBE than for EGME or EGEE. In hamster liver, the ALD activity was less than those of EGME and EGEE.

In a series of *in vitro* studies the metabolites of some glycol ethers, EGME, EGEE and EGBE were determined in human and rat hepatocyte cultures (Green *et al.*, 1996). Adult male Fisher 344 hepatocytes and human hepatocytes were incubated with EGBE (purity unknown) at doses of 0.02 – 0.2 – 2 and 10 mM for 4 hr.

Over 90 % of the radioactivity was detected in 3 peaks: EGBE – BAA and EG. BAA was the major metabolite formed in both species. More than 90 % of the 0.2 mM EGBE was rapidly converted to BAA by rats hepatocytes (40 % for human hepatocytes). The percentage of EGBE that was converted to BAA after 4 hr of incubation with rat hepatocytes was similar at 0.02 – 0.2 and 2 mM. In human hepatocyte the percentage of EGBE that was metabolised to BAA decreased between 0.2 and 2 mM. The percentage of EGBE metabolised to EG was greatest at the lowest substrate concentration for both human and rats suggesting that this pathway became saturated at relatively low glycol ether concentration. Another minor metabolite of EGBE was identified in this study: glucuronide conjugate of EGBE.

V_{max} values were 15–20 fold greater in rat hepatocyte than in human hepatocytes (741 nmol/h/10^6 hepatocytes in rat and 113 in human). The Km value was similar for rats and humans (1 mM).

The potential of EGBE to form fatty acids conjugates was studied in F344 rats and *in vitro* (Kaphalia *et al.*, 1996). Fatty acid ethyl synthase (FAEES) was extracted from rat liver microsomes. Different quantities of this enzyme were incubated with 2 µmol of [1-^{14}C] oleic acid and 750 µmol EGBE at 37 ° C for 2 hr. A control reaction mixture was also incubated without enzyme. After extraction radioactivity was measured.

Incubation of [1-^{14}C] oleic acid and EGBE in the presence of the FAEES results in the formation of 18:1 fatty acid 2-butoxyethyl ester (not detected in the mixture incubated without enzyme).

The role of liver alcohol dehydrogenase isoenzymes in the oxidation of glycol ethers in rats was explored (Aasmoe *et al.*, 1998). Wistar rats were killed to prepare liver homogenate. ADH activity was assayed by gel electrophoresis after addition of EGBE at the following concentrations: 0.05-20 mM. Km and Vmax were calculated. Activities were expressed as specific activity (the rate of NADH formation/min/mg protein). This assay was also performed concurrently with Methanol (MeOH), Ethanol (EtOH),BuOH, EGME, EGEE, Ethylene Glycol Propyl Ether (EGPE) and Ethylene Glycol Hexyl Ether (EGHE).

After electrophoresis, the liver homogenate revealed one enzymatically active zone on staining with MeOH, EtOH, BuOH, EGME, EGEE, EGBE and EGPE (this pattern was identical for male and females). This enzymatic form is probably identical with the ADH-3. The intensity of the ADH-3 increased with increasing chain length of the alcohol or glycol ether. The ADH-2 isoenzyme was only active with medium and long chain aliphatic alcohols (not with glycol ethers). The same ADH isoenzyme was active in males and females, however the activity of ADH is sex-dependent, with higher enzyme activity in females than in males. This experiment also showed that this difference was a result of higher amount of ADH in females rather than of sex-specific isoenzymes.

The substrate specificity (Vmax/Km) decrease in the following order: EGBE > EtOH > EGEE > EGME. The Km value for EGBE was approximately 10-fold lower than the other 2 glycol ethers, while the Vmax values were almost the same. Consequently, at low and identical glycol ether concentrations, the biotransformation of EGBE would be more efficient than that of the other two glycol ethers. At the same time, the low Km value for EGBE compared to EGME and EGEE indicates that metabolism of EGBE via ADH will be saturated at lower substrate concentrations than those of EGME and EGEE.

A PbPk model was developed with the results obtained from various studies (Johanson, 1986c). In his study, Johanson tried to extrapolate the *in vitro* rat data to man *in vivo*. Concentration-time curves are generated by computer simulation. The outcome of the simulation is compared with the results from the experimental exposure in male volunteers. The model used is designed for simulation of organic solvent kinetics in general. The organs are lumped into compartments according to blood flow and fat content. Muscles and skin form a separate compartment due to their wide variation in blood flow at different work loads. It is assumed that solvent uptake occurs only in the lungs and elimination only in the liver. It is further assumed that the solvent instantaneously distribute homogenously in each compartment, and as a consequence the solvent retained in the respiratory airways immediately reaches the arterial blood. The model structure is depicted in the figure 4.44:

Figure 4.44: First PBPK developed for EGBE

All partition ratios between blood and other compartments are set to 1. Strict flow limitation, i.e. diffusion equilibrium between end-capillary blood and tissue is assumed for all compartments. The relative pulmonary uptake of EGBE was set to 0.6. Volumes and blood flows to the different organ groups were computed from literature data.

There is a good agreement between simulated and experimental blood concentration curves, indicating that the assumptions made have a certain degree of validity. Increased physical activity increased the blood concentration of EGBE due to increased pulmonary uptake rate. Similarly, the co-exposure to EtOH caused elevated blood concentration of EGBE due to a decrease in the elimination rate. The simulation also suggested that venous blood samples are useful, if not slightly more useful, as arterial or capillary blood sample in the biological

monitoring of exposure to EGBE. In any case, blood samples should be collected during the exposure phase because of the rapid decay of EGBE. The risk of accumulation of unmetabolized solvent in the body appeared low. According to the model, non-linearities because of saturated elimination occur at concentrations well above 100 ppm, even in combination with physical exercise and ethanol, thus linear kinetics of EGBE may be expected at ordinary occupational inhalation exposure.

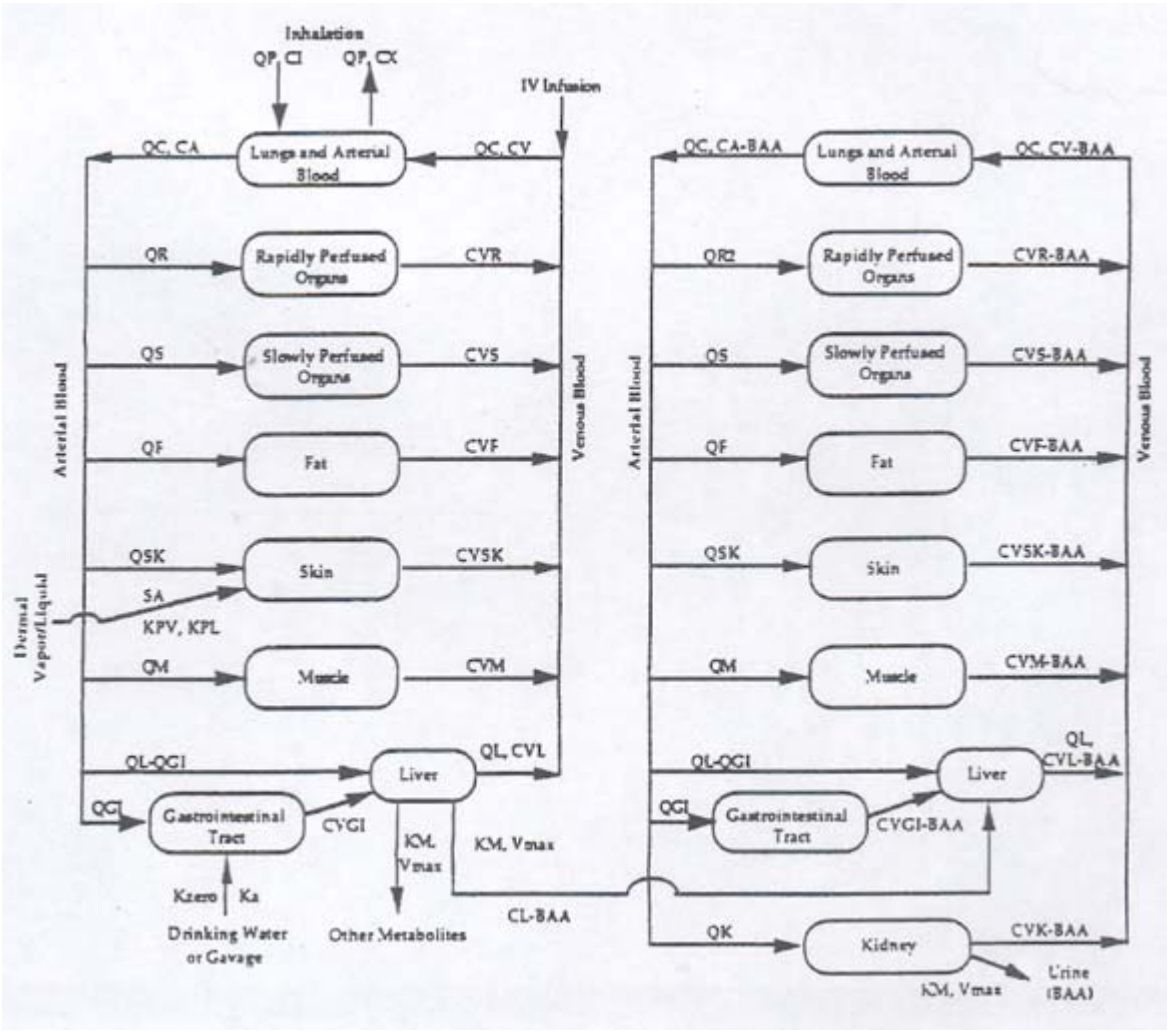
The model was improved by Shyr *et al.*, 1993. Improvements consisted in the modelisation of the metabolite formation by three routes of exposure (oral, dermal, inhalation).

In order to improve the PbPk model described by Johanson in 1986, a series of Good Laboratory Practice (GLP) studies were performed (Dow, 1993 – Corley *et al.*, 1994). The objectives were to :

- expand the PbPk model to include additional exposure routes (oral gavage, drinking water, IV infusion and dermal),
- determine the relative solubilities of EGBE and BAA in rat blood, rat tissues and human blood for use in estimating partition coefficients
- incorporate the disposition of BAA into the model
- add allometric scaling factors for rat and human physiological parameters
- add competing pathways for the metabolism of EGBE into the model.
- develop a method for analysing trace levels of both EGBE and BAA in blood
- validate the new analytical method by administering ^{14}C-labeled BE to rats and analysing blood for BE and BAA in urine for BE and major metabolites.
- confirm the presence of the metabolite, EG, in urine by mass spectroscopy
- validate the new PbPk model with data developed as a part of these studies and relevant data from the literature.

The model used was significantly different from that of Johanson (see figure 4.45):

Figure 4.45: Improvement of the PbPk model (from Dow, 1993)



Allometric-scalable physiological and biochemical parameters were used in place of standard values for a 70 kg man to normalise these parameters to the actual body weights reported in several human studies. The rat was added to expand the database for model validation and to assist interspecies comparisons. Additional routes of exposure were added for validation of the model simulations with data from published studies. A second model was linked to the EGBE model specifically to track the disposition of BAA. The kidney was added to the BAA model since it is an organ of elimination for BAA.

Nose only inhalation, IV infusion, oral gavage and drinking water simulations were modelled according to Andersen *et al*., 1987. Dermal exposure to EGBE were modelled according to Mc Dougal *et al* (1986 and 1990) for neat and aqueous solutions and according to Johanson and Boman, 1991 for vapour absorptions.

Physiological constants were taken from the literature (Anderson, 1987 ; Fiserova-Bergerova, 1983 ; Johanson, 1986c). The effect of exercise on the ventilation rates and blood flows were adapted from Astrand (1983) and Johanson (1986c).

Partition coefficients were available only for EGBE in human blood/air and saliva/air (Johanson and Dynesius, 1988). Partition coefficients for EGBE and BAA in the blood, liver, kidney, skin, fat, muscle, stomach, small intestine, caecum and lung tissues of male F344 rats and the blood of humans were determined experimentally.

After calculation of the partition coefficients the following assumptions were made for the PbPk model:

- the partition coefficients for EGBE and BAA for the rapidly perfused compartments were assumed to be equal to that of the liver
- the partition coefficients for the slowly perfused compartments were assumed to be equal to that of the muscle
- the average of the stomach, small intestine and caecum partition coefficients was used to represent GI tract
- the skin/air partition coefficient for dermal uptake of EGBE vapours was assumed to be equal to the blood/air partition coefficient
- the skin/liquid partition coefficient for dermal dosing of neat EGBE and solutions of EGBE was assumed to be equal to the measured skin/saline partition coefficient.

Biochemical constants : the metabolism of EGBE and BAA was assumed to occur only in the liver through a single metabolic pathway (alcohol / aldehyde dehydrogenase) following Michaelis-Menten kinetics. Other minor metabolic pathways have been reported by various authors, since there was insufficient kinetic data available to differentiate the parameters associates with each of these other pathways, a second saturable pathway was included in the model to simulate the disappearance of EGBE from the liver by all other pathways combined. Parameters for this pathway were estimated (based on Medinsky *et al.,* 1990 studies).

The model also assumed that BAA was bound to proteins in blood and was eliminated by a saturable process in the kidney. A model similar to that of Russel *et al*., 1987 was used for describing protein binding and saturable elimination of BAA.

C_{max} and AUC of the concentration for BAA in blood were used as an estimates of the "delivered dose" or "internal dose surrogate" for high dose/low dose, dose/route and rat to human extrapolation.

A metabolism study was conducted to assist in validation of model parameters studied (see § 4.1.2.1.1.1). These results with other data sets were compared with model predictions.

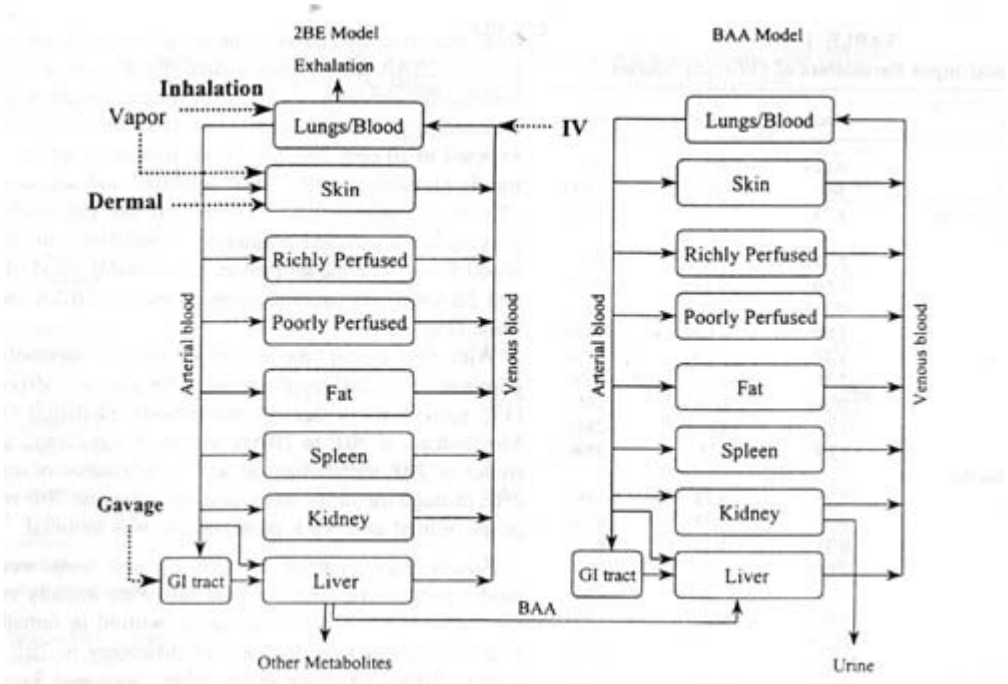
Data are generally in good agreement with the model except for dose levels which cause toxicity. The model has no systematic provision for correcting for potential diminished renal excretion and/or liver metabolism that arise secondary to the haemolytic activity of BAA and will therefore tend to overpredict the amounts of BAA excreted by the kidneys in urine. Nevertheless the model satisfactorily predicts concentrations of BAA in blood and amount of BAA eliminated in the urine at dose levels that do not cause toxicity.

The PbPk model of Corley *et al*.,1994 served as a background to develop a new model to take into account chronic inhalation and female rat and mice parameters. (Lee *et al.*, 1998). The 2-year toxicokinetic studies utilized in the model development were conducted as a part of the 2-year inhalation toxicity and carcinogenicity studies of EGBE (NTP, 1998) and are described previously in this report (Dill *et al.*, 1998).

Compared to the previous model, the muscle compartment was included in the poorly perfused compartment, a kidney compartment was added for EGBE and spleen was added as a separate compartment. The same assumptions than before were made. It was also assumed that partition coefficients of EGBE and BAA in male rats were representatives of those for other species regardless of the sex. Some adjustments have been made due to chronic modifications induced by EGBE:

- cardiac output start to diminish after 6 month exposure (down to 2/3 of the estimate used for young animals by the end of the 18 months of exposure).
- volume of the fat compartment starts to increase after 6 month of exposure (up to 3 fold at the end of the 18 months of exposure).
- Total plasma protein binding sites in mice start to increase after 6 months of exposure (up to 2 fold at the end of the study)
- The maximum rate of EGBE metabolism to BAA for a male rat starts to increase after 12 months of exposure (2 fold at the end of exposure).
- The maximum rate of 2BAA renal elimination starts to decrease after 6 months of exposure (down to ¾ of the estimate for young animals at the end of exposure).

Figure 4.46: Last PbPk model (Lee *et al.*, 1998)



In human via inhalation and dermal route, the study of the PbPk model demonstrated that it is not possible to reach a BAA blood concentration that might be able to cause even a slight haemolysis in humans (Corley, 1996). This is based on the assumption that a concentration of 8mM of BAA in blood is needed in human to be able to develop sign of haemolysis (only 0.5 mM in rats) and was calculated for theoretical exposure of 6 hr regardless of the concentration (even up to the saturated concentration of 1160 ppm EGBE) or inclusion of the dermal absorption of vapors.

Recently, the model was improved to include key studies that independently determined sex, age and species differences in partition coefficients, metabolism and renal clearance (Corley *et al*., 2005).

Summary other data :

In vitro studies have showed that EGBE transformation in BAA depended on ADH (isotype 3). This enzyme is more active in females than in males. Moreover, it has been demonstrated than metabolic rate in rat was 10 to 20 fold higher than in human.

A PbPk model has progressed with experimental data collected in humans and in animals. This model included at the present time various exposure route (inhalation, oral, dermal, IV), the differences between humans and animals, the kinetic parameters of the main metabolite BAA and allow also the modelling of repeated exposures. Recent studies have given results which are consistent with the current PbPk model.

4.1.2.1.4 Summary of toxicokinetics, metabolism and distribution

Oral administration of EGBE leads to a quite complete absorption of the substance. Via inhalation route, a "wash in / wash out" mechanism limits the absorption to 55 – 60 % of the administrated concentration. Via dermal route, uptake of liquid EGBE depends on the administration mode, the species and the concentration of EGBE in the final product. An occlusive administration will be responsible of a great percutaneous uptake whereas a non-occlusive administration will minimize absorption due to the volatility of EGBE.

Rat skin seems to be readily permeable compared to pig or human skin (2 or 3 fold more). Percutaneous uptake also depends on the EGBE concentration in the tested product : for 40 and 80 % aqueous solutions of EGBE, absorption was demonstrated to be maximum. In two rat studies, dermal absorption of liquid EGBE was estimated to be between 20 and 30 %.

In human, dermal studies with liquid EGBE give penetration uptakes which vary by a factor of 10 between different subjects exposed to EGBE with the same experimental conditions. A percentage of absorption of about 12 % was calculated in one study using EGBE at 5 and 10 % in water.

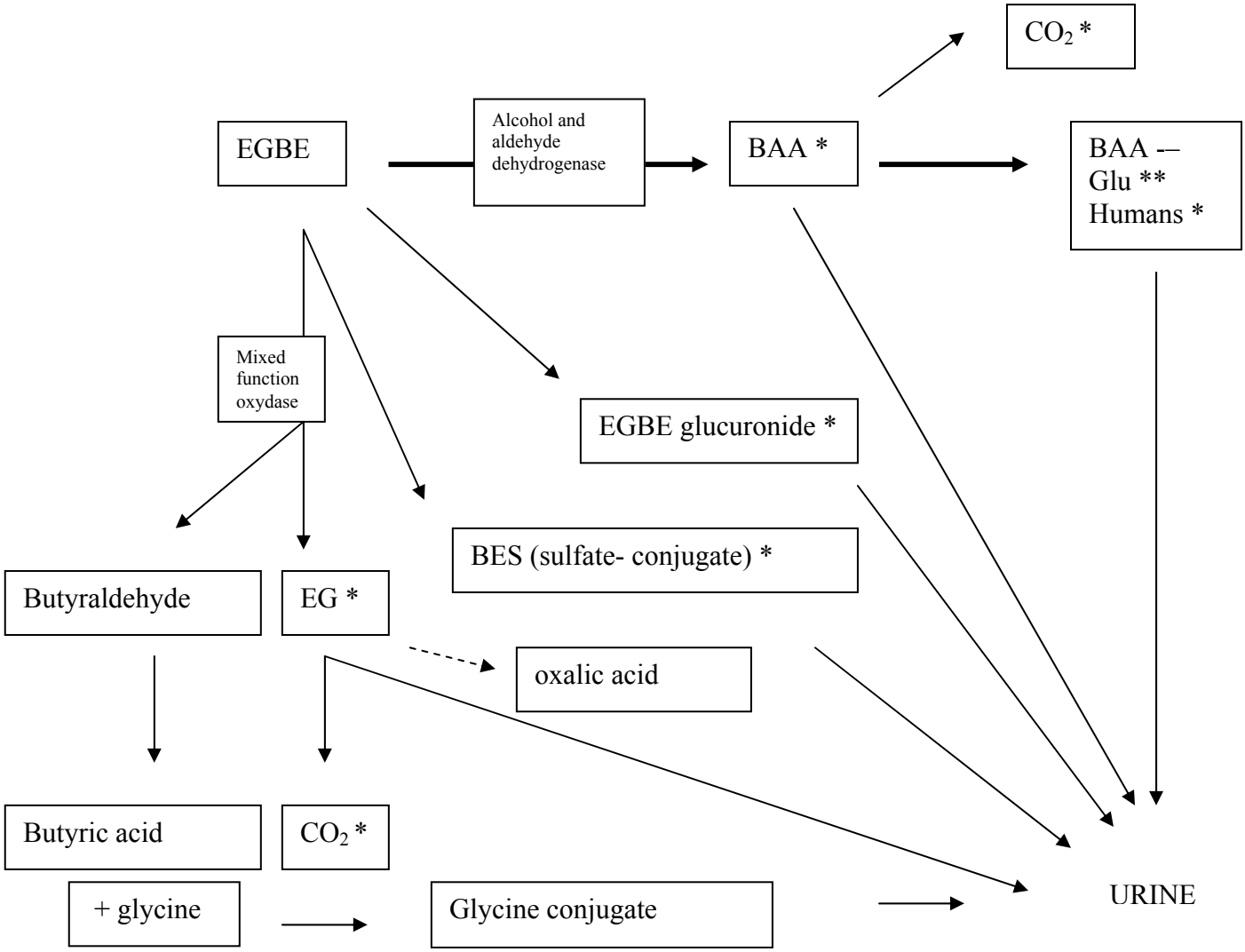
Dermal penetration of vapour EGBE was studied in humans. Depending on the experimental conditions, the percentage of the absorbed substance due to dermal uptake varies between 11 and 39 % of the total body burden.

EGBE reaches a maximum blood concentration rapidly after exposure whichever the route of exposure. EGBE is rapidly metabolised (with a plasmatic half life of about an hour).

Target organs are the liver, kidneys, thymus and stomach, in particular forestomach in the rat whichever the route of administration (oral and inhalation route, no data for dermal route).

The main metabolism pathway leads to the formation of BAA via Alcohol dehydrogenase and Aldehyde dehydrogenase in a saturable mechanism. With increasing doses of EGBE, the formation of glucuronide conjugate of EGBE or BAA is enhanced. Minor metabolites of EGBE are also reported depending on the species used (see figure 4.47).

Figure 4.47: Metabolism of EGBE (Patty, 2001)



Materials identified with an asterisk (*) have been identified in either rodents or humans.

**: BAA-Blu = N butoxyacetyl glutamine conjugate

Elimination is rapid and mainly via urinary route (80 to 90 % of the metabolites). The plasmatic half-life of metabolites is about 4 hours. A small amount is eliminated as CO_2 by the respiration (10 to 20 %). Normal renal excretion is conditioned by physiological state of the kidneys: females excreted less rapidly BAA than males and aged animals have a trend to eliminate metabolites with more difficulties than young animals. Any renal injury will enhance BAA toxicity by increasing its blood persistence.

However if renal integrity is respected, a repeated administration of EGBE lead to an adaptation of the metabolism. In this case elimination of BAA occurred more rapidly. This mechanism of extra hepatic adaptation is also described for action of EGBE on red blood cells, especially on erythrocyte deformability (see § 4.1.2.2.3).

For risk characterisation, if absorption factors are needed, the following values will be taken :

- Oral route: 100 % absorption
- Inhalation route: 60 % absorption
- Demal route: distinction should be made between dermal absorption of EGBE vapour (in the atmosphere) and dermal absorption of liquid EGBE. For these two cases, the absorption factors should be discussed on a case by case basis because a lot of factors can modify these factors (temperature and humidity, clothing, concentration of EGBE if diluted, duration of the exposure...).

  Overall, dermal absorption of EGBE vapour will contribute for 27 % of the total EGBE body burden in normal uses and 39 % if extreme conditions are expected. Dermal absorption of liquid EGBE will vary in function of the duration of exposure and the concentration of EGBE in the formulation, internal doses will be calculated on a case by case basis using the dermal flux calculated in the Jakasa study. Where required, a permeation rate of 0.63mg/cm^2/hr will be used. This is the upper figure from the *in vivo* data and is consistent with the *in vitro* data.

It is considered that the PBPK model for EGBE is sufficiently well developed to justify its used to derive animal to man toxicokinetic extrapolation factors for the inhalation route. These factors are based on the toxokinetics of BAA since this is the metabolite that causes the critical toxic effects. A detailed description of how the model is used is given in the General Aspects introduction to the risk characterisation. Where required, the half life of BAA will be assumed to be 5 hours.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

Inhalation

Rats

Carpenter *et al.* 1956 and the report of the Mellon Institute of Industrial Research (1952) reported some experiments concerning acute toxicity of EGBE in various animals. For rats via inhalation route, a LT50 of 9 hours was reported for a concentration of 1300 ppm. At 800 ppm, mortality was 3/6 rats for a 8-hr exposure period and 0/6 rats for a 4-hr exposure period. At 500 ppm no rats out of 6 were dead for a 8 hr exposure period and only one out of 6 for a 4 hr exposure period. When older rats were exposed to 375 ppm (age not reported) 11 out of 13 and 23 out of 23 died following a 7 hr exposure period.

Saturated vapour of EGBE caused no mortality among 6 adult female rats exposed for 8 hr and observed for a 14-day observation period. Clinical symptoms were limited to bloody urine and poor coordination. Concentration was measured to be 475 ppm.

Earlier results (1940 and 1943 cited in Carpenter *et al.,* 1956) with saturated vapour give the following results: 4 out of 6 rats died following a 9 hr exposure, 2 out of 6 rats died after a 4 hr exposure and no rats died after a 2 hr exposure.

In a poorly reported study (Gage, 1970), rats (4 males and 4 females) were exposed whole body to EGBE (purity unknown) at an unique concentration of 2400 ppm (13 mg/l) during 5 hr. At this concentration test substance was in aerosol form.

All rats died within 2 days. Clinical symptoms before death were a comatose state and haematuria. Blood Hb was measured 35 to 50 % of the normal.

Groups of Fisher 344 rats (6/sex/dose) were exposed whole body to EGBE at doses of 867, 523 or 202 ppm (purity 99.4 %) for 4 hr (Bushy Run Research Center, 1980a). EC50 was calculated at the end of the 14-day observation period. Clinical observations and gross macroscopy was recorded during and at the end of the study.

In rats exposed to 867 ppm, loss of coordination was observed concurrently with laboured breathing and red fluid discharge around the urogenital region. All animals were dead 2 days after exposure. In rats exposed to 523 ppm, coordination loss was also observed and red fluid discharge around the urogenital region. 2 males and 3 females died during the 14-day observation period. At the end of the study, the tail of 2 males and 2 females appeared necrotic. In rats exposed to 202 ppm, except a slight amount of dried red material observed on the tail the day following exposure, no other sign of toxicity was observed. Body weight of the 523 and 202 ppm groups were depressed 1 day after exposure but a normal body weight was recovered by 4 days. Animals that died had enlarged and discoloured kidneys with urinary bladder filled with red stained urine.

In this study, a LC50 of 486 ppm was calculated for males and 450 ppm for females.

In a validation study for the OECD method 403 (ring test), 8 chemicals including EGBE were tested (Shell Chemicals, 1982). Wistar rats (3/sex/group) were exposed whole body to saturated vapour concentration (theoretically 617 ppm) for a variable period of time. The maximum exposure time was 7 hr, if deaths occurred during either the exposure period or the observation period, exposures were repeated for shorter intervals (eg. 3 hr, 1 hr, 30 min…).

For Butyl oxitol (EGBE – purity 99 %) durations of exposure were 7 hr, 3 hr and 1 hr. Measurements of the atmospheric concentrations in the exposure chamber gave values between 750 and 910 ppm.

During exposure animals exhibited signs of lethargy. For rats exposed during 7 hr, mortality was 1/3 males and 3/3 females at the end of the observation period. Clinical signs were necrosis of the end of tails, blood in urine and paleness of eyes and feet. For the 3 hr exposure group mortality was 0/3 males and 1/3 females. Except for necrosis of the tail, the same symptoms than the 7 hr group were observed. For the 1 hr group, no mortality was observed, symptoms were limited to the presence of blood in urine and paleness eyes and feet. Recovery was full 2 days after exposures.

The results of other laboratories participating at the ring test were published (Klimisch *et al.*, 1988). The nominal concentration at which animals were exposed varied from 3.3 mg/l to 4.2 mg/l (mean 3.7 mg/l). The LT_0 (time for which at least one death were found) was 3 hr for 5 laboratories and 1 hr for only one laboratory.

Mice

In a poorly reported inhalation study in mice,, the effects of EGBE and other glycol ethers was studied (Werner *et al.*, 1943a). Swiss mice were exposed in groups of 16 for 7 hr periods.

At 1210 ppm all animals died within 32 hr. At 920 ppm 11/16 animals died within 32 hr. At 770 ppm 14/16 animals died within 1 week after exposure. At 670 and 560 ppm, 2/16 animals died in 32 hr and 1 week respectively. No animals died with exposure to 390 ppm of EGBE.

Dyspnoea was the major toxic symptom described moreover when doses were near the lethal level, severe haemoglobinuria was seen. Moderate to marked follicular phagocytosis and congestion of the cavernous veins were frequent findings in spleens of exposed animals. Focal necrosis and lymphoid hyperplasia was also seen in 3 surviving animals. Interstitial nephritis and typical pictures of bronchopneumonia was seen in a few animals.

Guinea pigs

Carpenter *et al.*, 1956 reported some experiments concerning acute toxicity of EGBE in various animals. A LT50 of 7 hr was reported for guinea pigs for an exposure of 1300 ppm (Union Carbide Corp., 1943). Guinea pig mortality was 1/6 in a 4 hr exposure (Mellon Institute of Industrial Research, 1952).

In a GLP study, EGBE (99.87 % purity) was administrated via inhalation route (whole body) to Guinea Pigs for a single hr of exposure (Bushy Run Research Center, 1994). Target concentration for the exposure was the highest attainable EGBE concentration (saturated vapour concentration) at 20°C and 740 mm Hg: 791 ppm. Study design was identical to OECD guideline 403 except for the use of GP and for the during of the exposure (1hr).

Real concentration obtained were 633 and 691 ppm for the females and the males respectively.

No death nor clinical signs were seen during the exposure or during the 14-day post-exposure period. No changes in body weight or bodyweight gain were observed. Gross findings at necropsy were observed in 4/5 males and 3/5 females, all of these findings were considered to be incidental.

For this study, it can be concluded that LC50 in GP in greater than 633 ppm in females and greater than 691 ppm in males.

Summary inhalation route:

Table 4.48: Summary inhalation route

Lethal Concentration LC50 (mg/kg)	Mortality and treatment related effects in function of exposure time	Validity *	Reference
Rats inhalation route			
	Exposure to 800 ppm during 8 hr caused 50 % mortality whereas an exposure time of 4 hr did not cause ant death (6 animals in each group) Exposure to 500 ppm (2.45 mg/l) for 8 hr – did not cause any mortality (0/6) wherease a tratment period of 4hr caused one death (out of 6) If Older rats are exposed to 375 ppm:	2	Mellon Institute of Industrial Research, 1952 Carpenter *et al.*, 1956

	11/13 and 23/23 died after 7 hr of exposure		
LC50: 486 ppm (2.38 mg/l) for males and 450 ppm for females.	4hr exposure Laboured breathing, loss of coordination, tail necrosis, renal toxicity, haemolysis. Mortality from 523 ppm (2.56 mg/l)	2	Bushy Run Research Center, 1980a
	Saturated vapours (617 ppm – 3 mg/l) and exposure time variable. Mortality 4/6 for 7 hr exposure, 1/6 for 3 hr exposure and no mortality for 1 hr exposure. Lethargy, necrosis of the tail and haemolysis.	2	Shell Chemicals, 1982
	2400 ppm (11.76 mg/l) aerosol for 5 hr exposure. All rats died within 2 days. Comatose state and haemolysis.	3	Gage, 1970
Mice Inhalation route			
	Mortality from 670 ppm (3.28 mg/l) and 560 ppm (2.74 mg/l) for exposure of 32 hr and 1 week respectively. No dead for 390 ppm (1.91 mg/l). Dyspnea and haemolysis.	3	Werner *et al.*, 1943a
Guinea Pigs Inhalation route			
	LT50: 7 hrs at 1300 ppm (6.37 mg/l)	2	Carpenter *et al.*, 1956
> 633 ppm (3.1 mg/l) (females) > 691 ppm (3.39 mg/l) (males)	1 hr exposure. No effects	1	Bushy Run Research Center, 1994.

* 1: valid (performed according to GL and GLP) – 2: valid with restriction (not performed according GL and/or not GLP but scientifically acceptable – 3: not valid or not assessment possible.

In a good quality study performed on rats, LC50 was calculated to be comprised between 450 and 486 ppm (for a 4 hour exposure (Bushy Run Research Center, 1980a). Other studies give results quite consistent with this one. In these studies, females and old animals were more sensitive than males or young animals.

Clinical symptoms and pathology were: lethargy, ataxia, laboured breathing, loss of coordination, haemolysis and tail necrosis. Renal injuries were commonly seen during pathological examinations.

According to the data available, a classification R20 is needed.

Dermal

Rats

EGBE (purity unknown) was applied dermally (occlusive) on rats for 4 hr (Mellon Institute of Industrial Research, 1961). Animals were observed during 14 days after application.

The calculated LD50 was 2275 mg/kg (1680 – 3079 mg/kg).

The dermal toxicity of EGBE (purity unknown) was assessed in rats in a GLP study (Safepharm laboratories, 1993a) according to OECD guideline 402. SD rats (5/sex) were given a single 24 hr, semi-occluded dermal application of undiluted test substance and then observed for 14 days.

No deaths were reported. No signs of systemic toxicity or irritation were noted during the study. All rats gained body weight as expected during the study. No abnormalities were noted at necropsy. The LD50 in this study was greater than 2000 mg/kg for both males and females rats.

The dermal toxicity of EGBE (purity unknown) was assessed in rats in a GLP study (Safepharm laboratories, 1993b) according to OECD guideline 402. SD rats (5/sex) were given a single 24 hr, occluded dermal application of undiluted test substance. Animals were observed for 14 days following application.

One female was found dead 2 days after dosing. Comment signs of systemic toxicity noted were ataxia, pallor of the extremities, emaciation, lethargy, decreased respiratory rate, laboured respiration and tiptoe gait with incidents of ptosis and red/brown staining around the eyes. A full recovery was seen before the end of the study. No signs of irritation were noted during the study. Surviving animals showed expected gain in bodyweight during the study except for one female which showed bodyweight loss during the firs week. No abnormalities were noted at necropsy in surviving animals. Female that died during the study showed haemorrhagic lungs, dark liver, dark kidneys, sloughing of the non-glandular epithelium of the stomach and haemorrhage of the small and large intestines. The LD50 in this study was greater than 2000 mg/kg for both males and females rats.

Guinea pigs

Results of studies were reported briefly (Mellon Institute of Industrial Research, 1952). Dermal LD50 was found to be 6411 mg/kg (5400 to 7765 mg/kg).

LD50 in rabbits and in guinea pigs were compared (Roudabush *et al.*, 1965). Groups of Hartley guinea pigs and white rabbits (4 animals / dose) were dosed (at least 3 doses) with EGBE (purity unknown) in intact skin for GP and rabbits and also in abraded skin for GP only.

The LD50 values were614 mg/kg in rabbits and 208 and 271 mg/kg for intact skin and abraded skin respectively in guinea pigs.

A comparative percutaneous toxicity study of 10 industrial solvents (including EGBE) was performed in guinea pig (Wahlberg and Boman, 1979). A fixed volume of EGBE (purity > 99 %) was administrated occlusively to 20 guinea pigs for a period of time which allow the test substance to be totally absorbed (between 5 and 7 days). Animals were then observed for 35 days for clinical symptoms and were weighted once daily except on week end. For comparison the same amount of EGBE was injected IP and the animals were followed in the same manner as the epicutaneously exposed guinea pigs. The applied volumes of EGBE were 450 and 1800 mg.

No deaths were seen for the 450 mg dose. For the high dose, 5 animals died on day 3 cumulative death reached 11 on day 4 and finally 13 on day 7 and after. No changes on bodyweight compared to controls were seen for the 450 mg dose. With IP injection of 1800 mg, 100 % mortality was reached in 6 hours, and with 450 mg dose, 70 % of the mortality was reached in 48 hr.

In a GLP study performed according to the OECD guideline 402, Hartley guinea pigs were exposed to 2000 mg/kg of EGBE (purity 99.8 %) via dermal route (Eastman Kodak, 1994b). The test substance undiluted was applied under occlusive conditions to the guinea pigs (5 /sex) for a test period of 24 hr.

No death was reported. All animals appeared clinically normal during the 14 day post exposure period. No effects were seen on body weight, bodyweight gain and no treatment related changes were noted at necropsy. Based on this assay, the dermal LD50 of EGBE can be considered greater than 2000 mg/kg.

Rabbits

New Zealand White rabbits were dosed with EGBE (purity unknown) via dermal route (Mellon Institute of Industrial Research, 1952 ; Carpenter *et al.*, 1956). Test material was applied occlusively during 24 hr at different doses. LD50 was calculated after a 14-day observation period.

Extreme congestion of the kidneys, bloody urine, pale liver and engorged spleen were noted in dead animals. The LD50 calculated was 560 mg/kg (480 to 640 mg/kg).

In the same report, previous studies were reported: in 1947, 10 rabbits were doses with 505 mg/kg undiluted EGBE and all died within 48 hr. Bloody urine was a common finding. In 1948, 505 mg/kg was applied to 6 rabbits and 3 out of 6 survived but had bloody urine. Similarly in 1951, 4 out of 6 rabbits died when dosed with 505 mg/kg EGBE.

Ten New Zealand White rabbits were exposed dermally to Polysolv EB (EGBE – purity unknown) at dose of 2000 mg/kg during 24 hr (MB research laboratories, 1976). Epidermal abrasions were made every 2-3 cm over the exposed area (sufficiently deep to penetrate the stratum corneum but not deep enough to produce bleeding). The wrapping was occlusive. After the 24 hr exposure period, animals were observed for 14 days.

At 24 hr, all rabbits exhibited lacrimation, bloody urine, flaccid muscle tone and anorexia. No spontaneous movement were noted. All animals died during the second day of observation. It can be concluded that LD50 is lesser than 2000 mg/kg.

In order to estimate more precisely the value of the LD50, another study was performed with the same experimental conditions except for the dose level chosen and the number of animals. Four doses were applied to three groups of four NZ white rabbits: 250, 500 and 1000 mg/kg.

In this second study, mortality was seen at 500 mg/kg (1/4) and 1000 mg/kg (4/4). No toxic signs were reported for the 250 mg/kg group, for 500 and 1000 mg/kg group blood was found in urine and for the 1000 mg/kg group animals were flaccid. In died animals, necropsy findings were blood in urine and sometimes liver and renal injuries. The calculated LD50 based on this study was 580 mg/kg (0.31 – 0.85).

The percutaneous toxicity of butyl cellosolve was determined on rabbit skin (Duprat and Gradiski, 1978). Groups of 6 New Zealand White rabbits were exposed to undiluted EGBE (purity > 99.5 %) at doses of 72, 90, 108, 135, 180 and 225 mg/kg for a 8 hr period. Animals were observed daily over 15 days. Macroscopic and microscopic examination were performed on dead animals and those surviving on the 15th day.

At the end of the exposure period, the total amounts of EGBE applied were absorbed. Mortality was 2/6 animals for 72 and 90 mg/kg, 4/6 animals for 108 mg/kg, 5/6 animals for 135 and 180 mg/kg and 6/6 animals for 225 mg/kg. Death occurred between day 1 and day 8. Clinical signs were prostration, hypothermia and hemoglobinuria especially in the groups receiving the highest doses. Early death were considered to be caused by narcosis or failure of respiration and delayed death were probably due to renal impairment. Lungs appeared modified. Histopathological investigation in dead animals showed congestion of the liver, necrotic foci with mesenchymatous reactions and inconstant steatosis, passive congestion of the spleen, enlarged kidney with haemoglobinemic nephrosis, cutaneous lesions including necrosis. There were no evident dose-effect relationship since the findings were exhibited at all dose levels. In surviving animals no effects were seen for 90 and 72 mg/kg dose. Other animals showed signs of renal toxicity and skin irritation.

In this study, the calculated LD50 was 100 mg/kg bw.

In a briefly reported study, groups of male New Zealand rabbits were exposed occlusively to EGBE during 24 hr at concentrations of 900 and 450 mg/kg (Bushy Run Research Center, 1980b). Mortality was 4/4 animals for the high dose and 1/4 for the low dose. Signs of erythema and necrosis were seen at the administration point. In dead animals necropsy showed orange red lung and liver, dark spleen, dark red kidneys, orange peritonea and intestine, blood in urine. In this study LD50 was 569 mg/kg (348 – 931 mg/kg).

Nine glycol ethers including EGBE were tested for determination of acute dermal LD50 (Eastman Kodak, 1981b). EGBE (purity > 99.5 %) was administrated dermally to groups of New Zealand White rabbits (5 animals/group) at doses of 153, 307, 614 and 1239 mg/kg. After 24 hr of occluded exposure, the wrap was removed and animals were observed during 14 days for clinical and irritation signs. Body weights, gross pathology were also checked.

Clinical signs were anorexia, depression, cyanosis and ataxia for the lowest doses. Salivation, nasal discharge, iritis, depression, laboured breathing and prostration were observed at higher doses. No gross pathological effects were seen at the 153 and 307 mg/kg doses. At the two highest doses, however, adverse and dose-related effects on kidneys were recorded. Reddish coloured fluid was observed in the urinary bladder of animals. Some effects were also seen in liver and in thymus. Calculated LD50 was 435 mg/kg (range 330 to 578 mg/kg).

A GLP study was performed to assess the acute dermal toxicity of EGBE (purity unknown) in the New Zealand White rabbit using a method described in the OECD guideline 402

(Safepharm laboratories, 1994a). 2 groups of 10 animals (5 males and 5 females) were given a single 24 hr semi-occluded dermal application to intact skin at dose levels of 1000 and 2000 mg/kg. Animals were observed for 14 days after the day of treatment and were killed for gross pathological examination.

There were no death for the 1000 mg/kg dose. For the high dose, one female was found dead 2 days after dosing and 2 animals (1 male and 1 female) were killed in extremis one day after dosing. No signs of toxicity were seen for the low dose, for the high dose, common signs of systemic toxicity related to EGBE administration were reported: lethargy, red stained urine, laboured breathing, hunched posture and isolated incidents of loss of righting reflexes, hypothermia, ataxia and diarrhoea. Surviving animals recovered before the end of the observation period. Very slight to well-defined erythema with or without very slight or slight oedema was noted at treatment sites of all animals and at all doses, in high dose animals, some signs of necrosis were seen in 5 animals. All surviving animals except one female in the low dose group and one male in the high dose group showed expected gain in bodyweight during the study. For surviving animals, no abnormalities were reported at necropsy (1000 and 2000 mg/kg), only the animals dead in the high dose group showed signs of hepatic and renal toxicity and haemorrhage of the gastric mucosa, of the non glandular epithelium of the stomach, of the small and large intestine and red fluid present in the bladder.

In this study, it can be conclude that LD50 is greater than 2000 mg/kg.

A GLP study was performed to assess the acute dermal toxicity of EGBE (purity unknown) in New Zealand White rabbit using a method described in the OECD guideline 402 (Safepharm laboratories, 1994b). 3 groups of 10 animals (5 males and 5 females) were given a single 24 hr occluded dermal application to intact skin at dose levels of 500, 707 and 1000 mg/kg. Animals were observed for 14 days after the day of treatment and were killed for gross pathological examination.

One female treated with 500 mg/kg and 3 animals treated with 1000 mg/kg were killed in extremis. Common signs of toxicity noted in all groups were ataxia, hunched posture, lethargy, laboured breathing, diuresis, red-coloured urine and skin and eyes pale yellow in appearance. Yellow coloured eyes was commonly noted in animals treated with 707 or 1000 mg/kg. Isolated incidents of toxicity noted in animals treated with 1000 mg/kg were diarrhoea, hypothermia and loss of righting reflexes. Common signs of skin irritation noted were very slight to well defined erythema, very slight to severe oedema, scattered areas of grey/green-coloured dermal necrosis, desquamation, slight haemorrhage of dermal capillaries, small superficial scabs. Animals that died or were killed in extremis during the study showed haemorrhagic lungs, dark or pale liver, dark kidneys and red liquid present in the bladder. In dead animals, slight haemorrhage of the gastric mucosa and haemorrhage of the large and/or small intestines. No abnormalities were noted at necropsy of animals that were killed at the end of the study. The calculation of LD50 gives an overall value of 841 mg/kg, LD50 for males only was 1060 mg/kg and LD50 for females only was 667 mg/kg.

Summary acute toxicity dermal route

Table 4.49: Summary acute toxicity via dermal route.

LD50 (mg/kg)	Experimental conditions	Effects	Validity *	Reference
Rat dermal route				
2275 mg/kg	4 hours occlusive		2	Mellon Institute of Industrial Research, 1961.
> 2000 mg/kg	24 hours, semi occlusive	No irritation, no sign of toxicity	1	Safepharm laboratories, 1993a
> 2000 mg/kg	24 hours, occlusive	Ataxia, pallor of extremities, lethargy, laboured breathing. No signs of irritation.	1	Safepharm laboratories, 1993b
Guinea-pigs Dermal route				
6411 mg/kg			2	Mellon Institute of Industrial Research, 1952
208 mg/kg (intact skin) 271 mg/kg (abraded skin)			2	Roudabush *et al.*, 1965
450 – 1800 mg/kg		Mortality at 1800 mg/kg only (13/20 animals)	2	Wahlberg and Boman, 1979
> 2000 mg/kg	24 hours, occlusive	no irritation, no signs of toxicity	1	Eastman Kodak, 1994b
Rabbits Dermal route				
560 mg/kg 505 mg/kg	24 hours occlusive	Haemolysis, renal – hepatic – splenic toxicity	2	Mellon Institute of Industrial Research, 1952 Carpenter *et al.*, 1956
580 mg/kg	24 hours	Anorexia, depression,	2	MB Research

	occlusive, dermal abrasion	cyanosis, ataxia, laboured breathing, renal – hepatic and thymic toxicity. Mortality from 500 mg/kg.		Laboratories, 1976
100 mg/kg	8 hours	Haemolysis, narcosis, laboured breathing, skin irritation. Liver, splenic and kidney toxicity.	2	Duprat and Gradiski, 1978
569 mg/kg	24 hours occlusively	Mortality from 450 mg/kg.	2	Bushy Run Research Center, 1980 b
435 mg/kg	24 hours occlusively	Anorexia, depression, cyanosis, ataxia, laboured breathing, renal – hepatic and thymic toxicity.	2	Eastman Kodak, 1981b
> 2000 mg/kg	24 hours, semi occlusive	Lethargy, haemolysis, ataxia, signs of irritation mild to severe at high doses (necrosis). Hepatic and renal toxicity.	1	Safepharm laboratories, 1994a
841 mg/kg 1060 mg/kg for males 667 mg/kg for females	24 hours, occlusively	Mortality from 72 mg/kg. Signs of irritation mild to severe at high doses (necrosis). Very slight systemic effects.	1	Safepharm laboratories, 1994a

* 1: valid (performed according to GL and GLP) – 2: valid with restriction (not performed according GL and/or not GLP but scientifically acceptable – 3: not valid or not assessment possible.

In rats, three studies showed LD50 greater than 2000 mg/kg. In the most recent studies, performed according to the same experimental protocol except for occlusion (one occlusive and the other ½ occlusive) animals exhibited clinical signs only when exposed to EGBE under complete occlusion. Clinical signs were the same than those seen with inhalation administration: mainly haemolysis, lethargy, ataxia, hepatic and renal toxicity. No signs of irritation were noted in any of the studies.

In guinea-pigs, great variations were seen depending on the studies. LD50 ranged from 208 mg/kg to 6411 mg/kg. Only one recent study were performed according to guidelines. This study gave a LD50 of greater than 2000 mg/kg. For this study, no adverse effects were described (local or systemic). Very few details are available about the local or systemic toxicity for the other studies.

In rabbits, except for one study (Duprat and Gradiski, 1978), results were quite consistent. When EGBE was applied occlusively, calculated LD50 for a 24-hour application ranged from 435 to 841 mg/kg in 6 studies. When applied semi-occlusively, LD50 was greater than 2000 mg/kg, showing the importance of evaporation. Common systemic signs of toxicity usually seen with EGBE were described: ataxia, laboured breathing, depression, cyanosis, renal – hepatic, thymic and splenic toxicity. Local signs of irritation were seen in some studies, mild irritation for lower doses and sometimes severe irritation , even necrosis for the higher doses.

Overall, for dermal toxicity, great differences were seen between the tested species and the mode of occlusion. When EGBE was administered non-occlusively or semi-occlusively the LD50 was much higher than when administered occlusively. As for acute oral toxicity rabbit seems to be the most sensitive species with LD50 of about 500 mg/kg when administered occlusively whereas other species exhibited LD50 greater than 2000 mg/kg in the same experimental conditions. Clinical signs were the same than those observed via oral route. Local signs of irritation were noted, mild for low doses and sometimes necrosis for the high doses. By dermal route, depending on the application (occlusive or not) the LD50 was 500 mg/kg or > 2000 mg/kg respectively.

According to the data available, a classification R21 is needed.

Oral

Rat studies

Oral acute toxicity of 69 glycols and glycols derivatives was assessed (Smyth *et al.*, 1941). The substance was administrated diluted in water via oral route. Ten Wistar male rats were used for each dosage and enough dosages were administrated to include those at which all died. Observation period after dosing was 14 days. The maximum tested concentration was 2 %.

Most deaths occurred within the first 2 days after dosing Lethal Dose: LD50 was 1260 mg/kg (1120 to 1420).

In the publication of Carpenter *et al.,* 1956 and Mellon Institute of Industrial Research, 1952, some LD50 results were reported. These tests were performed according to a standard procedure (exceptions were specified) on non fasten rats (5-6 weeks old) with dose levels differing by a factor of 1.26 or 2 in a geometric series. A 14-day post exposure observation period was made. Wistar rats were used until 1942, then Sherman rat from 1942 to 1952 and Carworth – Wistar from 1952.

Symptoms were sluggishness, ruffling of coats, prostration, and narcosis. Autopsies on dead rats revealed congested of haemorrhaged lungs, mottled livers, severely congested kidneys and haemoglobinuria.

Results are summarised in the table 4.50:

Table 4.50: Summary of the LD50 reported in Carpenter *et al.*, 1956

Sex	Weight (g)	Concentration EGBE in Water (%)	LD50 and range (mg/kg)	Year
M	90-120	10	1480 (1150 to 1910)	1938
M	90-120	10	2600 (2300 to 2900)	1951
F	90-120	10	2300 (1900 to 2800)	1951
M	90-120	5	2800 (2400 to 3300)	1952
F	90-120	5	1600 (1200 to 2100)	1952
M	90-120	5	1900 (1300 to 2600)	1953
F	90-120	5	1600 (1400 to 1900)	1954
M	30-60	5	3000 (2600 to 3400)	1954
F	30-60	5	2300 (2100 to 2600)	1954
M	335-460	5	560	1954
F	260-320	5	530 (380 to 750)	1954
M	90-120	5	2400 (2100 to 2700)	1954
F	90-120	Undiluted	2800 (2200 to 3700)	1954
M	90-120	5	2100 (1400 to 3100)	1955

In a range-finding test performed on rats (poorly described), EGBE (purity unknown) was administered orally in a water vehicle to groups of 5 animals at doses of 252, 500 and 1000 mg/kg (Dow, 1959).

Mortality was 3/5 animals at 500 mg/kg and 2/2 at 1000 mg/kg. From the lower dose, animals appeared sick and drowsy. Signs of haematotoxicity appeared at the dose of 500 mg/kg (haematuria). From this study a LD50 of 470 mg/kg can be calculated.

EGBE (purity unknown) was administered by oral route to groups of 10 female rats at doses of 1000 – 1600 – 2000 – 2500 and 4000 mg/kg in a 10 % solution in corn oil. Rats were fasted 12 hours before administration. Post exposure observation period was 7 days (Hoechst, 1966).

Mortality occurred from 1600 mg/kg bw dose and higher and between 1h30 and 24 hrs after treatment. In this study the calculated LD50 was 1950 mg/kg.

Male Wistar rats were administrated Polysolv EB (EGBE – purity unknown) orally by intubation (MB research laboratories, 1976). Doses of 670, 1310, 2560 and 5000 mg/kg were used. Animals were observed for mortality, toxicity for 14 days and the LD50 calculated at the end of this period.

No mortality was found for the 670 mg/kg group, 3/10 animals died in the 1310 mg/kg group, 9/10 animals died in the 2560 mg/kg group and 10/10 animals died in the 5000 mg/kg group. Signs of toxicity were lethargy in all groups and ataxia in the high dose group. At necropsy, signs of liver and kidney toxicity were seen in the 1310 mg/kg group. Blood in bladder was

seen in all animals of the 2 highest dose groups. The LD50 calculated in this study was 1590 mg/kg.

Male Wistar rats (5/group) were dosed with EGBE at doses of 9030- 4515 - 2257 - and 1128 mg/kg (Bushy Run Research Center, 1980b). Animals were observed during 14 days before calculating the LD50.

All animals died at doses of 9030 and 4515 mg/kg during or the day after administration. At 2257 mg/kg two out of 5 animals died on day 5 after administration. No deaths were seen at 1128 mg/kg dose.

For the 2 high doses, clinical symptoms were difficult breathing, sluggish and bloody salivation. For the 2257 mg/kg dose, animals were sluggish and had an unsteady gait. Nothing remarkable was seen on animals of the lowest dose. On dead animals, necropsy revealed dark livers, distented stomach, filled with liquids and gaz. Red kidneys, intestine containing blood, red adrenals were also reported. Nothing was seen in survivors. In this study, calculated LD50 was 2420 mg/kg (1670 - 3504 mg/kg).

EGBE undiluted (purity unknown) was administered orally to groups of females CDF rats (3/group) at doses of 130, 250, 500, 1000 and 2000 mg/kg (Dow, 1981).

Mortality was seen at 200 mg/kg (2 out of 3animals). Clinical signs were lethargy and respiratory difficulties at 2000 mg/kg and necrosis of the tails in all surviving rats of the 1000 and 2000 mg/kg dosed groups. For this study, the LD50 was between 1000 and 2000 mg/kg.

The acute oral LD50 was determined in fasted and fed rats (5/group) for 9 chemicals including EGBE (purity > 99.5 %) (Eastman Kodak, 1981a). Male CD/BR rats were given EGBE undiluted by gavage (five different doses progressing by a factor of 2).

The LD50 calculated for fasted or fed rats was 1746 mg/kg. For both groups (fed and fasted) clinical signs were: inactivity, laboured breathing, anorexia, tremors and death. Haematuria was seen at high dose. In dead animals, blood was found in stomach and intestines.

Some LD50 studies were reported without details concerning the method used (Tyler, 1984). A LD 50 of 620 mg/kg was reported in rats (Rowe and Wolf, 1982).

Mouse

Carpenter *et al.,* 1956 reported some experiments concerning acute toxicity of EGBE in various animals. A LD50 of 1230 mg/kg was reported for mice via oral route.

The acute oral LD50 was determined in fasted and fed mice (5/group) for 9 chemicals including EGBE (purity > 99.5 %) (Eastman Kodak, 1981a). Male CD1 mice were given EGBE undiluted by gavage (five different doses progressing by a factor of 2).

The LD50 calculated for fasted mice was 1519 mg/kg, whereas LD50 for fed mice was 2005 mg/kg. For both groups (fed and fasted) clinical signs were: inactivity, laboured breathing, anorexia, tremors and death. Haematuria was seen at high dose. In dead animals, blood was found in stomach and intestines.

Some LD50 studies were reported without details concerning the method used (Tyler, 1984). Two LD 50 of 1170-1700 mg/kg and 1000-1600 were reported (Rowe and Wolf, 1982 and Saparmamedov, 1974 respectively).

Rabbit

Carpenter *et al.,* 1956 reported some experiments concerning acute toxicity of EGBE in various animals. A LD50 of 320 to 370 mg/kg was reported for rabbits via oral route.

Guinea-pig

In a GLP study (performed according to OECD GL 401), guinea pig were given orally EGBE (purity 99.8 %) in a distilled water vehicle at dose of 500, 1000 and 2000 mg/kg (Eastman Kodak, 1994a).

Mortality was 3/5 in males and 5/5 in females at the top dose. At 1000 mg/kg, the mortality was 1/5 for males and females. Clinical signs were slight to severe weakness, sialorrhea and staining of the hair of the face and/or abdomen for the 2000 mg/kg dose. At 1000 mg/kg animals evidenced weakness and prostration the day of administration. Only a slight weakness was noted for the 500 mg/kg group. Sign of necrosis and minor haemorrhage in the gastric mucosa was seen after termination in animals doses with 1000 and 2000 mg/kg. For this study, the LD50 was calculated to be 1414 mg/kg.

Carpenter *et al.,* 1956 reported some experiments concerning acute toxicity of EGBE in various animals. A LD50 of 1200 mg/kg was reported for guinea-pigs via oral route.

Summary oral route:

Table 4.51: Summary of LD50 values after oral route administration

LD50 (mg/kg)	Toxicological effects	Validity *	Reference
Rats Oral route			
1260 mg/kg		3	Smith, 1941
560-2800 mg/kg for males 530 and 2300 mg/kg for females	Narcosis - prostration	2	Mellon Institute of Industrial Research, 1952 Carpenter *et al.*, 1956
470 mg/kg	Mortality from 500 mg/kg. Haematuria.	3	Dow, 1959
1950 mg/kg	Mortality from 1600 mg/kg	2	Hoechst, 1966
1590 mg/kg	Lethargy, laboured breathing, haemolysis, liver and kidney toxicity. Mortality from 1310 mg/kg	2	MB Research Labs, 1976
2420 mg/kg	Laboured breathing, sluggish and bloody salivation. Haemolysis, dark liver and red	2	Bushy Run Research Center, 1980 b

	kidneys.		
1000-2000 mg/kg	Lethargy, laboured breathing, necrosis of the tail. Mortality from 2000 mg/kg.	2	Dow, 1981
1746 mg/kg (fasted or fed)	Inactivity, laboured breathing, anorexia, tremors, haemolysis.	2	Eastman Kodak, 1981a
620 mg/kg		3	Rowe and Wolf, 1982
Mice Oral route			
1230 mg/kg		2	Carpenter *et al.*, 1956
1170 – 1700 mg/kg 1000 – 1600 mg/kg		3	Rowe and Wolf, 1982 Saparmamedov, 1974
1519 mg/kg (fasted) 2005 mg/kg (fed)	Laboured breathing, anorexia, tremors, haemolysis.	2	Eastman Kodak, 1981a
Rabbits Oral route			
320 – 370 mg/kg		2	Carpenter *et al.*, 1956
Guinea pig oral route			
1200 mg/kg		2	Carpenter *et al.*, 1956
1414 mg/kg	Weakness, prostration, necrosis and haemorrhage of gastric mucosa. Mortality from 1000 mg/kg	1	Eastman Kodak, 1994a

* 1: valid (performed according to GL and GLP) – 2: valid with restriction (not performed according GL and/or not GLP but scientifically acceptable – 3: not valid or not assessment possible.

In rats, numerous studies has been performed to assess the LD50 via oral route. Results varies a lot between 470 mg/kg and 2600 mg/kg. Recent studies (performed according to well defined experimental methods) have given results between 1000 and 2600 mg/kg. Clinical signs noted were: lethargy, laboured breathing, ataxia. For pathology, haemolysis was seen in the majority of the studies, sometimes accompanied with renal and hepatic lesions (certainly a consequence of haemolysis).

In mice, available studies exhibited LD50 ranging from about 1500 to 2000 mg/kg (these results are more consistent than those obtained in rat studies). Clinical symptoms similar than those of the rat studies were seen.

One study was performed in rabbits showing a LD50 ranging from 320 to 370 mg/kg. This value seems very low compared to other studies on other species via oral route. The rabbit can be considered to be the most sensitive species concerning acute oral toxicity of EGBE.

Considering that this study is an old study, and that only the result is available (no details on purity or experimental conditions), it is difficult to take this value into account for the risk characterisation or classification purposes.

Two studies are available in Guinea-pigs. The LD50 calculated were 1414 and 1200 mg/kg. The same clinical signs and pathology than other species tested were seen in these studies. Necrosis and haemorrhage of the gastric mucosa was also seen.

According to the data available, a classification R22 is needed.

Other routes

Rats

Various results concerning IV administration of EGBE were reported (Mellon Institute of Industrial Research, 1952 ; Carpenter *et al.*, 1956): LD50 in female rats (170-230 g) when administrated as 3 % dilution was 380 mg/kg (290 – 500 mg/kg) – LD50 in female rats (90-120 g) when administered undiluted was 340 mg/kg (300 to 380 mg/kg).

In a non-GLP study, unique IP injection of 2 different EGBE (n butyl oxitol ® 98-99 purity and Dowanol EB ® > 99 % purity) was performed on female SD rats (4/group). Doses of 200, 252, 316, 398 were tested for both substances with an additional dose of 500 mg/kg for Dowanol EB ® (Dow, 1972).

For both substances, mortality was seen from 252 mg/kg. In all dose groups and with both substances blood was seen in urine. A LD50 of 252 mg/kg was calculated for n butyl oxitol ® and a LD50 of 317 mg/kg was calculated for Dowanol EB ®.

Ten female SD rats were injected IV with 0.034 ml EGBE/kg (Freundt and Helm, 1986). Twenty four hour urine samples were collected during the next 4 days. The following parameters of kidney function were examined in the urine: volume, osmotic pressure (by means of freezing point depression), haematuria (using a semi-quantitative test), albumin, Lactate DeHydrogenase (LDH). Gel filtration of the urine was carried out before enzyme and albumin analyses.

After treatment, the following parameters were found to be changed significantly: the albumin level and the LDH activity was augmented only on the 2nd day, and the GAL activity was decreased only in the 24-h urine samples from the 4th day. The urinary LAP activity, the volume, and the osmotic pressure of the urine remained unchanged. EGBE caused a microhaematuria (approximately 50 erythrocytes/µl) in 2 (of 10) rats on the 1st dose day after its application.

SD rats were injected IV with 0.034 ml/kg EGBE (purity unknown) (Freundt *et al.*, 1993 – abstract). The activities of lactate dehydrogenase (LDH), leucine aminopeptidase (LAP) and beta-galactosidase (GAL), the concentrations of albumin and creatinine, the volume, the specific gravity and the pH, leucocytes, erythrocytes, nitrite, total protein, ketone, bilirubin and urobilinogen were analysed in the 24 hr urine.

A significant increase of albumin and LDH activity was seen in the female rats at the 2nd day after administration. All other parameters remained normal. Because these changes were small and transient, the authors concluded that EGBE have a slight nephrotoxic potential.

Mice

SC injection of 0.5 cc/kg to white mice produced severe toxicity (Von Oettingen and Jirouch, 1931). For 0.25 cc/kg no mortality was seen and one out of two animals died with 0.5 cc/kg dose. The only animal treated with 0.75 cc/kg died. Due to the small number of animals treated no LD50 was calculated.

One study concerning IV administration of EGBE to mice was reported (Carpenter *et al.*, 1956): LD50 in male and female mice when administrated as 3 % dilution was 1130 mg/kg.

Acute toxicity was assessed *in vitro* and *in vivo* in mice (Tanii *et al.*, 1992). Male ddY mice (4 animals/dose – 4 doses) were given IP 2000 mg CCl_4 in olive oil or olive oil only 24 hr before dosing with EGBE. CCl_4 is known to inactivate the hepatic microsomal monooxygenase system and may influence the detoxication of EGBE. It also inhibits hepatic non-microsomal enzymes including ADH. Animals were observed 1 week and after termination the LD50 was calculated. Concurrently a cytotoxicity test was performed to determine ED50.

LD50 without CCl_4 was 6.77 mmol/kg whereas it was equal to 4.99 mmol/kg with CCl_4. As CCl_4 at the dose used appears to inhibit all hepatic enzyme activities (microsomial and non microsomial), the authors concluded that it is unlikely that the metabolite contribute to the acute toxicity of EGBE.

There was no significant correlation between ED50 for the different cells used in the cytotoxicity studies and LD50. A correlation was found between LD50 and logP for the majority of the tested substances. No quantitative relationship was described.

Rabbits

The LD50 for male rabbits by IV route of undiluted EGBE is 0.14 ml/kg (Mellon Institute of Industrial Research, 1952). Death occurred precipitally after 0.2 ml/kg while after 0.1 ml/kg the rabbits were prostrated and rapidly recovered. At the 0.05 ml/kg dosage level, the only symptoms were increased rate of respiration.

In the same report, various results concerning IV administration of EGBE were reported: 3 % EGBE in 0.75 % NaCl solution was not haemolytic to rabbit red blood cells. LD50 of a 3 % EGBE solution was 500 mg/kg (380 – 650 mg/kg).

Table 4.52: Summary other routes

LD50 (mg/kg)	Experimental conditions	Effects	Validity *	Reference
Rats				
380 mg/kg	IV route in 3 % water dilution		2	Mellon Institute of Industrial Research, 1952
317 mg/kg	IP route.	Mortality from 252 mg/kg. Haemolysis	2	Dow, 1972
	IV route	Increase of LDH activity and decrease of	3	Freundt and Helm, 1986

	0.034 ml/kg	GAL activity. Microhaematuria		
	IV route 0.034 ml/kg	Increase of albumin and LDH activity.	3	Freundt and Helm, 1986
Mice				
About 0.5 cc/kg	SC 0.25, 0.5 and 0.75 cc/kg	0.5 cc/kg produced severe toxicity (1 death out of 2 treated). 0.75 cc/kg: death of the only treated animal.	3	Von Oettingen and Jirouch, 1931
1130 mg/kg	IV, 3 % dilution.		3	Carpenter *et al.*, 1956
6.77 mmol/kg without CCl4 4.99 mmol/kg with CCl4	IP		3	Tanii *et al.*, 1992
Rabbits				
500 mg/kg	IV route		2	Mellon Institute of Industrial Research, 1952

* 1: valid (performed according to GL and GLP) – 2: valid with restriction (not performed according GL and/or not GLP but scientifically acceptable – 3: not valid or not assessment possible.

Some studies performed via IV, IP and SC routes in various species gave different results. These studies are not suitable for RA because these routes of administration are not relevant with exposure scenarios. However, some of them give valuable data about the mode of action of EGBE for renal toxicity and valuable information about hepatic metabolism.

4.1.2.2.2 Data from human exposure

Inhalation

2 males and 1 female were exposed to 200 ppm two 4-hr periods separated by 30 minutes lunch (Mellon Institute of Industrial Research, 1955). Erythrocyte fragility test, blood pressure and pulse-rate were determined the exposure day (3 measure before - during lunch and after exposure - for fragility test, one measure was also made during exposure). Urine analysis for glucose and albumin were conducted the following morning, and the BAA levels were determined in 24-hour urine samples first collected at the end of the exposure day.

One male and the female excreted considerable amount of BAA in the two 4 hrs periods following exposure but the other male excreted only trace amount of this metabolite for the same period. Clinical symptom (subjective) were: immediate irritation of the nose and throat,

followed by ocular irritation and disturbed taste was experienced by all three subjects. The female subject, who excreted the largest amount of BAA, reacted most adversely to the exposure, and acquired a headache which lasted about 24 hr. Unlike rats, no effects were seen in the fragility test in human tested.

Another study was performed on human with lower doses: 2 females and 1 male and the male of the precedent study whom had only traces of BAA in urine were exposed to 100 ppm EGBE in the same conditions that the precedent study.

The only objective finding of significance in the exposed subjects was the urinary excretion of BAA. Other measured parameters did not shown significant differences. Even one subject who had not excreted significant quantities of the metabolite after the 200 ppm exposure, did eliminate 75.5 mg in 24 hours. The urinary BAA levels of the other subjects were similar to that found at the 200 ppm exposure. Surprisingly, the subjective response of the humans exposed to 100 ppm EGBE was at least as great, if not greater, than that elicited at the 200 ppm exposure level. The subject whom participated at the former experiment reported that the EGBE odor was not as strong as at the 200 ppm level, although he did have a slight cold at the time of exposure. The two female subjects, apparently experienced greater distress during and after exposure than did the males. One of them vomited after 7 hours of exposure and several times during the day after while the other contracted a headache the following day. The former subject stated that high air temperatures often cause emesis, and was convinced that the present attack was caused by the relatively high chamber temperature (28.5° in the afternoon), rather than the solvent vapours. The other subject also though that the chamber temperature caused her headache.

Oral

In a suicide attempt, a 50-year woman ingested 250-500 ml of a window cleaner containing 12 % of EGBE, corresponding to about 0.5 to 1 g/kg bw (Rambourg-Schepens *et al.*, 1988). On admission to the intensive care unit, the patient was comatose. Biochimically she presented with metabolic acidosis, hypokalaemia, a rise in serum creatinine level and a markedly increased urinary excretion of oxalate crystals.

In another case of attempted suicide (Gijsenbergh *et al.*, 1989), a 23-year-old woman had ingested about 500 ml of a mixture containing EGBE (12.7 %) and of EtOH (3.2 %) (about 57 g of EGBE corresponding to a dose of about 1g/kg bw).

Upon admission, she was in a comatose state, had breathing difficulties was in metabolic acidosis and had a concentration of EGBE in blood of 432 mg/l. 2 hr after admission, the blood concentration of EGBE was 304 mg/l . A fall in Hb from 11.9 g/dl on admission to 8.9 g/dl on the second day, together with the appearance of haematuria was noticed. The half-life of EGBE in the blood was 210 min (greater than the commonly values described in the literature probably due to the presence of EtOH in the mixture). BAA concentration in the urine was maximum 4 hr after dialysis treatment was initiated.

A case of acute poisoning with EGBE was reported (Bauer *et al.*, 1992). A 53-year-old man ingested intentionally 500 ml of a mixture containing 9.1 % of EGBE (45.5 g – about 750 mg/kg bw) and 2.5 % EtOH.

Clinic signs were coma, tachycardia, metabolic acidosis and hypoxemias, pulmonary oedema and Adult Respiratory Distress Syndrome (ARDS). By 36 hr after admission, the subject

showed signs of non haemolytic hypochromic anaemia with thrombopenia. No signs of haemolytic anaemia were noticed.

Some cases of children poisoning which have occurred in the Pittsburgh Poison Center were summarized in a publication (Dean and Krenzelok, 1992). The ingested products contained EGBE in concentrations ranging from 0.5 % to 9.9 %. The children poisoned ages ranged from 7 months to 9 years. The quantities ranged from 5 to 300 mL - 24 children were studied - 2 of them were treated by gastric gavage followed by a 24 hr hospitalisation. The other children were treated at home with simple dilution. Common symptoms seen in acute animal dosing (haemolysis, CNS depression, metabolic acidosis and renal compromise) did not occur.

A 18-year-old male consumed between 360 and 480 ml of a glass cleaner containing 22 % EGBE (maximum 95 g of EGBE – about 1,25 g/kg bw) (Gualtieri *et al.*, 1995 –and Gualtieri *et al.*, 2003).

He was hospitalised with metabolic acidosis and hepatic biochemical abnormalities (Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and hepatic bilirubin). After treatment with EtOH and haemodialysis, the patient returned home without sequelae. Nine days following his initial discharge, he was again admitted after ingestion of 480 ml of the same cleaner. The second hospitalisation was absent of any significant metabolic or hepatic abnormalities. Haematological and renal abnormalities were absent in both hospitalisations. Quantitative analysis for BAA has given a maximum concentration of 4.86 mmol/L approximately 16 hours after ingestion.

A 19 year old man ingested about 20-30 ounces of a product containing 25-35 % EGBE (maximum 336 g of EGBE – about 4.5 g/kg bw) (Burkhart and Donovan, 1998). The product had a pH of 13 and was therefore considered as an alkaline corrosive. The patient developed characteristic features of coma, acidosis and haematuria which were previously reported following EGBE ingestion. After treatment, the patient still exhibited neurologic sequelae (difficulties with fine motor skills and stop speaking). However the patient medical history was significant for mental retardation, depression, hyperactivity and he was treated with various strong psychiatric medecine stopped after the ingestion. This weakens the direct relationship between EGBE and the sequelaes reported.

A case report of a massive ingestion of a mixture containing 10-30 % of EGBE and 10-40 % of isopropanol was reported (McKinney *et al.*, 2000). A 51-year-old woman ingested 8 ounces of this mixture (corresponding to an estimate dose of 24-72g of EGBE corresponding to a range of 0.4 – 1.2 g/kg bw) in a suicide attempt.

Clinical symptoms were metabolic acidosis and mental status depression. She was treated with EtOH therapy and recovered without apparent sequelae. No signs of haematotoxicity were noticed.

Table 4.53: Summary human acute toxicity data

Estimation of absorbed dose	Patient history	Patient pathology	Reference
Between 0.5 and 1 mg/kg bw	50-year woman. Suicide attempt with glass cleaner.	Coma, metabolic acidosis, hypokaliemia, increase in serum creatinine level and urinary excretion of oxalate crystals	Rambourg-Schepens *et al.*, 1988.
About 1 g/kg bw	23-year woman. Suicide attempt with mixture containing EGBE.	Coma, breathing difficulties and metabolic acidosis. Haematuria and decreased Hb for 2 days.	Gijsenbergh *et al.*, 1989.
About 750 mg/kg bw	53-year man. Suicide attempt with mixture containing EGBE.	Coma, tachycardia, metabolic acidosis, hypoxemia, pulmonary oedema and ARDS. Non haemolytic anaemia with thrombopenia.	Bauer *et al*., 1992.
About 1.25 g/kg bw – 2 times separated by 9 days	18-year man. Ingestion of a glass cleaner.	Metabolic acidosis and hepatic biochemical disorders. Nothing after the second ingestion.	Gualtieri *et al.*, 1995, Gualtieri *et al.*, 2003.
About 4.5 g/kg bw.	19-year man. Ingestion of a mixture containing EGBE.	Coma, acidosis and haematuria.	Burkhart and Donovan, 1998
Between 0.4 and 1.2 g/kg bw	51-year woman. Ingestion of a mixture containing EGBE.	Metabolic acidosis and mental status depression.	McKinney *et al.*, 2000

According to this data, a LOAEL of 400 mg/kg bw can be taken into account for acute toxicity by oral route in humans. It should be noted that this is a worst case estimation derived from the McKinney paper in which the possible range of exposure was between 0.4 and 1.2 g/kg bw.

Summary human data:

Acute human toxicity data comes from children accidental ingestion or adult suicide attempts made with mixtures containing EGBE and from one study on human volunteers by inhalation. For oral route case reports, ingested doses are difficult to evaluate because of the lack of data concerning the body weight of all patients and the exact ingested dose, but a semi-quantitative estimation of the ingested doses was made for each case (see table 4.7). The range of doses which lead to clinical symptoms varies between 0.5 and 4.5 g/kg bw. In all cases, patients exhibited SNC depression and metabolic acidosis. Signs of haemolysis were seen in some cases but this finding was not systematic (this showed that human is much more resistant to haemolysis than rodents). After a first acute ingestion, a second administrator some days later did not exhibit the same symptoms, this finding was also seen with animals in some studies. In these cases, EGBE was ingested together with other substances (ethanol and/or unknown substances) that could have some influence on the symptoms seen. Between 0.5 and 1.5 g/kg bw the patients totally recovered after treatment. According to this data, a LOAEL of 400 mg/kg bw can be taken into account for acute toxicity by oral route in humans in the risk characterisation section. If a risk characterisation by dermal and inhalation route is needed for acute effects, kinetic data is sufficient to extrapolate this oral LOAEL to inhalation and dermal LOAEL. Human data is preferred for risk characterisation, especially for EGBE because of its haematotoxicity more marked in animals than in humans. It should be noted that this is a worst case estimation derived from the McKinney paper in which the possible range of exposure was between 0.4 and 1.2 g/kg bw.

By inhalation route, symptoms reported by the three volunteers were signs of irritation (throat and ocular) and headache. These symptoms did not seem to be dose-related.

Summary acute toxicity

For the inhalation route, the 4 hour LC_{50} in rats, which are susceptible to haemolysis (see next section), was of the region of 450ppm (2214mg/m^3). Higher values are seen in other species.

For the dermal route, great differences were seen between the tested species and the mode of occlusion. The rabbit seems to be the most sensitive species with LD_{50} of about 500 mg/kg when administered occlusively.

For the oral route, available studies considered as reliable show LD_{50} values upwards from 1000mg/kg.

According to the data available, a classification R20/21/22 is needed for all three routes of exposure.

A number of human case studies are available from attempted suicides which suggest that the human LOEL is in the region of 400 mg/kg bw. Human data is preferred for risk characterisation, especially for EGBE, because its haematotoxicity is more marked in animals than in humans. There is sufficient kinetic data available to enable route to route extrapolation to be carried out to risk assess the more relevant dermal and inhalation routes. These extrapolations are carried out in the risk characterisation section. It should be noted that this is a worst case estimation of the LOEL derived from the McKinney paper in which the possible range of exposure was between 0.4 and 1.2 g/kg bw.

4.1.2.2.3 Specific toxicity: haematotoxicity

Some studies were performed especially to assess the haematotoxic potential of EGBE in various conditions.

The studies described above are but a few that indicate that EGBE causes acute haemolytic anaemia in rats and mice. This response is consistent and probably forms the basis for establishing an No Observable Adverse Effects Levels (NOAEL) in species used experimentally. It has also been suggested that it is an essential, early step in the development of haemangiosarcomas of the liver in male mice (described in 4.1.2.8.3). Clearly, if the haemangiosarcomas arising in male mice (see Section 4.1.2.8.3) are ultimately a consequence of anaemia induced by EGBE, the species and sex specificity of the neoplastic response must be due to some factor(s) subsequent to the anaemia, since this also occurs in rats and female mice. Possible elements contributing to the specificity of the response will be examined and the strength of the evidence evaluated in order to reach a conclusion on the human relevance of the haematological effects and the neoplastic responses observed in rodents.

In vitro

In vitro and *in vivo* response of mammalian erythrocytes to EGBE and BAA was assessed (Carpenter *et al.*, 1956). The results are summarized in the table 4.54:

Table 4.54: Summary of the erythrocyte toxicity in Carpenter *et al.*, 1956.

In vitro response						*In vivo* response	
		2.5 % EGBE		0.1 % BAA			
Animal	Sex	Max.time without haemolysis, Min	Max. time to haemolysis, Min	Max.time without haemolysis, Min	Max. time to haemolysis, Min	Osmotic fragility upon inhalation of EGBE	Haemoglobinuria upon inhalation of EGBE
2 rats	F	46	60-67			Significant ↑ at 62 ppm x 4h	Seen at 203 ppm x 7h but not at 107 ppm x7h
3 rats	M			35-40	40-60		
2 rats	F			35-40	40-46		
2 mice	M	22-30	35-40			Significant ↑ at least as low as 100 ppm x 7h	Seen at 200 ppm but not at 100 ppm x7h
2 mice	M			40-45	50-60		
1 mouse	F			45	55		
2 rabbits	M	110.113	123-125			Significant ↑ at 125 ppm x 7h	Not seen as high as 197 ppm x 7h
4 rabbits	M			60-90	80-100		
2 rabbits	F			80-90	85-100		
1 monkey	F			120	130	No Significant ↑	Not seen as high as

1 monkey	M			103	120	at 200 ppm x 7h	200 ppm x 7h
2 dogs 2 dogs	F F	20	40-86	89-120		No Significant ↑ at 665 ppm x 7h x 2 days	Not seen as high as 665 ppm x 7h
1 human 2 humans	F M	80 60-83	100 80-94	147-268		No Significant ↑ at 200 ppm x 8h	Not seen at 200 ppm x 8h
2 guinea pigs 2 guinea pigs	M M	60-68	80	360		No Significant ↑ at 665 ppm x 8h	Not seen at 665 ppm x 8h

Effects of EGBE and BAA on human, rat, dog and rabbit blood were assessed *in vitro* (ICI, 1985). EGBE and BAA solutions were prepared with veronal buffered saline. pH was adjusted to 7.2. One ml of erythrocyte suspension was added to 3 ml of the test solution. The haemolytic activity was calculated using positive (totally lysed sample) and negative (no test substance) controls. Concentration range of 0.01 to 2 % BAA and 0.05 to 0.5 % EGBE were tested in the main study. With 2.5 and 1 % EGBE solutions, no analysis could be performed due to a discoloration of the test medium. The problem was solved by employing 0.5 % EGBE as the maximum permissible concentration for incubation.

With BAA, in the preliminary study, no effects were seen on human erythrocytes whereas marked haemolysis was noted in rat erythrocytes (38.4 %).

With BAA only rats erythrocytes readily lysed and a very marked haemolysis was seen from 0.05 %. With EGBE dog erythrocytes seemed to be very sensitive to haemolysis and various effects were seen from 0.4 % in other species (see tables 4.55 and 4.56).

Table 4.55: Haemolytic activity of various concentrations of butoxyacetic acid incubated with erythrocytes from rat, human, dog and rabbit

Species	Haemolysis (%)								
	Overall concentration (%)								
	0.01	0.02	0.03	0.04	0.05	0.1	0.2	0.5	2
Rat	0.8	4.4	8.4	11.1	53	91.9	97.9	98.8	24.9
Human	0.8	1.4	-	-	0.2	0	1.2	0.8	0
Dog	0	13.6	-	-	0	0	0	0	2.6
Rabbit	4.9	0	-	-	3.2	1.2	0	1.1	1.7

Table 4.56: Haemolytic activity of various concentrations of EGBE incubated with erythrocytes from rat, human, dog and rabbit.

Species	Haemolysis (%)				
	Overall concentration (%)				
	0.05	0.1	0.25	0.4	0.5
Rat	-	0	2.5	51.5	62
Human	-	0	1.5	20.5	70.9
Dog	46.8	36.2	-	41.2	62.3
Rabbit	-	0	2.8	83.7	72

A single dose of EGBE was applied dermally (semi-occlusively) to groups of 3 female Wistar rat at doses of 500, 375 and 260 mg/kg and to a group of 6 female rats at 200 mg/kg (Bartnik *et al.*, 1987). Six hours after dosing, blood was sampled retro-orbitally. Animals were then killed and an additional blood sample was taken by heart puncture. Concurrently EGBE was injected intravenously to groups of 4 male rats at doses of 25, 37.5, 50, 62.5 and 75 mg/kg. At 3 and 6 hr following application blood was collected retro-orbitally. After termination, blood was also collected by heart puncture.

In this study, *in vitro* haemolysis was also studied, male Wistar rats and humans red blood cells were treated with various concentrations of EGBE and BAA. Positive and negative controls were performed, haemolytic activity was expressed as percentage of totally hemolyzed.

In vitro, BAA showed higher haemolytic potency than EGBE. Hemolytic activity of EGBE and BAA was markedly lower on the human erythrocytes compared with rat erythrocytes (see tables 4.57 and 4.58).

Table 4.57: Haemolysis of human and rat erythrocytes by EGBE

Percentage haemolysis												
	15 min		30 min		45 min		60 min		120 min		180 min	
EGBE (mmol/liter)	R	H	R	H	R	H	R	H	R	H	R	H
100	0		0		0		0		0		0	0
125	0		0		0		0		10		64	0
150	0	0	0	0	0	0	8	0	62	3	92	11
175	0	0	6	0	41	0	91	2	100	23	100	89
200	5		100	0	100	2	100	8	100	100	100	100
225	96	0	100	14	100	89	100	100	100	100	100	100
250	100	8	100	100	100	100	100	100	100	100	100	100

Table 4.58: Haemolysis of human and rat erythrocytes by BAA

Percentage haemolysis												
	15 min		30 min		45 min		60 min		120 min		180 min	
BAA (mmol/liter)	R	H	R	H	R	H	R	H	R	H	R	H
1.25	0	0	0	0	0	0	0	0	6	0	25	0
2.50	0	0	0	0	4	0	20	0	79	0	95	0
3.75	0	0	0	0	17	0	49	0	86	0	100	0
5.0	0	0	13	0	59	0	88	0	100	0	100	0
6.25	0	0	17	0	59	0	89	0	100	0	100	0
7.5	0	0	25	0	69	0	100	0	100	0	100	0
10		0		0		0		0		0		0
15		0		0		0		0		0		0

In vivo, a dermal dose of 200 mg/kg did not cause any haemolytic effect. Other tested doses caused haemolytic effects indicated by increase in mean cell volume, lower erythrocyte count and haemoglobin level and haemoglobinuria. No dose response relationship was found but according to the authors it might be due to inherent biologic variation in percutaneous absorption and haemolytic susceptibility and also to fewer animals per dose group. Following a IV injection of 62.5 mg/kg, no haemolysis was detectable whereas a dose of 75 mg/kg produced slight effects.

The effects of EGBE and its metabolites on human and rat erythrocytes were investigated *in vitro* (Ghanayem, 1989). Heparinised blood was collected from a male and a female volunteer and cardiac blood was collected from male F344 rats, using EDTA as the anticoagulant. Blood-chemical mixtures were incubated in a shaking water bath at 37° for up to 4 hr. Incubation of whole blood from rats with 20 mM EGBE was associated with a slight, but significant decrease in haematocrit values and significant haemolysis, as determined by free plasma haemoglobin concentrations. No significant changes were observed with 5 or 10 mM EGBE. Similar incubations, but with 2-(1-[^{14}C]butoxy)ethanol, followed by High Perfomance Liquid Chromatograpy (HPLC) analysis for metabolites failed to demonstrate the presence of either Butoxy Aldehyde (BAL) or BAA. Incubation of rat blood with BAL at 0.5, 1.0 or 2.0 mM resulted in both time- and concentration-dependent swelling of erythrocytes, as shown by an increase in haematocrit values, although significant haemolysis occurred only after incubation with 2 mM BAL for 4 hr. Similar incubations of rat blood with BAA at 0.5, 1.0 or 2.0 mM also induced swelling of erythrocytes and haemolysis, but the highest concentration induced its maximum haematocrit response in 2 hr, which was followed by a rapid fall as haemolysis was essentially complete by 4 hr. Repetition of the incubations of rat blood with BAL, but supplemented with aldehyde dehydrogenase and NAD^+, resulted in a significant potentiation of the effect of BAL, whereas addition of cyanamide (an inhibitor of aldehyde dehydrogenase) significantly decreased the activity of BAL in both the presence and the absence of the exogenous enzyme. Therefore, it appeared that BAA was the haemolytic metabolite and that haemolytic activity of BAL was due to its metabolism to BAA by

dehydrogenases present in rat blood. It was also found that reduction of blood ATP concentrations was more effectively produced by the acid than by the aldehyde, while EGBE itself had no effect on ATP concentration, even at a 10-fold higher concentration. It can be concluded that erythrocyte swelling precedes haemolysis, strongly suggesting that the erythrocyte cell membrane is the target of the haemolytic species. It was not clear, however, whether cell swelling was mediated by loss of ATP, or whether this loss was a secondary effect.

In contrast to these data with rat blood, incubation of blood from volunteers with BAA at concentrations up to 2 mM had no effect on haematocrit or plasma free haemoglobin. Small, but significant increases in haematocrit were observed at 4 and 8 mM after 4 hr incubation and there was minimal, but significant haemolysis at 8 mM after 4 hr. A slight decrease in blood ATP was observed with 8mM BAA, but there was no time dependent relationship.

SD rats were administrated EGBE by gavage at 50 and 500 mg/kg (Kurantsin-Mills *et al*., 1992). Blood samples were taken at 0.5 – 2 and 4hr after dosage for determination of the physical characteristic of RBCs by scanning microscopy (SEM) and frequency distribution of their indices by flow cytometry.

SEM revealed discocyte-spherocyte transformation in treated rats. Flow cytometric analysis of the frequency distribution of the RBCs showed that the MCV and Mean Cell Haemoglobin Concentration (MCHC) increased and decreased, respectively compared to the controls. Whole blood viscosity was evaluated by microviscometry at 37°C over a wide range of shear rates. The deformability of RBCs was estimated as the resistance of a cell flowing through microchannels that simulated microvessels. Whole blood viscosity increased at shear rates of 5.75 to 230/s for doses 50 and 100 mg/kg, and then decreased thereafter due to haemolysis. *In vitro* simulation of shear-dependent haemolysis using the microviscometer at shear forces of 0.62 to 13.20 dynes/cm^2 indicated that the RBCs of EGBE-treated rats hemolyzed significantly more than the controls. Flow studies in the microchannels also showed that the deformability of RBCs of EGBE-treated rats was significantly less than the control cells due to the decreased surface area to volume ratio. These results demonstrate that membrane and cellular changes in the RBC due to EGBE and/or its metabolites alter its rheological properties, and therefore compromise transit through the microvessels of the microcirculation resulting in haemolysis.

In vitro haematopoietic toxicity of some glycol ethers and derivatives (EGEE, EGME, EGBE, EGEEA, EGMEA, EGBEA, diEGBE, EGBEdiA and PGME) was assessed using haematopoietic culture assays in murine, rat and human species (Ruchaud *et al.*, 1992).

Acute and subacute toxicities of the compounds were analysed: on established haemopoietic cell lines (either leukemic or growth-factor-dependent cell lines), on haemopoietic progenitor growth and differentiation (CFU-c assay) and in bone marrow long-term cultures (Dexter type cultures) to determine the effects on both the hemopoietic and the stromal cell tissues.

Acute and subacute toxicity on haematopoietic cell line.

EGBE was tested for 48 hr at concentrations between 0.1 M and 0.1 μM. Typical dose response curves were obtained after treatment of NB4 cells. For all compound tested dose-responses show the common feature of a very steep slope on a semi-log plot, indicating that cell viability went from normal to nil by increasing concentrations by only a factor of 4 to 8. This steep slope and the sigmoid shape of the curve suggested that the substance hindered some cellular mechanisms essential for cell survival. Combined dose-response/time-course analysis were carried out on the human promyelocytic leukaemia celll line NB4 show that the

toxicity increased regularly with time over 96 hr treatment. Compared to other glycol ethers, EGBE caused a distinct toxicity feature for short incubations (0 to 40 hr) indicating that injuries causing cell death had probably multiple origins. The first component of the toxic phase worked at doses > 1mM and was over by 48 hr incubation. The second component probably worked from the beginning of the incubation, when its effects accumulate with the previous ones, but persist for longer incubation times (> 91 hr) and at lower doses. No differences were found for the same cell line when growth was supported by one or the other of the two growth factors tested, IL3 and G-CSF.

Acute toxicity on Haemopoietic Progenitor Cells (CFU-c): human umbilical cord blood cells were treated with EGBE for 15 hr before CFU-c assay plating, then incubated for 14 days with 5637 CM. Growth of haemopoietic colonies was dramatically reduced after treatment. Virtually no growth was observed at concentrations > 20 mM. Similar results were found with murine bone marrow cells.

Toxicity on haemopoietic cells and microenvironment in Dexter Cultures: effects of EGBE were evaluated on Dexter type long-term cultures two weeks after their establishment. Haemopoiesis was followed for 2 months with a constant exposure to various concentrations of compound. An acute toxicity of EGBE became obvious from 4 days of exposure at 7.5 mM. Virtually no haemopoietic cells were found in the culture medium while only a few haematopoietic foci were still present within the layer of stromal cells. Increasing the concentrations definitely eradicated haemopoiesis, but stromal cells remained unaffected. Large macrophages like cells in the adherent layer where more sensitive than fibroblastic or preadipocytic stromal cells. After 2 weeks of exposure, aplastic cultures were obtained for doses between 7 and 15 mM. In these cultures, the stromal cell layer was apparently unaffected but no haemopoiesis persisted. Doses exceeding 30 mM also destroyed the haemopoietic microenvironment. Haemopoietic cell production in the liquid phase of cultures was evaluated twice a week for 2 months. Results after 4 or 8 days of treatment showed that haemopoiesis was very rapidly destroyed within a narrow dose range of the compound: for low doses low injury was apparent as judged by cell counts or cell morphology.

In summary, of these studies, EGBE was the most toxic compound. The IC50 after 96 hr of exposure was in the range 50-100 μM. The pattern of toxicity suggested that cell death resulted from several mechanisms that had cumulative effects during the earliest period of exposure. This result is reminiscent of the haemolytic effect of EGBE reported by the others. By targeting the leucocyte membrane, EGBE could be responsible for both irreversible damage and cytolysis.

In vitro haemolytic effects of BAA were assessed on human and rat erythrocytes (Udden, 2002). Human blood was sampled from healthy volunteers, hospitalised patients and children and rat blood from Fisher 344 rats (9-11 weeks old). RBC were isolated, adjusted to a Packed Cell Volume (PCV) of 10 % and incubated in the presence of BAA (99 % pure) for 1-4 hours at 37° C. Various parameters were assessed: microhaematocrit, haemoglobin, RBC count, % haemolysis, erythrocyte deformability, erythrocyte osmotic fragility, density and Na and K^+ concentrations in erythrocytes. A microscopic examination of erythrocytes was also performed.

A time dependent increase of deformability was seen after exposure of human erythrocyte to 10 mM BAA compared to controls. No haemolysis was observed. To compare sub-haemolytic effects of BAA, human RBC were challenged with 0-10 mM BAA for 4 hours while rat erythrocytes were exposed to BAA at 100 fold lower concentrations. A comparable increase in cell rigidity with haemolysis was seen in both species. At the highest concentration

of BAA used, there was a 64 % increase in Po of rat RBCs and an increase in Po of 40 % for human RBCs. A significant change in human erythrocyte deformability occurred at 7.5 and 10 mM BAA whereas decrease deformability in the rat erythrocyte was noted first at 0.05 mM BAA. In Mean Cellular Volume (MCV), there was a small but significant increase in human red blood cell volume after exposure to the highest concentration of BAA. For the size distribution, a main peak (very close to the MCV) was seen. A shoulder was also present. Both rats and human erythrocytes appeared to shift in size to a larger cell volume, as the number of smaller red blood cells diminished. For osmotic fragility, no significant changes in human erythrocytes were seen until they had been exposed to either 7.5 or 10 mM. Rat erythrocytes demonstrated a remarkable increase in osmotic fragility after exposure to lower concentrations of BAA. For erythrocyte density, human red blood cells showed only a very small shift toward lower density, however, the rat erythrocytes showed a marked shift to lower cellular density. For erythrocyte cations contents, there was a significant increase in both human and rat erythrocyte Na concentration with exposure to high and low levels of BAA respectively. However, human red blood cell K^+ was slightly decreased and this occurred after incubation in the highest concentrations of BAA. Rat red blood cell K levels decreased after exposure to 0.025 mM BAA, the lowest concentration used. Although there was a trend towards increased total cation contents in the human, only the rat erythrocytes showed a significant net change in total cation content, a small net increase after their incubation with 0.1 mM BAA for 4 hours. The morphology of human erythrocytes was not changed after exposure to 10 mM BAA for 4 hours. However, rat erythrocytes exposed to 0.1 mM BAA showed increased numbers of spherocytes compared to the controls.

After corrections, there was no difference in haemolysis of RBCs obtained from hospitalised adults and children compared to well adults when exposed to 10 mM BAA. MCV did increase as a result of BAA exposure, but no individual had a significant difference in the pattern of the frequency distribution of cell size.

In vivo

A fragility test was performed by adding a drop of whole rat blood delivered with a needle into an agglutination tube containing 1 ml of saline (Mellon Institute of Industrial Research, 1952) . The saline concentrations ranged from 0.36 % to 0.72 % in steps of 0.04 %. After addition of the blood the tubes were incubated at room temperature for 2 hr and read. The supernatant liquid is examined for any indication of haemolysis. Initial haemolysis was read as the highest concentration of saline in which haemolysis occurs and complete haemolysis the nearest in which there were no intact red cells visible. Cell detritus will not settle out in the 2 hr interval of the test. Initial haemolysis occurred at 0.44 % NaCl and complete haemolysis at 0.36 % NaCl in a remarkable consistent manner with rat blood. The exceptional normal reading was 0.48 % initial and 0.40 % complete.

Fifteen males and 15 females were exposed 7 hr/d to 500 ppm of EGBE. 13 out of 15 females died during or shortly after the first exposure. Lung congestion and haemorrhage and generalised congestion of other organs was found at autopsy. The 2 other rats died after the second and the third exposures respectively. Seven out of 15 males died during or after the first exposure, one each after 2 and 3 exposures, 3 after 4 exposures and 3 survived 30 exposures. Two of these survivors had moderate cloudy swelling of convoluted and loop tubules of the kidney, one had moderate cloudy swelling of the liver and one had completely normal kidneys, liver, lung, spleen and testis. Blood studies after 30 exposures revealed anaemia and increased fragility of the red cells.

Regarding the high mortality at 500 ppm, another study was carried out with lesser concentrations. Animals were exposed to 314 ppm but lethality was found within 3 days of exposure in the 15 females exposed. Pathology was comparable to that described for the 500 ppm dose.

Another study using 200 ppm doses was performed to the fragility test. Fragility of red cells on a group of rats was followed before and after exposure each day for a period of 9 days. Each day fragility of the red cells increased from initial haemolysis in 0.48 % saline before exposure to initial haemolysis at 0.52 % to 0.68 % after the exposure (following an overnight or a 17 hr rest period red cell fragility had returned to the normal value.

Another group of 6 rats concurrently exposed at 200 ppm was used for enumeration of red blood cells and haemoglobin determinations. The protocols showed that the rats became anemic after 26 hr of exposure during 4 days. The red cell count was about half of pre-exposure level and haemoglobin had dropped from about 90 to 65 %. After 5 exposures of 6 or 7 hours duration followed by a 7-day although they were still below the pre-exposure levels in most cases. Thereupon the rats were again subjected to 3 more exposures. Again the red counts were depressed and the haemoglobin values lowered even more so than after the first 5 exposures. A subsequent rest period of 8 days resulted in a measurable increase in both values but the red cell counts were still subnormal. Fragility values were normal.

Further study on rats revealed that at a concentration as low as 25 ppm of EGBE a 7 hr exposure produced a rise in red cell fragility whereas after 10 ppm for 7 hr only 1 of 4 rats showed any increase.

Rats fed orally 1.48 g/kg as a 10 % aqueous dilution showed a relatively enormous increase in red cell fragility. The results on these four rats are as follows:

Table 4.59: Effects on rat blood

Time after dosing	Initial and complete haemolysis (% Na Cl)
15 min	0.48 – 0.40
50 min	0.72 – 0.60
130 min	0.72 – 0.60
4 hr 1/2	0.72 – 0.60

2 Guinea pigs and 2 rabbits were followed for 3 days while being similarly exposed to 200 ppm EGBE. Guinea pigs were not affected whereas rabbits responded like rats.

One rabbit was exposed for 10 hours to 200 ppm of EGBE and the fragility or red cells was determined periodically until the blood had returned to normal.

Table 4.60: Effects on rabbit blood

Time after dosing	Initial and complete haemolysis (% Na Cl)
Pre-exposure	0.48 – 0.40
3 – 180 min inclusive post-exposure	0.60 – 0.44
240 – 420 min inclusive post-exposure	0.52 – 0.40
420 min post exposure	0.48 – 0.40
25 hours post exposure	0.48 – 0.36

Two dogs were exposed to concentrations ranging form 200 to 665 ppm to determine the sensibility of their red blood cells to EGBE. No effects on RBC fragility were evidenced in these studies.

Two men were exposed to 100 ppm EGBE for a 4 hr period. Rats were concurrently exposed to serve as positive controls. RBC fragility tests were performed 4 times in men and twice in the rats. No response was seen in humans whereas rats elicited expected positive response showing that they are much more sensitive as humans to the haemolytic properties of EGBE.

Groups of 24 male F344 rats were dosed Per Os (PO) to EGBE (purity 99 %) at doses of 500 and 1000 mg/kg (Grant *et al.*, 1985). Six rats/group were bled and killed on day 1, 4, 8 and 22 after the last treatment. Analysis of RBC, White Blood Cell (WBC), Hb, MCV, Haematocrit (HCT), reticulocytes and differential white cell count were performed. After termination, spleen, thymus, liver, testes and kidneys were weighted and were examined by microscopy. Cervical and mesenteric lymph nodes were also examined by microscopy. Femoral bone marrow was also examined by electronic microscopy. This study was concurrently performed with EGME to compare haematological effects caused by the two glycol ethers.

Body weight gain was affected for high doses of EGBE. There was also an increase of the relative weights of spleen, liver and kidneys and a decrease of thymus weight. These changes returned to normal by day 22 apart for liver and kidneys for which the weight was still slightly raised. Hyperplasia of marrow without evidence of haemorrhage was seen. Splenic extramedullary haemopoiesis was marked in both groups on day 1 but returned to normal by day 8. A transient lymphocyte depletion of the thymic cortex was also noted and returned to normal by day 4. Animals treated with the high dose had reduced RBC, HCT, HB and elevated MCV, reticulocytes counts and Mean Corpuscular Haemoglobin (MCH). Most of these changes disappeared over the recovery period, although the MCV and MCH remained slightly elevated on day 22. The reticulocytosis was particularly striking on day 1 and 4 but had returned to normal by day 8. In this group increased number of normoblastes, pronounced anisocytosis, polychromasia and presence of Howell Jolly bodies were found but had resorbed by day 8. Leucocytes were also depressed on day 1 only due to a decrease number of circulating lymphocytes. These changes were also seen with the lower doses, but less in severity.

The role of Alcohol and Aldehyde dehydrogenases in the haematotoxicity of EGBE was assessed (Ghanayem *et al.*, 1987b). Three groups of F344 rats (9-12 weeks old) were submitted to different treatments: rats of the first group were treated IP with pyrasole (250 mg/kg) one hour before gavage administration of water (control group) or EGBE. For the

second group, cyanamide (50 mg/kg) was administrated IP to animals 0.5 hr before water (control) or EGBE administration by gavage. The third group received only a single dose of EGBE by gavage (500 mg/kg). Animals were placed immediately in metabolism cages after EGBE treatments to collect urine and faeces.

Concurrently, BAA or BAL was administrated to rats by gavage at the molar equivalent of 125 mg/kg EGBE. Rats were also placed in metabolic cages.

After EGBE administration, blood samples were collected during termination of the study. Evaluated parameters were: RBC, Haemoglobin (Hb) concentration, PCV, HCT, MCV, MCH, MCHC and platelet. Free Hb was also quantified in the plasma and urine of these rats. After collection of blood samples, liver, kidneys and spleen of treated animals were collected and weighted. Tissues were collected 24 hr and 48 hr after treatments.

Gavage administration of EGBE caused severe haemolysis accompanied by an increase in the relative spleen weight. The increase was significant at all time points and was maximum 2 hr after EGBE administration. Recovery from treatment was not complete even 48 hr after dosing. A significant decrease in the number of circulating RBCs and Hb concentration and HCT was seen in treated animals. These effects were maximum within 8 to 24 hr after dosing and showed a rebound at 48 hr. These finding were accompanied by increase of the free Hb in the plasma and haemoglobinuria. In the liver, histopathologic evaluation made 24 hr after dosing showed coagulative necrosis and phagocytosis of Hb by hepatocytes and Kupffer cells. In contrast, pre-treatment of rats with pyrasole resulted in complete prevention of the increase of the relative spleen weight and of the haemolytic effects caused by EGBE alone. Liver effects were also completely prevented by pyrasole pre-treatment. With cyanamide treatment, significant increases of the relative spleen weight, however the time to maximum effects was approximately doubled in rats treated with cyanamide and EGBE as compared to rats treated with EGBE alone, a significant decline in number of RBCs, HCT and Hb concentration in cyanamide treated rats was also seen but was significantly less than what was found after EGBE treatment only.

After BAA or BAL administration, the same effects were seen on relative spleen weight than after EGBE administration. The maximum effects were observed at 4hr after dosing. No differences were seen on blood parameters between the three chemicals. Treatment with cyanamide before BAL administration resulted in a significant protection against BAL induced toxicity.

Effect of age on the toxicity was assessed (Ghanayem *et al*., 1987c). EGBE (purity > 99 %) was administered orally to F344 rats (16 months old) at doses of 32, 63, 125, 250 and 500 mg/kg. Immediately after dosing rats were placed in metabolic cages and urine was collected at different times. Rats were killed at various time points after administration and blood was collected by cardiac puncture. Spleen, liver, kidneys, testes and urinary bladder were collected and examined histologically. The following measurements were done on blood samples: RBC, WBC, Hb, HCT, MCV, MCH, MCHC and platelet count. Free concentrations of Hb were also determined in urine and in plasma.

Gavage administration of EGBE in young or adults rats resulted in a significant dose related increase of the spleen weight evidenced from 125 mg/kg. Young rats were less sensitive than older ones. Maximum effects on relative spleen weight were observed at 2 and 4 hr after the administration of 500 mg/kg to rats (young or old with a more marked effect in old animals). At 125 mg/kg dose, effects were maximum 4 hr after gavage in both age groups. After reaching the maximum, controls levels where obtained about 48 hr after treatment. On

haematologic parameters, EGBE caused a significant decrease in the number of circulating RBCs, Hb concentration and HCT. Other blood parameters showed minimal changes. These effects were dose dependent and older rats were significantly more susceptible than younger rats. At 125 mg/kg, effects on RBCs, Hb and HCT were only seen in old animals 8 and 24 hr after administration. Young animals were not affected by treatment at this dose level. The decline in RBCs, Hb and HCT was accompanied by a significant dose dependent increase in the concentration of free Hb in the plasma of treated rats. The peak level of free Hb in the plasma was lower in young animals compared to the older ones at all doses investigated. Maximum free plasma Hb levels were seen after the administration of 500 mg/kg EGBE and were approximately 5 times higher in the plasma of the older rats than those of measured in the plasma of the younger rats. For the 125 mg/kg dose, no effects were seen on free plasma Hb of young animals whereas a significant increase was detected 8 hr after administration of the same dose to older rats. Haemoglobinuria was observed and was concomitant with the decline of free Hb in the plasma. A difference between old and young rats was also noted (see table 4.61).

Table 4.61: Incidence of haemoglobinuria induced in rats of various ages by gavage administration of EGBE.

	Dose (mg/kg)				
Age	32	63	125	250	500
4-5 weeks	0/6	0/6	1/11	6/6	12/12
9-13 weeks	0/6	0/6	12/12	6/6	12/12
5-6 months	0/6	6/6	6/6	ND	ND
16 months	6/6	6/6	6/6	ND	ND

Histopathologic evaluation of tissues of rats of various ages examined 24 hr after EGBE administration revealed that EGBE causes dose and age dependent liver changes. In 9 to 13 week old rats, EGBE caused focal disseminated coagulative necrosis of hepatocytes. In addition there was evidence of phagocytosis of dark pink protein compatible with Hb by Kupffer calls and hepatocytes. These effects were observed at an incidence of approximately 16 and 83 % at 250 and 500 mg/kg doses, respectively. Evidence of regression of these liver lesions was seen at 48 hr after EGBE dosing. No liver changes were observed at the same doses in 4 to 5 week old rats. In the kidneys, small and dark pink protein droplets, compatible with Hb, were seen in the proximal tubular epithelium of treated rats. In addition Hb casts were observed in the proximal tubules. The incidence and severity of these effects were dose dependent and were observed in 9 to 13 week old rats but not in 4 to 5 week old rats. Similar to the liver, kidney lesions were more severe at 24 hr after EGBE administration and exhibited signs of regression when examined at 48 hr after dosing.

The influence of the type of haematology analyser on measured parameters was assessed (Ghanayem *et al.*, 1990b). Groups of male F344 rats (9-11 weeks old, 3-5 animals per group) were treated by gavage with 125, 250 or 500 mg/kg of EGBE (purity 99.9 %). Animals were held for 1, 2, 4, 8 or 24 hr after treatment. Control rats received the vehicle (water). At the end of each holding period, blood samples were collected and analysed using both a laser-based and an impedance-based haematology analyser. The following parameters were measured : RBC and WBC counts, Hb, HCT, MCV, MCH, MCHC and platelet counts. In addition PCV were determined by centrifugation.

Parameters obtained with the laser-based analyser indicated dose and time-related decreases in RBC and Hb. Similar effects were observed with the impedance based analyser. Striking differences were seen in HCT MCVs MCH and MCHC of EGBE treated rats. While a dose and time dependant increase in HCTs was recorded early after treatment (followed by a decline as haemolysis occurs) using the impedance based analyser a dose-dependant decrease was consistently recorded using the laser-based analyser.

By 24 hr HCT significantly decreased in all three dose groups and whichever the analyser used. The impedance based analyser also detected marked doses related early increases in MCVs followed by a decline to near normal by 24 hr after EGBE treatment. In contrast a small decrease in MCV was detected with the laser-based analyser. PCV confirmed the results obtained from the impedance-based analyser showing an early increase in PCVs followed by a decline as haemolysis of RBCs occurs.

In the 125 and 250 mg/kg dose groups, changes in MCHs recorded by both types of instruments were the same. However 4 hr after the high dose, a much greater increase was seen in MCHs from the impedance-based than from the laser-based analyser. Finally, while the laser-based analyser recorded a dose-dependent increase in MCHCs the impedance-based analyser recorded a dose-dependent decrease in MCHCs in the same blood samples.

This study demonstrated the importance of the analyser used to determine the early effects described for EGBE haematotoxicity: increase of HCT and MCV. The impedance-based analyser is therefore more appropriate.

EGBE (99 % pure) was administrated via oral route to groups of male SD rats at doses of 50 – 100 - 250 and 500 mg/kg (CMA, 1990). Blood samples were collected at 0.5 – 2 and 4 hr after treatment for various analysis:

- Cell morphology was examined by microscopy and red cell indices were recorded: RBCV and Hb concentration distribution. Plasma and urine Hb concentrations were also measured.
- The measure of the erythrocyte density distribution was performed. This parameter reflect changes in the volume of the red cells and the profile of the changes (entire population or a fraction of them).
- The ATP content of the red cells was determined to assess the ability of EGBE to perturb the energy state of the RBC and consequently the function of ionic pumps.
- Effects on red cell lipid auto-oxydation was measured to see if haemolysis could be produced by a mechanism of peroxidation of unsaturated fatty acids of the cell membrane, MDA (malonyldialdehyde) served as a marker of lipid auto-oxidation.
- Viscosity and deformability of RBC was also measured after treatment.

The morphology of RBC showed a dose related trend to the formation of spherocyte. For RBC indices, the MCV frequency distribution curve broadened significantly. The mean cell volume increased from 53.8 fL to 86.9 fL. The frequency distribution of MCHC in contrast narrowed, and the mean value decreased from 35.2 gm/dl to 25.1 gm/dl. Similar data were obtained for all animals studied, and the MCV, MCHC, RDW and HDW values, as well as the other indices showed similar trends depending on the dose of EGBE administered and time of exposure prior to the sampling of blood. The red cell MCV increased steadily whereas the MCHC decreased as a function of the dose of ingested EGBE, suggesting an increase in cell water content. The mean value of MCV increased with increasing dose of ingested

EGBE. The EGBE-treated animals had significantly higher MCV than the control group. The dose-response relationship noted for MCV was most apparent for 500 mg group which showed a significantly higher mean MCV values among the EGBE-treated rats as compared with the control group. Erythrocytes from the control animals had higher MCHC values than all EGBE-treated animals, with higher doses of the glycol ether resulting in lower MCHC values. No clear dose response relationship was observed for other parameters. Using a 2-way ANOVA with time and dose as factors, a significant difference between 50 and 500 mg/kg dose group was observed in the following parameters: MCV, MCHC, HCT, RDW, HDW, Hb and RBC at 0.5 and 2 hr observation times. Haemoglobin in the plasma and urine was demonstrated 2 hours after EGBE administration. Dose-dependency was demonstrated 2 hours after the EGBE administration.

The median density of rat RBCs treated with EGBE decreased as a function of the dose of ingested compound. These changes in the profile indicated an increase in cell volume and a decrease in cell Hb concentration (perhaps as a result of an increased water cell content). There was no specific effect on any subpopulation of cells according to the density profiles.

No differences in the RBC ATP levels were seen in EGBE treated animals.

The MDA concentration decreased to about 50 % regardless of the administered dose. The decline in MDA concentration is suggestive of the arrest of autooxidative processes in the cells by EGBE or its metabolites. There was no evidence for an increase in membrane lipid peroxidation.

An increase of 72 % of the plasma viscosity was seen in treated animals (at dose of 500 mg/kg EGBE). At equal haematocrit of 40 %, the mean blood viscosity of the rats that received 50 and 100 mg/kg was 2 to 3-fold higher than that of he control rats. The mean viscosity of the rat that received 250 and 500 mg/kg was lower than that at the other two doses, but higher than that of control rats.

The effects of a daily dose of EGBE on haematological parameters were assessed in rats (Ghanayem *et al*., 1992). EGBE (purity 99 %) was administrated by gavage to groups of male Fischer 344 rats (10-14 weeks old – 6 rats/group). Five groups of animals received 125 mg/kg EGBE during 1, 2, 3, 6 or 12 consecutive days. Twenty four hours after the last dose animals were killed and 2 blood samples were collected, one with EDTA/dipotassiom salt as anticoagulant and the other with an acetate citrate dextrose (ACD) solution (to be used for the determinaion of blood ATP concentration). Livers and spleens were collected and weighted. Blood samples were analysed for the determination of RBC count, HB, HCT, MCV, MCH, MCHC and platelet counts. In addition PCV were determined on all blood samples. Other mechanistic studies were performed both *in vitro* and *in vivo*.

Some studies were performed in parallel to try to draw a mechanism of action of RBC toxicity:

- Experiment 1: Haematotoxicity of EGBE in pretreated/recovered rats *in vivo* was assessed: one group of rats received 125 mg/kg/d for 3 consecutive days and another group of untreated rats served as controls. Animals were then allowed to recover for a period of 7 days. Treated and untreated rats were divided into 3 groups, the first receiving only water, the second and the third receiving EGBE at doses of 125 and 250 mg/kg respectively. Blood samples were collected 2, 8 and 24 hr after dosing (the same analysis than the previous described study were performed). Animals were killed after blood collection and the spleen was collected and weighed.

- Experiment 2: The effects of BAA on blood from EGBE pretreated/recovered rats were also studied *in vitro*. Rats were treated with 125 mg/kg for 3 concecutive days and allowed a 7-day recovery period. Blood was incubated with BAA at concentration of 0 – 0.5 – 1 or 2 mM for 0.5 – 1 – 2 or 4 hr. At the end of the incubation period, PCV and blood ATP concentrations were measured.

- Experiment 3: The effects of EGBE in bled/recovered rats *in vivo* were also studied. Rats were bled and allowed a 7-day recovery period. After this period, treatments with 250 and 125 mg/kg via oral route were administered. Blood samples were collected 2, 8 and 24 hr after dosage and analysed as described below. After termination, the spleen was removed and weighted.

- Experiment 4: Effects of BAA in bled/recovered rats *in vitro* were also assessed. Rats were bled and allowed a 7-day recovery period. Animals were then killed and blood collected and pooled. The effects of BAA on PCV and ATP were investigated as described in the previous experiments.

Following repeated exposure (experiment 1), haemolysis evidenced by a decrease RBC, HB and HCT was seen and become more pronounced following the third day of dosing but gradually recovery was observed thereafter. With continued repeated exposures to EGBE beyond day 3, rats exhibited a gradually increase in RBC and HB and approached pre-treatment levels by day 12. HCT followed a similar pattern as RBCs and HB as dosing continued. On the other hand, MCV increased as the dosing regimen was extended to 6 days and declined to remain above control levels throughout the 12 day study. ATP concentrations, MCV and the number of reticulocytes increased after 6 days and then declined by day 12. Reticulocytosis and increased ATP concentration were evident after the second day of dosing and persisted in EGBE treated rats. The relative weight of the spleen increased to a maximum after 6 days from which it declined between days 6 and 12. Relative liver weight was minimally affected by treatment and following a moderate decline on day 3 and 6, it was increased on day 12 compared to controls.

In the second experiment, EGBE pretreated/recovered rats were found to be less sensitive to the haemolytic effects of EGBE than control rats. The RBC and HB declined in a dose and time dependent manner, moreover there was a minimal decline of these parameters in rats pretreated with the low dose of EGBE. HCT increased at 2 hr and remained near or slightly below controls in EGBE pretreated/recovered rats. In contrast, rast which were not previously exposed to EGBE exhibited an increase in HCT early after treatment which later declined in a dose and time dependent manner. Treatment of pretreated animals with the high EGBE dose (250 mg/kg) caused more pronounced haematotoxicity than the low dose (125 mg/kg), this effect remained less than that observed in untreated rats of the same dose level. Pretreated animals were also less sensitive to the increase in MCV and ATP depletion caused by EGBE than non exposed rats. In addition the number of reticulocytes was significantly higher in rats pretreated with EGBE than in controls. Comparison of the relative spleen weight showed that the increase caused by EGBE is less in pretreated animals than in untreated.

In vitro (experiment 3), blood from pretreated rats was significantly less sensitive to BAA than untreated rats: increase in PCV was greater in untreated rats than in treated rats. Similarly ATP concentrations in blood showed that pre-treatment decrease the susceptibility of erythrocytes to the effects of BAA *in vitro*. Both effects of BAA became more dramatic as the concentration of BAA and the incubation time increased.

Bled/recovered rats (experiment 4) were significantly less sensitive to the haemolytic effects of EGBE than controls. The RBCs and HB declined in a dose and time dependent manner, however the decline in bled/recovered rats treated with the 125 mg/kg dose was minimal. HCT increased at 2 hr and remained near pre-treatment levels in bled/recovered rats. In contrast, EGBE administration to controls resulted in an early increase in HCT and then declined in a dose and time dependent manner. Although the number of reticulocytes in bled/recovered rats prior to the exposure to EGBE was significantly higher than in the corresponding controls. Treatment of bled/recovered animals with the high dose of EGBE caused more pronounced haematotoxicity than the low dose, however these effects continued to be less pronounced than those observed in control rats receiving the same EGBE dose. Comparison of the relative spleen weights demonstrated that there was a significantly greater increase caused by EGBE in control rats compared to bled/recovered rats.

In vitro, blood obtained from bled/recovered rats was less sensitive to BAA–induced increases in PCV and ATP depletion. These effects were more pronounced with high BAA concentrations and with longer incubation times.

Some studies tried to demonstrate that a low dose of EGBE administrated a few days before the administration of a lethal dose of EGBE can protect from haematological effects and then from death (Sivarao and Mehendale, 1995). In these studies, 3 groups of 12 female SD rats were treated orally with EGBE 500 mg/kg for groups 1 and 2 whereas group 3 served as controls and was administrated with distilled water. On day 7 groups 1 and 3 were administrated LD50 dose of EGBE (1500 mg/kg) while group 2 received distilled water. Haematocrit of all rats was monitored on a daily basis as an index of toxicity. Rats were observed 14 days.

The effect of increasing the time gap between pre-treatment and lethal challenge on animal survival was studied by pretreating rats with 500 mg/kg EGBE on day 0. Rats were randomly divided into 3 groups (n=10, 8 and 6). On day 7, 14 and 21 days, one group of these rats received a single administration of 1500 mg/kg. Rats were observed twice daily for 14 days.

In vivo effects of pyrasole (ADH inhibitor) were tested with about the same experimental protocol (7 rats in each group): the treatment group received pyrasole 250 mg/kg one hour before treatment with 500 mg EGBE. HCT were recorded before pyrasole administration, 24 hr following EGBE pre-treatment and on day 7 of the experiment.

In these studies, the *in vitro* haematotoxicity of EGBE was also assessed. Dose response curves were constructed by incubating 1 – 10 mM of BAA in saline to rats blood. PCV (Packed Cell Volume – a marker of haematotoxicity found to be specific to BAA) were determined at various time points (1 - 4 hr). PCV are expressed as percent of controls.

Another experiment was carried out to investigate if the loss of autoprotection *in vivo* could be related to a corresponding loss in resilience of RBC *in vitro*. Rats were administrated a single dose of 500 mg/kg EGBE following which they were randomly divided in 2 groups of 4 rats each. On 7 and 21 days one of the groups was killed and blood was incubated with BAA and PCV was determined in different concentrations of BAA.

To test the hypothesis that newly formed RBCs are primarily responsible for the EGBE induced autoprotection, bled/recovered rats were used. Rats were bled 2.5 ml for 3 days. Blood letting was followed by a 7-day period of recovery. Recovery was ensured by the return of depleted haematocrit values to pre-bled levels. Control rats underwent a sham treatment without blood letting. Both the bled/recovered rats as well as their sham treated controls were challenged with a lethal dose of EGBE (1500 mg/kg), and their survival was

recorded, twice daily for 14 days. Incubation studies with BAA, were also conducted using RBC from these rats, to corroborate the *in vivo* studies.

Moderately toxic symptoms were seen with the "protective" dose of EGBE: ataxia, haematuria, paleness of skin and morbidity. Haematocrit values fell to 18.6 twenty four hr after treatment. Recovery was rapid. HCT reached control values level by 7 days. Rats received pre-treatment were fully protected against the subsequent lethal challenge with 1500 mg/kg EGBE while "non protected" rats experimented high (> 90 %) mortality. Survivors showed an average HCT of 13.5 whereas the "protected" rats experienced a much lesser fall in their HCT with no mortality.

Regarding the effects of pyrasole pre-treatment on autoprotection, the initial haemolytic episode due to EGBE pre-treatment was somehow attenuated (HCT recorded 24 hr after EGBE pre-treatment did not fall in the pyrasole treated rats) but following the administration, rats experienced profound ataxia that lasted several hours more than observed in control rats. Following the lethal challenge, pyrasole pretreated rats suffered high mortality (87 %).

With increasing time between the pre-treatment and the lethal dose, the percentage of survival decreased indicating that the autoprotection is gradually lost with time.

RBC isolated after EGBE pre-treatment showed a significantly higher susceptibility to BAA induced swelling than the cells isolated 7 days after EGBE treatment indicating that the resilience decrease with time.

When bled/recovered rats were challenged with lethal dose of EGBE, 87 % survived in sharp contrast to the 6 % survival of the corresponding sham treated controls. These findings suggested that newly formed RBCs were innately resistant to the haemolytic action of BAA.

Morphological changes of rat erythrocytes after administration of EGBE *in vivo* or BAA *in vitro* were assessed (Udden, 2000). Fisher 344 rats were dosed orally with 125 and 250 mg/kg EGBE. Blood samples were collected before and 30 minutes, 1 hr, 2 hr and 4 hr after administration. Approximately 75 µl of blood anticoagulated with EDTA was collected for microhaematocrit determination, fixation and for preparation of blood smears on glass slides.

For *in vitro* studies blood samples EDTA anti-coagulated were exposed to 1 or 2 mM BAA. Samples were taken at intervals for determination of a spun haematocrit, preparation of blood smear and fixation. Washed red blood cells were prepared by centrifugation followed by removal of the plasma and buffy coat and re-suspension in buffer. Cells were washed 4 times and then re-suspended to 50 % haematocrit. BAA was added to achieve a final concentration of 1 or 2 mM.

Table 4.62: *In vivo* and *in vitro* results

In vivo results	*In vitro* results
30 min / 125 mg/kg Erythrocytes with mouth shaped central pallor or stomatocytes. 2 hr / 125 mg/kg Increase of the stomatocytes nb and increase of sphero-echinocytes. 4 hr / 125 mg/kg Prevalence of stomatocytes and spherocytes 1 hr / 250 mg/kg Impressive spherocytosis HCT increased in a dose dependant manner.	30 min / both doses Cupping and spherocytic changes more pronounced after 1 hr similar than EGBE administrated PO. 2 hr / 1 mM Most of RBC were spherocytes. Wright stained: spherocytosis but only minimal stomatocytose. Sphero-echinocytosis was frequently observed after 1-2 hr of incubation. Effects were more marked with 2 mM BAA. Washed erythrocytes incubated with 1 or 2 mM BAA demonstrated haemolysis with ghost formation and agglutination. Increase of HCT comparable to EGBE treated animals (1 hr / 125 mg/kg equivalent to 1hr / 1mM BAA).
	No changes in human erythrocytes.

The difference between males and females Fischer 344 rats after exposure to an oral dose of EGBE was assessed (Ghanayem *et al.*, 2000). Fischer rats (12-16 weeks old) received an oral dose of 250 mg/kg EGBE (purity > 99 %). Control rats received 5 ml water/kg. Haematological parameters were analysed after termination (RBC – WBC – HB – HCT – MCV – MCH – MCHC – PCV). Blood smears were prepared from all samples. Morphological changes were evaluated by light microscopy.

The effects of BAA were also evaluated *in vitro*. Blood samples were exposed to concentrations of 0.25 – 0.5 and 1 mM. Samples of blood were also incubated with physiological saline to serve as controls. PCV was determined at 0.25 – 0.5 – 1 – 2 and 4 hr after incubation.

EGBE resulted in early swelling of erythrocytes as evidenced by an increase in HCT and MCV. In comparison to males, female rats exhibited a greater increase in MCV at all time points. However HCT declined in both genders at 8 and 24 hr after dosing. At 4 and 8 hr after dosing, RBC, HCT and HB were significantly lower in female rats than in males, however the

difference in these parameters were not statistically significant at 24 hr. This reflects a faster onset of erythrocytes haemolysis in female after dosing compared to males. This is also confirmed by the greater increase of the relative spleen weight in females compared to males. Other haematological parameters were also consistent with a faster onset of haemolysis in female rats. In parallel reticulocyte counts were greater in females than in males at all time points, indicating a faster regenerative response. Morphological alterations were seen in blood smears prepared from rats at 4, 8 and 24 hr after EGBE administration. At 4 hr after administration blood smears exhibited evidence of erythrocyte destruction, schistocytes, stomatocytes, ghost cells and other deformed erythrocytes. A decrease in the nb of RBC and absence of schistocytes with hypochromic RBC remaining at 8 and 24 hr was also observed. Aggregation of RBCs was evident by the formation of rouleaux at 24 hr after administration. In males alteration in RBCs were quantitatively similar than in females but these changes were observed more frequently and developed at a faster onset in female rats.

EGBE (purity > 99 %) was administered by gavage to groups of Fisher 344 rats at doses of 250 mg/kg for 1, 2 or 3 consecutive days (Ghanayem *et al.*, 2001). Animals were sacrificed 24 or 48 hr after the last dose. Blood samples collected at termination were analysed for RBC, WBC, HB, HCT, MCV, MCH, MCHC and platelet count. In addition PCV were determined on all blood samples. Erythrocytes morphological changes were evaluated by light microscopy. A complete necropsy examination was performed on all rats. The following tissues and organs were collected and examined: sternum, femur, vertebral column, tail, heart, lung, liver, spleen, kidneys, left eye and brain.

Severe acute regenerative anaemia in both sex in all treated animals were seen. However the progression of haemolytic anaemia as a function of the number of days of exposure varied and suggested a faster onset of haemolysis in females. After 24 hr following a single dose, the haematological parameters were relatively similar in both males and females except for the reticulocyte count. Female rats exhibited approximately a 2 fold increase in reticulocytes with a minimal increase in males. In addition, haemolysis was more severe in females at 24 and 48 hr after the second daily dose, as evidenced by the continued decline of the haematological parameters at these time points. Reticulocyte counts in females doubled and quadrupled at 24 and 48 hr after treatment respectively. The progression of haemolytic anaemia was also more severe in females after the second daily exposure. At 24 hr after the third dose, the haematological parameters calculated as percentages of controls, were relatively similar in both sexes of rats and were indicated severe regenerative anaemia.

Significant morphological changes in erythrocyte were also observed in treated animals. Slight macrocytosis with moderate rouleaux formation and stomatocytosis were observed in female rats at 24 hr after a single dose. These morphological changes became progressively more severe as dosing continued and included the occurrence of occasional schistocytes and ghost cells and an increased number of reticulocytes (micronuclei). Erythrocyte morphological changes were qualitatively similar in male rats after 1 or 2 doses and were most severe after 3 daily doses of EGBE.

Macroscopic abnormalities were noted in both males and females: watery blood, yellowish discoloured livers, dark brownish kidneys and multiple petechial haemorrhages on the sclera. In females, only bluish discoloration was noted on the tip of the tail. In the 2 females that died within 24 hr after the first dose, thrombosis was noted only in submucosa of the most anterior section of the nasal cavity. Other lesions noted in these rats included focal hepatocellular necrosis and renal tubular necrosis associated with haemoglobin casts. Thrombosis was evident in females sacrificed 24 hr after a single dose. The vascular thrombi were occlusive and consisted of amorphous granular material or partly organized fibrin with varying numbers

of red blood cells. Widespread thrombi formation noted in the ocular ciliary processes and in the limbus associated with retinal haemorrhage and necrosis were suggestive of an ischemic-infarctive process. Thrombosis was also observed in the submucosa of the most anterior section of the nasal cavity, mostly located in the nasal and maxillary turbinate as well as the septum. Evidence of EGBE-induced thrombi was also observed in the pulmonary small caliber vessels and the right ventricle of the heart in association with foci of myocardial necrosis and inflammation. In agreement with earlier findings, thrombosis also affected the dental pulp of the incisors in conjonction with focal stromal and odontoblastic coagulative necrosis. Lesions consistent with acute infarction were noted in the coccygeal vertebrae and femur, where histopathological changes included intramedullary thrombosis, and necrosis of bone marrow cells, bone-lining cells, and the cortical and trabecular osteocytes. Dying cells exhibited nuclear pyknosis and/or fragmentation to complete loss of nuclear staining, often appearing as ghost cells. Soi¯ne of the lacunae were empty. The changes in the femur were usually confined to the diaphysis (medullary cavity bone marrow and trabecular bone) and inner one-third of the cortex, while in the coccygeal vertebrae, the metaphyseal bony trabeculae and epiphysis were also necrotic. In rats sacrificed 48 hr after two or three daily EGBE doses, bone marrow regenerative erythroid hyperplasia was also observed in addition to the consistent presence of the fore mentioned lesions. Other noted changes included multifocal hepatocellular necrosis in the liver, extramedullary haematopoiesis in the spleen, and haemoglobinuric nephrosis.

In male rats treated with EGBE for one or two days, pathological changes were limited to those associated with haemolysis, consisting of renal haemoglobinuric nephrosis and splenic extramedullary haematopoiesis. However, the 3-day EGBE dosing induced thrombosis and tissue infarctions similar to those seen in female rats. In particular, disseminated thrombosis was noted in the longs, nasal submucosa, liver, heart, bones, tail and teeth. Degeneration, necrosis and/or infarction were noted in the heart, eyes, teeth and bones.

Three groups of 3 female GP were treated with a unique subcutaneous dose of EGBE at 0.1 – 0.5 or 1 ml/kg (Unilever, 2001). Blood samples were taken before dosing and on day 1, 2 and 5 after dosing prior termination. On blood sampled, the following parameters were measured:

* Haematology: PCV – Hb – RBBC – WBCC – MCHC – MCV – MCH and reticulocytes

* Biochemistry: K^+ - Mg^{2+} - Ca^{2+} - free haemoglobin

At necropsy the following organs were examined macroscopically: liver, spleen and kidneys.

No effects were seen on any measured parameters.

In a recent publication (Koshkaryev *et al.*, 2003), the mechanism of EGBE-induced haemotoxicity was studied. Male and female Fischer 344 rats were exposed to two, three or four daily doses of EGBE (purity > 99 %) at 250 mg/kg. Blood samples were taken from rats before sacrifice on days 2, 3 and 4 (groups 2, 3 and 4 respectively), each time 2h after administration and from group 5 before sacrifice 24 days after the last dosing. After isolation, RBC were subjected to determination of RBC flow properties on the day of collection (aggregability, adherence and deformability).

RBC aggregability was not affected by the treatment. The effects on deformability were inconclusive. The main effect was an increase of the RBC adherence to Extra Cellular Matrix.

There were no differences between male and female. The adherence was highest at day 2 (the first to be examined) after which it decreased sharply with time.

Further investigations were performed to elucidated the mechanism of thrombosis formation (Nyska *et al.*, 2003). Eyes from female Fischer 344 rats exposed to EGBE were studied histologically and immunohistochemically using specific monoclonal antibodies to determine the *in situ* protein expression of vascular cell adhesion molecule-1 (VCAM-1), endothelial intercellular adhesion molecule-1 (ICAM-1), and P-selectin. Groups of female rats (4 per treated groups, 8 for control group) were treated with 250 mg/kg EGBE (100 % purity) in drinking water for 2, 3 or 4 consecutive daily treatment. Sacrificeof the animals was done 2 hours after the last scheduled treatment.

Histopathologically, lesions in the retina consisted of retinal thrombosis, haemorrhage, degeneration and necrosis. Changes were mainly located in proximity to capillaries and consited of multifocal haemorrhage within the retinal pigment epithelium (RPE) and choriocapillaries and between the RPE and photoreceptors (leading to retinal detachement) and the photoreceptors and inner and outer nuclear layers. Haemorrhage in the RPE was associated with degeneration, exfoliation, loss of epithelial cells and presence of neutrophils. Haemorrhage whitnin the receptor layer caused organizational disruption and loss of photoreceptor cells. In the inner and outer nuclear layers, the haemorrhages and thrombosis were associated with degeneration and loss of cells resulting in thinning, disorganisation, and fusion of these layers. Thrombi were also noted within blood vessels of the ciliary processes, in the limbus and in the retina. Positive VCAM-1 expression was observed only in eyes of rats exposed to 3 and 4 doses of EGBE (mainly in the iris, ciliary processes and retina). Only weak effects were seen after one or two doses. No effects were seen for ICAM-1 and P-selectin.

Studies with metabolites

The effects of BAA on human and rat blood cells were investigated (CMA, 1993). Human erythrocytes were obtained from healthy volunteers and rats erythrocytes from Fischer 344 rats. Red cells were isolated and incubated in the presence of 0, 0.2 or 2mM BAA for 1-4 hr at 37 °C. Erythrocyte suspensions were sampled at intervals for determination of microhaematocrit, Hb, RBC count and percentage of haemolysis. Morphology of RBC was also observed. The deformability of erythrocytes was assessed.

A 30 % haemolysis was demonstrated in rat erythrocytes when incubated with 2 mM. For 0.2 mM only little haemolysis was seen (when incubated for 6 hr or longer, mild haemolysis was detected). Less than 1 % haemolysis was seen in human erythrocyte when incubated for 4 hr with 2mM. For deformability, exposure of rats erythrocytes to 2 mM for 1 hr resulted in a dramatic decrease in red blood cell deformability (increase in cell rigidity). A longer incubation (4 hr) in the presence of 0.2 mM BAA also produced significant changes in the deformability curve in the absence of significant haemolysis. No deformation was seen in human erythrocytes exposed to 2 mM for 4 hr.

Distinct changes in the morphology of rat RBCs exposed to BAA were observed. Rat RBCs incubated in buffer alone for 4 hr were found to be mostly normal appearing discocytes with occasional crenated cells identified. Incubation in the presence of 0.2 mM BAA resulted in striking spherocytic and spheroechinocytic RBCs. Also apparent was a tendency for BAA incubated RBCs to adhere to each other. Incubation with 2 mM BAA resulted in dramatic haemolysis with the appearance of RBC ghosts (which appear to be mutually adherent).

Another finding was the presence of precipitates of rat Hb. Human RBC morphology was unaffected by a 4 hr incubation in 2mM BAA.

Effects of BAA on RBCs from 10 mammalians were studied *in vitro* (Ward *et al.*, 1992 ; Ghanayem and Sullivan, 1993). To validate *in vitro* finding, the haematotoxic effects of EGBE was compared *in vivo* using one representative from each of the sensitive and less sensitive animals. Various animals were used to collect blood samples (see table 4.63)

Table 4.63: Effects of BAA on blood from different species

Species	Strain	Average age
Mouse	B6C3F1	9 wks
Rat	F344	10 wks
Hamster	Syrian (LVG:LAK)	27 wks
Rabbit	New Zealand	37 wks
Guinea pig	Hartley	28 wks
Canine	Hound	18 wks
Feline	Dom. Short Hair	7 yrs
Pig	Poland China	2 yrs
Baboon	P. cynocephalus	5 yrs
Human		Adult

Erythrocytes were incubated with BAA at concentrations of 0, 1 or 2 mM. Complete Blood Counts (CBCs) and packed cell volume (spun haematocrit) were measured at 0, 1, 2 and 4hr after incubation. CBCs included white blood cell count, platelet count, RBC, HB, HCT, MCV, MCH and MCHC.

Male rats and male Guinea Pigs were administrated EGBE at dose of 250 mg/kg. Blood samples were taken at 2, 8 or 24 hr after treatment for rats and 1, 2, 4, 8 hr for Guinea Pigs. CBC and spun HCT were performed at each time point.

All three rodent species were relatively susceptible to the effects of BAA: It caused a time and concentration dependant increase in MCV and HCT. At high concentrations, swelling of RBCs was occasionally followed by a decline in the number of RBCs. Haemolysis was most significant in mouse blood incubated with the high concentration of BAA. The 2 mM dose of BAA produced greater than 45 % increase above the corresponding control in MCV at 2 hr, with a 6 % decrease below control in the RBC count. At 4 hr after incubation, MCV declined from the 2 hr levels but remained significantly higher than controls. The number of RBCs decreased but 30 % below the corresponding control levels. HB concentration declined in a manner parallel, but to a lesser extend, to the number of RBCs. MCV increased by greater than 15 % and 35 % above controls after incubation with 1 or 2 mM BAA for 4 hr. In addition, HCT increased in parallel to MCV. Although significant swelling of RBC occurred, no significant haemolysis was observed in hamsters.

For guinea pigs, there was no significant changes in MCV, HCT, RBC count or HB concentration in the blood of animals with vehicle, 1mM or 2mM BAA over the 4 hr study.

Blood from rabbits exhibited an increase of MCV and HCT in a concentration and time dependent manner. At the 2 mM BAA, a greater than 20 % increase in the MCV was observed above the corresponding control at 2 hr which further increased to 39 % above control levels at 4 hr. HCT increased in a manner parallel to MCV and was time and concentration dependent. Despite this extensive swelling, the RBC count and HB concentration remained essentially unaffected throughout the incubation.

Two species of carnivores were investigated for the haematological effects of BAA. Both canine and feline RBCs were found to be essentially insensitive to BAA.

Pig cells exhibited no significant change in size or number.

Two species of primates were investigated: baboon and human. In baboon blood incubated with BAA, MCV increased in a time and concentration dependent manner approaching a 35 % above control increase after 4 hr incubation with 2 mM BAA. HCT increased in a fashion parallel to MCV and this increase was time and concentration dependent. The RBC counts declined slightly and this haemolysis was significant in blood incubated with either BAA concentration for 4 hr. In contrast, incubation of human blood with the high concentration of BAA demonstrated minimal or no effect. The changes in red cells size in the 2mM dose varied slightly from one human to another, with MCV increased of about 3.5 % above corresponding controls at 1, 2 and 4 hr. HCT also increased 2-4 % above controls in the 4 hr incubation period. The effects of BAA on MCV and HCT were not time dependent and were not statistically significant at any time point during the incubation. These minimal effects were not statistically significant and were not associated with any significant changes in RBC count or HB concentration.

In vivo, gavage administration of EGBE caused an early increase in MCV and HCT in rat blood followed by a decline as a function of time. These effects wee associated with haemolysis and a decline in HB concentration and the number of RBCs. MCH and MCHC were affected in a manner reflective of the changes in RBC and HB. In contrast to the effects in rats, EGBE caused no significant changes in any parameters of guinea-pigs. These findings were in full agreement with the effects seen in the *in vitro* experiments.

The effects of BAA on rat and human erythrocytes were also studied by Udden and Patton, (1994) and Udden (1994). Heparinised human blood was obtained from healthy volunteers and rat blood was obtained by cardiac puncture of male F344 rats. Erythrocytes were washed free of plasma, re-suspended in buffer to a packed cell volume of 10 % and incubated in buffer alone or buffer containing 0.2 or 2mM BAA (Shell Chemicals Development Co., purity 99 %) for up to 4 hr. BAA at 0.2 mM did not cause haemolysis of rat erythrocytes, but the 2mM concentration had a rapid effect resulting in ca. 30 % haemolysis after 4 hr. Addition of glucose to the medium gave no protection. In contrast, incubation of human erythrocytes for 4 hr in the presence of 2mM BAA resulted in no haemolysis over that slight amount (< 1 %) owing to incubation of the cells in buffer alone. Rat erythrocytes also demonstrated decreased deformability (or increased cell rigidity), using a nucleopore filtration technique, and an increased mean cellular volume in the presence of 0.2 or 2mM BAA, whereas these effects were not observed with human erythrocytes.

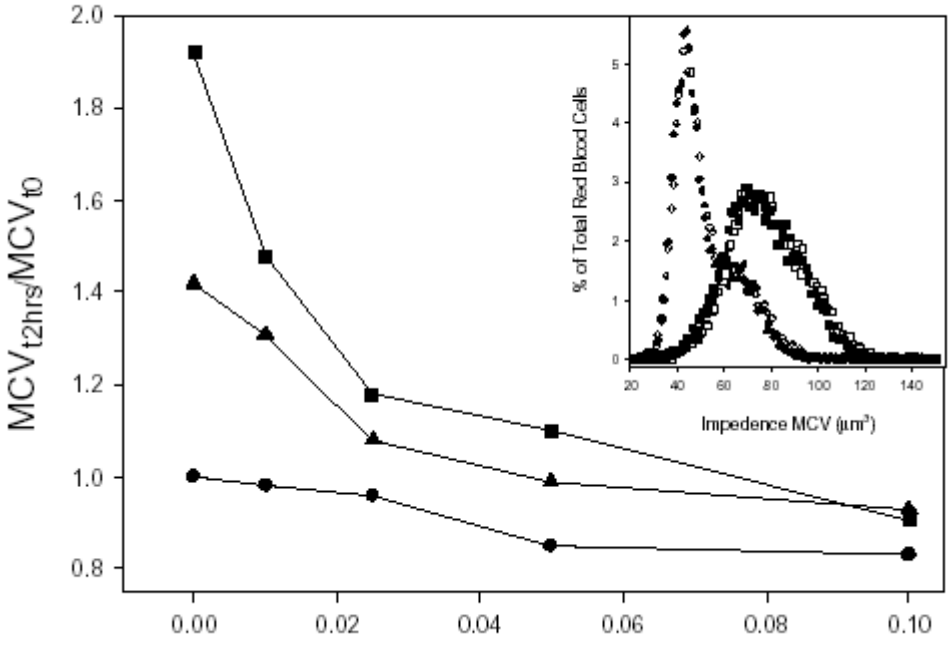
Study of the effects *in vitro* of BAA on human blood samples was extended to include heparinised blood obtained from 9 healthy adults (mean age 41.6 years, range 31 – 56; 5 men, 4 women), 9 older people (mean age 71.9 years, range 64 – 79; 5 men, 4 women), 7 patients

with sickle cell disease and 3 people with hereditary spherocytosis (Udden and Patton, 1994). Erythrocytes were washed free of plasma, re-suspended in buffer to a packed cell volume of 10 % and incubated in buffer alone or buffer containing 2mM BAA (Shell Chemicals Development Co., purity 99 %) for up to 4 hr. Erythrocytes from the younger healthy subjects showed no significant increase in haemolysis in presence of BAA compared to that observed in the incubations in buffer alone. There was a greater tendency for erythrocytes from the other volunteer groups to haemolyse more readily in buffer alone than erythrocytes from the younger healthy group. However, none of the groups showed an increase in haemolysis due to the presence of 2 mM BAA. Erythrocyte deformability was measured using a nuclepore filtration technique, but no changes due to BAA exposure was detected (Udden and Patton, 1994).

Mechanistic studies were performed by Udden and Patton, 2005 to elucidate the mechanism of BAA induced haemolysis. Red Blood Cells of Fischer 344 rats (9-11 week old) were added to the following culture medium: 10 mM TRIS, pH 7.4; 140 mM KCl and 10 mM glucose with or without 2 mM $CaCl_2$. The RBCs were incubated in the presence of BAA at 37° C. In some experiments the inhibitor charybdotoxin (inhibitor of the Ca activated potassium channel) was added 30 minutes before the addition of BAA.

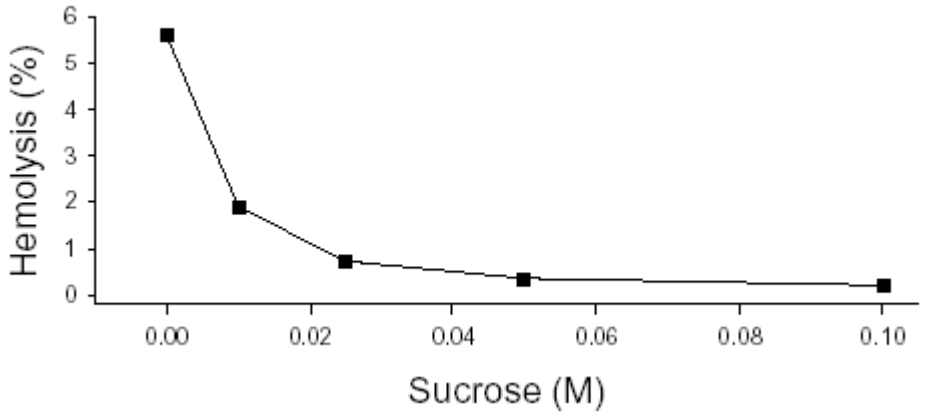
To determine the effect of osmolarity, RBC were incubated with 1 or 2 mM BAA for 2 hours in the presence of 0 – 0.1 sucrose. Results are summarised in the table 4.64.

Table 4.64: Effect of external sucrose on BAA-induced cell swelling



RBC was determined before (MCVt0) and after (MCV2hrs) a two hour incubation with 0 (●), 1 (▲) or 2 mM (■) BAA in the presence of 0-0.1 M sucrose. The ratio MCV_{2hrs}/MCV_{t0} is shown to demonstrate the relative decrease in cell swelling as a function of the increased external concentration of sucrose. The inset figure shows impedance size distributions determined by a particle counter for rat red blood cells incubated in buffer, buffer with 0.1 M

sucrose, 2.0 mM BAA, or 2.0 mM BAA with 0.1 M sucrose after 2 hour incubation and resuspension in isotonic saline without sucrose or additional BAA.

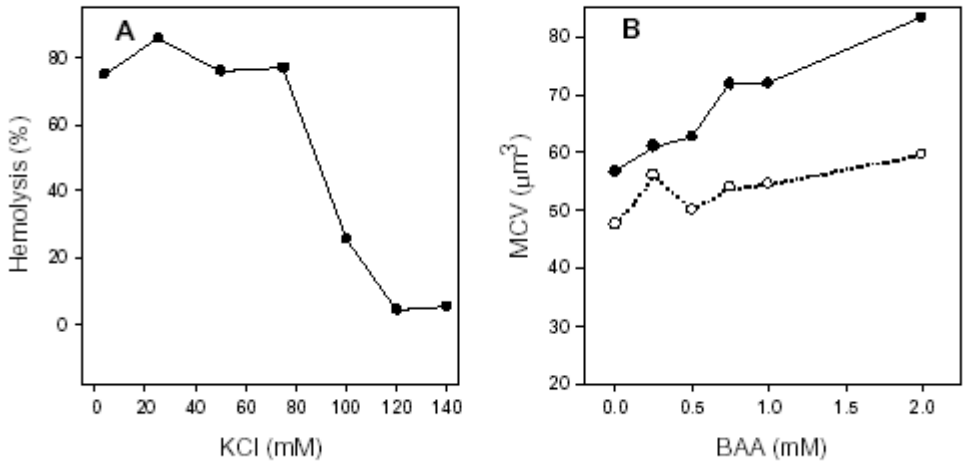


Haemolysis as a result of incubation with 2.0 mM BAA and 0-0.1 M sucrose.

Influence of K and Na content in the suspending medium was also assessed. Rat red blood cells were incubated for 4 hours in calcium free media with external sodium concentration varying from 0-140 mM. Potassium was added to achieve a total external cation concentration of 140 mM.Results are summarised in the figure 4.65.

Rat red blood cells were incubated for 2 hours in the presence of 0-2.0 mM BAA in low sodium (-o-) buffer containing 110 mM potassium and 30 mM sodium or normal sodium (-•-) buffer containing 140 mM sodium Potassium was added to achieve a total external cation concentration of 140 mM.Results are summarised in the table 4.65.

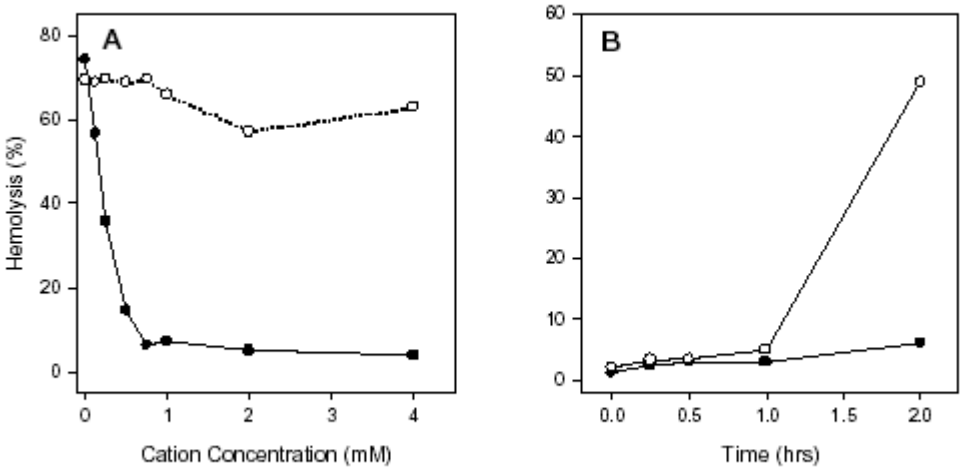
Table 4.65: Effect of replacement of external sodium with potassium on haemolysis and MCV changes induced by 2.0 mM BAA.



RBC were incubated in the presence of 2 mM BAA for two hours in the presence of 0-4 mM $CaCl_2$ (-o-) or $MgCl_2$ (-•-). Results are summarised in the table 3a. In another experiment

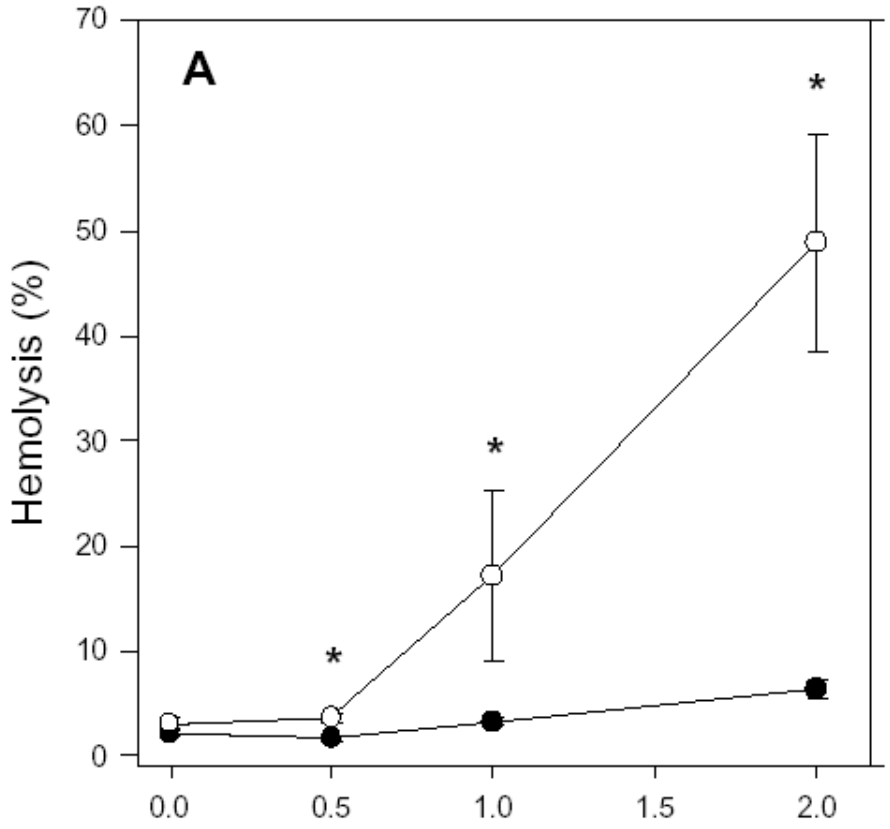
EGTA (Ca chelating agent) (-o-) was added to the medium containing 2 mM $CaCl_2$ (-•-). Results are summarised in the table 4.66.

Table 4.66: Effects of divalent cathions and Ca chelation on BAA-induced haemolysis



When rat red blood cells were incubated for 2 hours with BAA at concentrations between 0 and 2 mM there was a dose related increase in haemolysis and MCV when calcium was present (-•-) (Figure 4a). The dose response for haemolysis showed a greater effect of BAA when calcium was absent (-o-). However, a dose response effect for BAA for increased MCV was not apparent in the cells treated in the absence of calcium (Table 4.67).

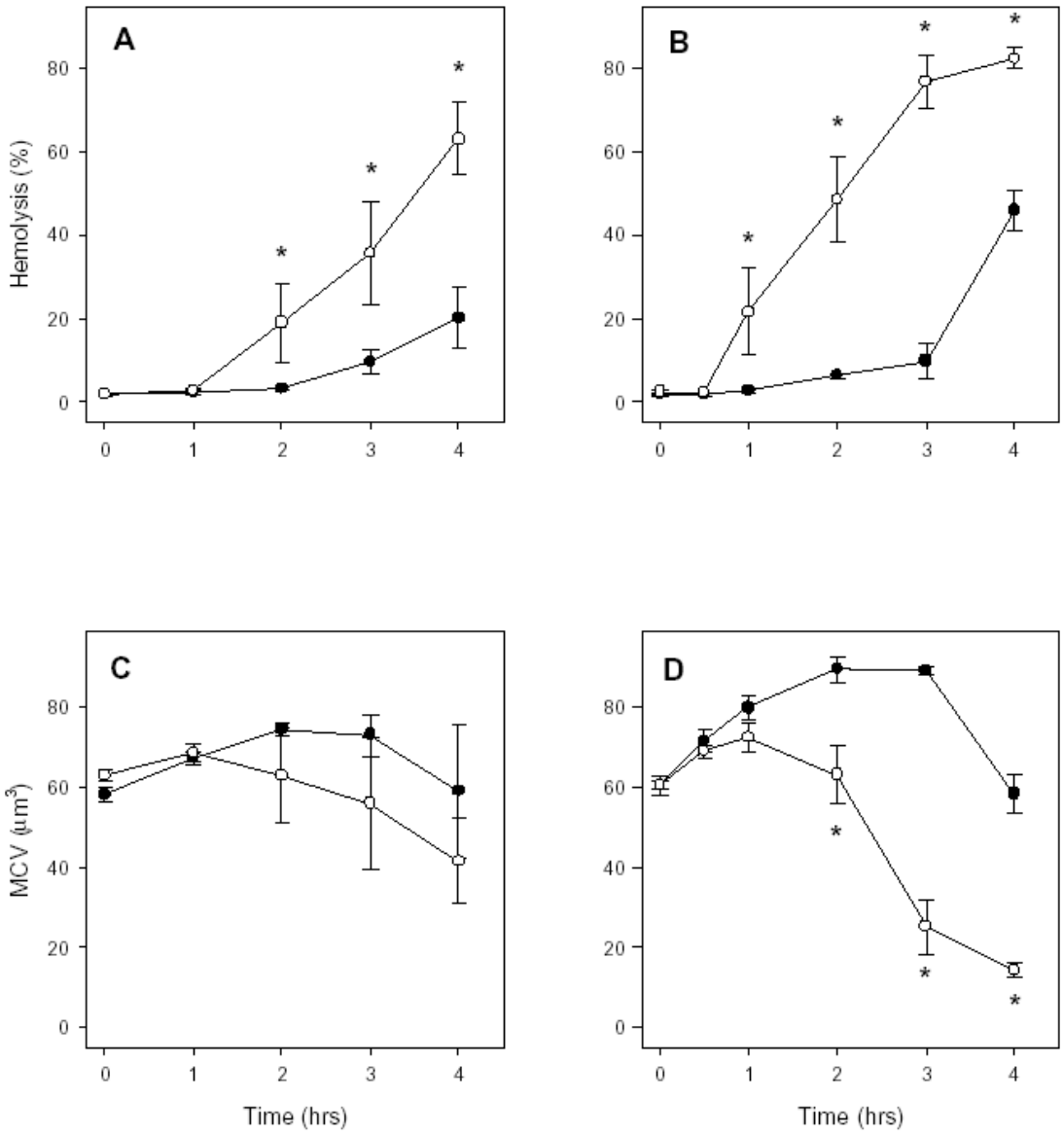
Table 4.67: Dose response relationship for BAA induced haemolysis and cell swelling



The morphology of rat red blood cells suspended in calcium free or calcium containing buffer was similar. Incubation with BAA in the presence of calcium produced spherocytes. In the absence of calcium, spherocytosis was more prominent as was ghost formation and fragmentation of the cells.

To confirm that the effect of Ca was to delay the onset of haemolysis, RBC were incubated with 1 (figures 68a and 68c) or 2 mM (figures 68b and 68d) BAA in the presence (-•-) or absence (-o-) of 2 mM Ca for four hours.

Table 4.68: Effect of Ca on haemolysis



These figures show that Ca delayed the onset of haemolysis caused by BAA. During the first hour of exposure, the initial increase in cell size did not depend on the presence of absence of Ca (fig c and d). At the onset of significant haemolysis more cells were destroyed when Ca was absent and the MCVof the remaining cells appeared to be much smaller.

An increased in RBC intracellular Ca was confirmed by measurements after exposure to 0-2 mM BAA in the presence of Ca for 4 hours.

To verify that effects were due to an activation of a specific Ca activated K channel (causing cells to lose K and prevent cell swelling – Gardos pathway), RBC were incubated with a specific inhibitor of this channel (charybdotoxin). Charybdotoxin in the absence of BAA did not cause haemolysis, but haemolysis was greatly increased in presence of BAA. When Ca was present in the middle containing charybdotoxin, the increase of haemolysis was

equivalent to the haemolysis seen in the previous experiment when there were no Ca in the middle (figures 4.68a and 4.68b).

From these experiments, the authors suggested that the mode of action of BAA begins with an influx of sodium that is not fully compensated by potassium loss and ends with osmotic lysis of the red cell because of an influx of water accompanying sodium. Our data also points to a role for calcium entry in the red blood cells.

Summary haematotoxicity

In studies performed *in vivo*, the same signs of toxicity seen in acute toxicity studies (LD50 studies) were recorded. Thrombosis were observed in various localisations sometimes leading to necrosis due to an infarction mechanism (tail, dental pulp, pulmonary, ocular).

Mechanistic studies have shown that EGBE causes haematotoxicity *in vivo* in rats and that BAA causes the same effects *in vitro* at very low concentration. When metabolic pathways leading to the formation of BAA were blocked, no effects were seen on RBC. It can be concluded that BAA is responsible of haematotoxicity *in vivo*.

Some species were very sensitive to EGBE- or BAA-induced haemolysis: rat, mouse, hamster baboon whereas other species were resistant to these effects: dog, guinea pig, pig, cat, rabbit and humans (30 x less sensitive than rats). In one study, dogs were very sensitive to EGBE but not to BAA.

In these studies, an increased sensitivity to haemolysis was seen in old animals and in females *in vitro* and *in vivo* with BAA, showing that the differences of metabolism between male and female could not explain totally sex difference.

In one study, it was demonstrated that the type of analyser was important to detect early changes in blood parameters (increase of HCT and MCV). An impedance based analyser was more sensitive than a laser based analyser.

In vivo or *in vitro*, haemolysis was due to a decrease of erythrocyte deformability due to erythrocyte swelling (this also explains the formation of thrombosis). Newly formed erythrocytes were more resistant than old ones. It was also showed that EGBE pre-treatment gave a relative "protection" against higher doses administered later. Moreover, a study (Lomonova and Klimova, 1977) showed that repeated exposure to EGBE, 3 hours a day, 6 days a week for 4 months was more haematotoxic than the exposure to the same dose of EGBE, 6 hours a day, 3 consecutive days a week for 4 month. This study demonstrates an adaptive mechanism of "protection" when animals have a period of recovery time before a re-exposure to EGBE.

The mechanism leading to erythrocyte swelling and loss of deformability is for the moment unknown. Apparently, there is no evidence of oxidative mechanism on erythrocyte membrane. Udden, 2002 showed that, at low doses of BAA in rats, the increase of Na^+ intra-erytrocytaire was not balanced by decrease in K^+. It has been demonstrated that Ca could also play a role in the mechanism. This mechanism lead to osmotic regulation causing an increase in the size and the cell volume of erythrocytes, a decrease of the density and of the deformability and an increase of the osmotic fragility.

In humans, slight effects were seen with doses of 8 mM and 4 mM of BAA *in vitro* (Ghanayem, 1989).

For the effects of EGBE in dog (and the absence of effects when treated *in vitro* with BAA), another mechanism could be involved in EGBE induced haemolysis.

4.1.2.3 Irritation

4.1.2.3.1 Skin

Studies in animals

Guinea pigs

Primary skin irritation was determined by application of the compound to the depilated abdomen of Guinea pigs at doses of 1, 5, 10 or 20 ml/kg (Eastman Kodak, 1981b).

Skin responses were compared with those seen in rabbits in the same study. A dose of 0.3 g/kg in rabbit lead to a moderate irritation whereas a dose of 4.5 g/kg in guinea pigs lead to a strong irritation.

Rabbits

A skin irritation study was performed according to the method described in the US code of Federal Regulations (Huntingdon Life science, 1979b). EGBE was administered on the abraded and intact skin of 6 rabbits during 24 hours under semi-occlusive conditions. After exposure, scoring was performed (according to the Draize method) immediately and 48 hr after the first reading.

At the first reading time, 5 out of 6 animals exhibited a slight to moderate erythema and 4 out of 6 exhibited a slight oedema. At the second reading time, 4 out of 6 animals exhibited a very slight to moderate erythema and 3 out of 6 exhibited a slight oedema.

Rabbit		24 hours		72 hours	
		Intact skin	Abraded skin	Intact skin	Abraded skin
1	Erythema	0	0	0	0
	Oedema	0	0	0	0
2	Erythema	1	1	2	2
	Oedema	0	0	0	0
3	Erythema	1	1	0	0
	Oedema	1	1	0	0
4	Erythema	2	2	1	1
	Oedema	1	1	1	1
5	Erythema	1	1	1	1
	Oedema	1	1	1	1
6	Erythema	1	1	1	1
	Oedema	1	1	1	1

According to Draize scoring.

In this study, EGBE can be considered to be slightly irritant to rabbit skin (primary irritation index: 1.5).

EGBE was only mildly irritating to rabbit skin when applied and left uncovered for a 4 hr period on rabbit skin (Bushy Run Research Center, 1980b). More prolonged exposure to the undiluted chemical (24 hr) can lead to moderate to severe irritation characterised by erythema, oedema and necrosis (as seen in acute dermal toxicity test).

EGBE and others glycol ethers were tested to assess their cutaneous irritation properties. (Zissu, 1995). Test substance (purity > 99 %) was administrated dermally to rabbits according 2 test methods for assessing cutaneous irritation: the EEC testing method and the Draize protocol. Three New Zealand white rabbits were used in the EEC protocol and 6 in the Draize protocol. 0.5 ml of the test substance was applied occlusively on the flank of each animal, 4 hr for the EEC protocol and 24 hr for the Draize protocol (for the later, EGBE was also applied on the second scarified flank). Animals were observed for erythema and oedema. At 72 hr, histopathological control of the skin at the site of application completed the macroscopic observation.

According to the EEC method, EGBE is classified irritant and according to the Draize method, EGBE is classified as severely irritant. These results were confirmed by histological observations. No detailed observations were available in this publication.

Within the framework of the elaboration of a new EEC directive covering all preparations, different classes of substances were tested in different dilutions according to the experimental protocols and interpretation criteria laid down in Annex V and VI, part ID of directive 79/831/EEC (Annex VI, 1983, Annex V, 1984) on dangerous substances. In Jacobs and

Martens, 1985 and 1987, EGBE and other ethylene glycols (purity > 99 %) were administrated dermally to rabbits according the EEC testing method. Five New Zealand white rabbits were used. 0.5 ml of EGBE was applied under an exposure chamber on the flank of each animal, during 4 hrs. Maximal mean erythema was 1.8 but the results per animal were very divergent. (From not irritating to very irritating). When diluted in polyethylene glycol 400 (50%), no sign of any irritation was observed.

Summary on skin irritation:

All the studies performed on rabbits and Guinea-pigs have shown that EGBE have caused moderate irritation (erythema and oedema) when applied occlusively on the skin for a period of 4 hours (in studies corresponding to the EU criteria for classification). If the substance was applied on scarified skin or for a longer period of time, signs of severe irritation sometimes leading to necrosis were reported.

Overall, in animals, EGBE can be considered to be a skin irritant. Classification R38 is needed.

4.1.2.3.2 Eye

Animal studies

Marked irritation (Erythema and oedema) was seen when rabbits were instillated with one drop of pure EGBE (Von Oettingen and Jirouch, 1931). The corneal reflex was absent after treatment.

In an old study poorly reported, EGBE produced only moderate irritation when 0.02 mL was instilled into a rabbit eye (Carpenter and Smyth, 1946).

Eye irritation was assessed after administration of EGBE into a rabbit eye, pure or diluted (1/10 and 1/100) (Hoechst, 1966). Animals were examined 1, 3, 7 and 24 hours after application.

For pure substance, corneal and conjuctival injuries were noted (severe redness and chemosis and corneal opacification). For the 1/10 dilution, only a slight conjuctival redness was seen. And for the 1/100 dilution, no effects were seen.

An eye irritation study was performed according to the method described in the US code of Federal Regulations (Huntingdon Life science, 1979a). Six animals were instillated with 0.1 ml of pure EGBE and observed during 72 hours. A scoring of the effects at 24, 48 and 72 hours according to the method of Draize was performed.

Effects on cornea, conjunctivae and iritis were seen in 5, 2 and 6 out of 6 animals at observation time of 24, 48 and 72 hours respectively and were not reversed after the 72 hr observation period. According to the EU classification system, EGBE could be considered as a severe eye irritant due to the irreversible effects seen at the end of the observation period although this period was only 72 hr. This classification rule is only relevant when no other data are available from other eye irritation tests for the period after 72 hours. This study cannot be relevant to support R41 classification.

Instillation of 0.005 mL of undiluted EGBE into rabbit eyes produced severe corneal injuries with iritis (Bushy Run Research Center, 1980b). Moderate corneal injury was observed when

0.5 mL of a 15 % aqueous dilution of EGBE was instilled into the eyes of rabbits and no injuries was noted for a 5 % aqueous dilution. No more details about this study are available, the value of 0.005mL reported in the study is quite surprising in relation with the effects observed and the other doses tested.

EGBE (99 % purity) was instilled in the right eye of 6 New Zealand white rabbits (Jacobs and Martens, 1987 "Evaluation of the enucleated eye test against the *in vivo* eye irritation test in the rabbit, Jacobs G. and Martens M." IHE-report 01/12/1987). - Parent, 1992). The test was performed in two phases. The test was performed according to OECD guideline 405 (version 1981). In the second phase, 3 other chemicals were tested and besides the clinical examination, corneal swelling was also measured.

Erythema, chemosis, iritis and corneal opacity were scored according to the Draize scores at 4, 24, 48, 72, 96 and 168 hr after treatment. The mean erythema, chemosis, iritis and corneal opacity values (average of the 24, 48 and 72 hour observation) are: 2. 59, 0. 78, 1.00 and 1. 33, respectively. The mean corneal upperlayer damage (loss of epithelium measured by fluorescein retention on the cornea using a hand-slit lamp) was 95% after 4 hrs. Pain response was measured and described as "a few blinks only, normal within one or two minutes; animals didn't squeal or rubbing the eyes". The substance caused marked pannus in all 6 rabbits.

EGBE, according to this study, should be classified as an irritant. (mean erythema score >2.5, iritis =1). Pain sensation after contact with the product seems to be low, what can lead to underestimation of the irritating properties. Studies with fluorescein retention revealed a 90% loss of the upperlayer of the cornea after instillation. Generally speaking, the pannus reported does not disappear in rabbits and remain through the lifetime. In contrast, pannus response when seen in humans is usually associated with chronic disease and is not an acute irritative effect. Pannus generally disappears in humans. For those reasons pannus was not considered in the past as an irreversible effect for EC-classification however it must be noted that under the GHS it will be considered as leading to a classification of "severe irritating".

The study was redone some years later under the same protocol (Jacobs, 1992). The authors came to nearly the same mean scores (average of the 24, 48 and 72 hour observation of: 2.47, 0.83, 0.83 and 1.73 respectively.), but since the mean erythema score and iritis score is lower than 2.5 and 1 respectively, no classification requirement is triggered.

Table 4.69: Irritation scores in the Parent, 1992 Jacobs, 1992 study

Time after end of application	4 hr	24 hr	48 hr	72 hr	96 hr
Mean score of conjunctivitis (3 max)	1.4	2.4	2.6	2.4	1.6
Mean score of chemosis (4 max)	1.7	1.0	0.8	0.7	0.2
Mean score of iritis (2 max)	1.0	1.0	1.0	0.5	0.2
Mean score of corneal opacity (4 max)	0.7	1.5	1.5	2.2	1.2
Mean surface of corneal damage (100 % max)	95	94	81	60	23

EGBE (99 % purity) was instilled in the eye of 3 rabbits (ECETOC, 1998). The test was performed according to OECD guideline 405 (version 1981) and GLP. Erythema, chemosis, iritis and corneal opacity were scored according to the Draize scores at 1, 2,3,7,14, 21 days

after treatment. The EC scores for erythema, chemosis and corneal opacity values (average of the 24, 48 and 72 hour observation) are 2.33, 2.78 and 2.33 respectively. Opacity resolved in all 3 animals althought very slight redness (score 1), resist in 2 of 3 animals and chemosis (score 1) in one animal. According to the scores a classification is needed as irritant.

Six New Zealand white rabbit were tested in a Draize irritancy test with about 100 chemicals including EGBE (purity > 99 %) (Kennah *et al*., 1989). The cornea, iris and conjunctivae were scored at 24, 48 and 72 hr and at 7, 10, 14 and 21 day. Ketamine was injected intramuscularly just prior to measurement at a dose of 40 mg/kg to sedate the rabbits. Corneal thickness was measured before instillation and thereafter at intervals coincident with the Draize scoring. Three measurements were made on each cornea and averaged. Results were expressed as swelling: corneal thickness on observed day / corneal thickness prior to instillation x 100. (a 100% swelling value represents no change).

Draize score obtained for EGBE are summarized in table 4.70

Table 4.70: Draize scores in the Kennah study

concentration	Draize score	% swelling (%- value untreated cornea)
100	66, severely irritating, resolution of ocular irritation within 14 days.	181 (81)
70	49, moderately irritating	146 (46)
30	39, moderately irritating; relative high scores are due to conjunctival damages	114 (14)
20	2, minimal irritating	113 (13)
10	1, minimal irritating	91 (-9)

Based on all the substances studied, a significant linear correlation was established between Draize score and % swelling (r = 0.86). A great majority of chemicals showed agreement between the Draize score and the corneal swelling when compared with class irritancy rating (mild, slight, moderate, severe, extreme).

For EGBE, Draize scoring was more severe than % of swelling. These differences are due to conjonctival damage, more heavily weighted by Draize scoring. But, swelling seems to detect more persistent irritation than Draize score. Resolution of ocular irritation was within 14 days.

Draize score is a “weighted scoring system involving multiplication factors” on a mix of all effects reported (e.g. discharge+chemosis+redness+opacity,+area of opacity and iritis). It is not possible to convert this scoring as such to the EC system.

In a similar study over 32 substances (Jacobs and Martens, 1989) using the EC method of calculation, a better linear correlation was obtained when comparing specific effects like corneal swelling and corneal opacity (r=0.94, n=32) as well as corneal swelling and erythema (r=0.93, n=32). When all eye lesions are taken into account, a threshold value of corneal

swelling of 60 (untreated cornea is set to 0%) can be accepted for classification purpose. For EGBE, the mean value of corneal swelling over 3 animals and 24,48,72 hrs was 70%. (In this study the observed pannus was not taken into consideration for the threshold value).

Application of this threshold value for classification (60% of corneal swelling) to the Kennah study results in:

EGBE (>99% pure) ; Irritating (Xi, R36)

EGBE (70%), EGBE (30%), EGBE(20%), EGBE (10%) not classified

In a paper reporting only the results of some studies, the instillation of a 5 % solution of EGBE according to Draize procedure did not exhibited any irritant properties (Bagley, 1994)

A GLP study was performed to assess the eye irritation potential of EGBE according to OECD guideline 405 (Safepharm laboratories, 1994b). 6 New Zealand white rabbits were instilled 0.1 ml EGBE into the conjunctival sac of the right eye. Amounts of ocular damage/irritation were noted 1, 24, 48 and 72 hr following treatment. Additional observations were made on days 7, 14 and 21 to assess the reversibility of the ocular effects. No information on test substance purity was included in the study report. The eyes were not rinsed 24 hours following treatment.

Areas of corneal opacity were noted in all treated eyes on 24 and 48 hr after treatment. Reversibility was not obtained in 21 days for 1 animal. Iridial inflammation was noted in all treated animals 1, 24 and 48 hr after instillation. Reversibility was obtained in 14 days. Moderate conjunctival irritation was observed at 1, 24 and 48 hr. Effects were present till D14. Petechial haemorrhage was seen in some animals at each observation time from 1 to 72 hr. Ectropion was noted from 72 hr observation time in some animals and not reversible for 1 animal at the end of the study. One animal showing signs of discomfort was therefore killed for humane reasons immediately after the 14th day. The scores are shown in the table below.

Table 4.70 bis: Individual score for Safepharm laboratories study, 1994b)

Animal	Time (hrs)	Opacity	Iritis	Redness	Chemosis
1	24	1	1	2	2
	48	1	1	2	2
	72	1	1	2	2
	7 day	0	0	0	0
2	24	1	1	2	2
	48	1	1	2	2
	72	1	1	2	2
	14 days*	2*	1	2	2
3	24	1	1	2	2
	48	1	1	2	2
	72	1	1	2	2
	7 days	0	0	0	0
4	24	1	1	2	2
	48	1	1	2	2
	72	2	1	2	2
	21 days	2*	0	0	0
5	24	1	1	2	2
	48	1	1	2	2
	72	2	1	2	2
	14 days	0	0	0	0
6	24	1	1	2	2
	48	1	1	2	2
	72	1	0	2	1
	7 days	0	0	0	0

* vascularisation. Animal 2 sacrificed after observation at 14 days

EGBE is considered to be a severe irritant to the eye in this study.

EGBE 5 % in water solution was tested in various *in vitro* assays: SIRC cytotoxicity assay, Neutral red uptake assay (48hr), cell protein assay, fibroblast cytotoxicity assay, corneal plasminogen activator assay (CEPA) (Harbell *et al.*, 1997).

Given that these assays are not yet (or will never be) validated, the results obtained cannot be used in this risk assessment. (However, the average Draize score obtained *in vivo* can be used: 2.7).

In a GLP study performed according to OECD guideline 405, 3 New Zealand white rabbits were instilled with EGBE (purity 99.6 %). Animals were observed 21 days (BASF, 2000). Eye washing was performed about 24 hours after treatment, before the 24 hour reading.

One animal exhibited a slight corneal opacity reversible in 21 days. Signs of a slight iris injury was described, reversible in 7 days. A medium to severe irritation of the conjunctivae was also observed and was reversible in 21 days. According to criteria for assessment of ocular lesions, EGBE can be considered as an eye irritant. The individual animal scores are shown below:

Table 4.70 ter: Individual score for BASF study, 2000

Animal	Time (hrs)	Opacity	Iritis	Redness	Chemosis
1	24	1	0	2	2
	48	1	0	2	2
	72	1	1	3	2
	14 days	0	0	0	0
2	24	0	1	3	2
	48	1	1	3	2
	72	1	1	3	2
	21 days*	0	0	0	0
3	24	1	1	3	2
	48	1	0	2	1
	72	1	0	2	1
	21 days	0	0	0	0

Human data:

After exposure to 200 ppm of EGBE, immediate irritation of the nose and throat, followed by ocular irritation and disturbed taste was experienced by all three subjects (Mellon Institute of Industrial Research, 1955).

Summary of eye irritancy data

A lot of studies have been performed to assess the eye irritation properties of EGBE. Most of them were not performed according to guidelines, but overall, all studies have shown that EGBE is irritant or severely irritant to the eyes of rabbits with effects both on conjunctivae, iris and cornea. In one well performed study EGBE produced irreversible effects on the conjunctivae and on the cornea in at least one treated rabbit. According to the EU classification system, these effects would trigger a classification R41, "severely irritant to eyes" because of the irreversibility of the effects at the end of the observation period. But these studies were performed according "old" guidelines. In the new guidelines, it is possible to rince the treated eye for animal welfare purposes and to more closely simulate the human situation where lacrimation would clear the substance from the eye. In a test, performed

according the recent guidelines, EGBE was only irritant (R36). This result was given precedence by the C&L group who confirmed a classification of R36.

It was also demonstrated that dilution of EGBE in water decrease its irritant properties as well as rinsing of the eyes in case of exposure.

4.1.2.3.3 Respiratory tract

Animal data:

Sensory irritation was evaluated using an *in vivo* method with mice (Kane *et al.*, 1980). Male Swiss-Webster mice (4/group) were exposed whole body to a series of chemicals (including EGBE). The measured response was the maximum percent decrease in respiratory rate, averaged over 4 mice, simultaneously exposed for 10 minutes. The responses obtained for various concentrations of solvents were utilized to develop a concentration-response relationship, for this, the concentration associated with a 50 % decrease in respiratory rate (RD50) was calculated.

Time response relationship: after an initial decrease observed within a few seconds and characterized by a pause during the respiratory rate, a phase of recovery occurred but respiration rate was lesser than normal until the end of the exposure period.

The response obtained within the concentration range tested was less than 50 % decrease in respiratory rate. The RD50 calculated by extrapolation was 2825 ppm (confidence limit 1695 – 7278). These values are well in excess of the saturated vapour pressure. When compared with the criteria of evaluation, 0.01 RD50 (about 28 ppm) would cause minimal or no sensory irritation whereas 0.1 RD50 (about 280 ppm) would cause definite but tolerable sensory irritation.

An estimate of the RD50 of EGBE was reported using modelling based on physicochemical properties (Alarie *et al.*, 1995). Calculated log RD50 was 3.45 (2818ppm). This is in good agreement with the experimental data.

Human data:

Three volunteers were exposed to 100 and 200 ppm of EGBE for periods of 2 or 4 hours, separated by a 2 hour period of non-exposure (Mellon Institute of Industrial Research, 1955). Immediate irritation of the nose and throat, followed by ocular irritation and disturbed taste was reported by all three subjects Whether such ‘irritation’ was physiological or merely discomfort is not clear..

Some human studies by inhalation for toxokinetic studies (Johanson *et al.*, 1986a, Kumagai *et al.*, 1999) have shown that EGBE was not irritant to the eyes or respiratory tract at doses of 20 ppm during 2 hours or 25 ppm for exposure periods of 10 minutes. A study performed by Johanson and Bomanin 1991 in which human volunteers were exposed to 50 ppm EGBE whole body (but breathing pure air) and mouth only did not describe irritant effects, but according to the description of the study, this kind of effects were not recorded.

In a recent study (Jones *et al.*, 2003) no signs of irritation were reported after exposure to 50 ppm EGBE (see § 4.1.2.1.2: Four volunteers (2 males and 2 females, aged 28-33) were exposed on 9 separate occasion, each occasion separated by at least 3 weeks. The exposure was 50 ppm EGBE for 2h. To record any physiological changes in the volunteers under the different conditions, physiological monitoring equipment was used.). The published study

paper does not mention if these signs were checked except the recording of the physiological changes. However, the author did indicate in a written communication that the volunteers were asked to report any adverse effects and none were reported (Jones, personnal communication).

Summary respiratory irritation:

Animal studies available (including repeated dose toxicity studies performed by inhalation on rats and mice) did not show any signs of significant respiratory irritation. No classification is required for this end point.

From the human data it is apparent that the NOEC is >50ppm whilst the NOEC (based on effects of discomfort) is <100-200ppm. A NOEC of 50ppm is taken forward for risk characterisation.

4.1.2.4 Corrosivity

According to EU classification criteria EGBE cannot be considered as a corrosive substance. Some signs of necrosis were seen in some studies but only when animals were exposed intra-dermally or for a long period of time by occlusive wrapping.

4.1.2.5 Sensitisation

4.1.2.5.1 Studies in animals

Skin

In vivo studies

In a GLP study performed according to OECD guideline 406, EGBE was tested in Guinea Pigs for sensitisation potential (Unilever, 1994). Induction was made with a dermal injection of 0.5 % EGBE in 0.9 % saline and a topical application of a 25 % EGBE in 0.9 % saline. Challenge was made with a topical application of 10 % EGBE in saline. All topical applications were made occlusively. Concentrations tested were based on results found in preliminary studies (showing a Maximal Concentration with No Irritation (CMNI) of 10 %). Two challenge applications were made at one week interval.

No evidence of sensitisation was seen in treated animals both at challenge and re-challenge.

EGBE was tested in a Maximised Magnusson & Kligman test (Zissu, 1995). After induction (test substance concentrations not reported) a 1 % concentration of EGBE was used for challenge.

No animals exhibited signs of sensitisation.

4.1.2.5.2 Studies in humans

Skin

In a GCP study, the sensitising potential of 10 % (v/v) EGBE in aqueous solution was assessed in human on 201 volunteers (TKL Research, 1992). Induction phase consisted in 9 consecutive occlusive application of EGBE. Patches were removed 24 hr after application. Assessments of the sites of application were made at 48 hr intervals and after new patches were reapplied. Following the ninth evaluation, the subjects were dismissed for a 14-day rest period. The challenge phase was initiated during the sixth week of the study, identical patches were applied to sites previously unexposed to EGBE. They were removed after 24 hr of exposure and the sites graded after additional 24 hr and 48 hr periods. Re-challenge if required was to be performed whenever there was evidence of possible sensitisation. The re-challenge was conducted on naïve sites on the back under both occlusive and semi-occlusive conditions approximately one or two weeks after challenge had been completed. Patches were applied for 24 hr, the patches removed and the sites evaluated 48, 72 and 96 hr after patches applications.

Only 7 of the 48 hr evaluations and 12 of the 72 hr evaluations showed slight erythema. One subject at the 72 hr reading had definite erythema. More severe responses which would have been expected if delayed contact sensitisation had occurred were not observed. In conclusion, according to the authors, none of the 201 subjects completing the study showed evidence of sensitisation. Re-challenge was not required for any subject.

Two cases of dermatitis herpetiformis (DH) temporally related to the use of a cleaning solution containing EGBE (Snider and Maize, 1993). Analysis of the mixture showed the presence of EGBE, trisodium phosphate, nonylphenol polyethylene glycol ether, anthraquinone, lemon fragrance and an antifoam agent. Patch testing with the individual chemicals at the same concentration than in the mixture (except for EGBE which was diluted to avoid irritation) and at the same time precipitated a flare of DH in both patients. Causal agent remained unknown because each individual ingredient was not separately patch tested. These results are hardly reliable for EGBE risk assessment because of co-exposure.

Respiratory tract

Considering SAR in the glycol ether family, the wide dispersive use of them and that no glycol ether has even been associated with cases of respiratory sensitisation, it can be considered that this toxicological property cannot be expected and is not relevant for risk assessment.

4.1.2.5.3 Summary of sensitisation

No signs of skin sensitisation were seen in two animal studies or in a human patch test. Moreover, considering Structure Activity Relationship (SAR) in the glycol ether family, the wide dispersive use of EGBE and that EGBE has never been associated with cases of skin sensitisation, it can be considered that skin sensitisation cannot be expected and is not relevant for risk assessment. No classification is needed for this end point.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Studies in animals

Inhalation

Rat

Three groups of 23 Wistar rats were exposed to EGBE in an inhalation chamber at concentrations of 0, 135 and 320 ppm (0, 652 and 1546 mg/m^3), 7 h/day, 5 day/week for 5 weeks. Eight animals/group were used for pathological examination and were sacrificed by pair after 1, 3 and 5 weeks of exposure (Werner *et al*., 1943b). The remaining animals were used for growth and haematological studies. Haematological examinations were made before – during and after the exposure (week 0-2-4-6-9) and consisted of red and white cell counts – differential counts, reticulocytes count and Hb estimations. Three weeks after the end of exposure and after a last blood analysis animals were sacrificed and organs examined (liver, kidney, heart, spleen and lung).

No effects on body weight gain were seen in either group of EGBE treated animals. No mortality or signs of systemic action were seen in the group of treated animals. The erythrocyte counts and haemoglobin concentration were significantly decreased at the end of 1 week of exposure to the high concentration of EGBE. Circulating reticulocytes were also increased over the same period.

For this study, transient effects on haematological parameters were seen at the high dose. The No Observable Adverse Effect Concentration (NOAEC) was 135 ppm based on transient haematological effects at 320 ppm.

Mortality of rats after two 8-hr exposures to 500 ppm (2415 mg/m^3) of EGBE was 3/6 animals (Mellon Institute of Industrial Research, 1952). At 250 ppm (1208 mg/m^3), 0/6 animals died after or during six 8 hr exposure whereas 1/5 animals died after or during four 8-hour exposure. No deaths occured when 6 animals were exposed to six 8-hour exposure of 125 ppm (604 mg/m^3) EGBE. No effects were seen in the control group. Due to the experimental conditions, this study cannot be taken into account for the Risk Assessment.

Groups of Alderly Park rats (4 males and 4 females/group) were exposed (whole body) to various concentrations of EGBE and for different periods of time (Gage, 1970). Rats were maintained in the exposure chamber for periods of up to 6 hours, and between repeated daily exposures they were returned to their cages where food and water were freely available. In the initial experiments the concentrations were selected to produce, if possible, acute effects after short exposures. Thereafter the exposure period was extended and the concentration lowered until the animals could survive 6-hour exposures, five days a week, for up to four weeks. With liquids, the concentrations which could be tested in these acute and subacute experiments. The rats were weighted and examined each morning, and their conditions and behaviour were recorded throughout the exposure period. Urine was collected overnight after the last exposure day for biochemical tests. Blood was collected during euthanasia for haematological tests. After a gross examination of the organs, the lung were collected and examined. The following organs were also taken for microscopic examination: lungs, liver, kidneys, spleen, adrenals and occasionally heart, jejunum, ileum and thymus.

Table 4.71: Experimental pattern of exposure for Gage study, 1970

Dose	Number of exposures	Symptoms
250 ppm (1208 mg/m^3)	4 x 6-hr exposures	initial haemaglobuniria and lethargy, low HB and MCHC, weight loss
100 ppm (483 mg/m^3)	15 x 6-hr exposures	no toxic signs, urine and blood tests normal apart from increased red cell osmotic fragility: autopsy, organs normal
50 ppm (242 mg/m^3)	15 x 6-hr exposures	as 100 ppm experiment
20 ppm (97 mg/m^3)	15 x 6-hr exposures	no toxic signs, blood normal: autopsy, organs normal

In this study, the Lowest Observable Adverse Effect Concentration: LOAEC based on haematological signs was 50 ppm (15 exposures) leading to a NOAEC of 20 ppm. Other effects than those seen on haematological parameters were seen only at 250 ppm (4 exposures)

Eighty five rats divided in three groups were exposed to EGBE (purity unknown) at doses of 0 or 17 ppm (84 mg/m^3). The first group was exposed 3 hr day on 6 days per weeks and the second group was exposed to 6 hr/day on 3 consecutive days per week (Lomonova and Klimova, 1977). The third group served as control. It is noted that this study was not conducted according to GLP ans was poorly reported. Body weight of animals was measured regularly. A standardised battery of general parameters and the level of blood cathecholamines were checked. For specific toxicity, blood parameters and erythrocyte fragility tests were performed. Catalase activity was also measured. Some functional tests were also performed: hexenal test, "cold test" and water maze. After the 4 months of exposure, an unique dose of 800 mg/m^3 of EGBE was administered to assess the adaptative effects (if any) of regular administration of small doses of EGBE.

Overall, the results of all parameters assessed has shown that the toxicity of EGBE was more severe in the first group (3 hr/d 6 days/week) than in the group 2. Moreover, the charge dose of 800 mg/kg administered at the end of the 4 month exposure period enhanced less effects in the second group than in the first one. Significant toxic signs were limited to haemolysis and narcosis. Due to the experimental conditions and the specificity of the test, data obtained can only be qualitative and therefore is not suitable for risk characterisation. No NOAEC can be derived.

Groups of Fisher 344 rats (8 animals per sex in each group with an addition of 7 females to the high exposure and control groups, 7 males to the control group and 8 males to the high exposure group - the additional animals were used to determine, if necessary, the reversibility of any effect - these animals were observed during 14 days after the last exposure) were exposed to 0, 20, 86 and 245 ppm (0, 97, 415 and 1183 mg/m^3) of EGBE 6 hr/day for 5 consecutive days –followed by 2 days without exposure and then 4 days of additional exposure (Bushy Run Research Center, 1981a). All animals were observed before, during and following exposure for any signs indicative of toxic effects. The appearance and demeanor of

all animals were recorded at these times. The modified Irwin Screen test was performed on five rats per sex from the 245 ppm exposure group and five rats per sex from the control group for signs indicative of behavioural and/or neuromuscular abnormalities. Animals were observed on exposure days 1, 2, 5, 6 and 7 prior to following exposure and preceding sacrifice. During the post-exposure period, reversibility groups were screened on days 4, 7 and 14. Evaluations were made on the following: corneal response, pupil response, tail pinch, toe pinch, righting reflex, locomotor activity, impaired gait, respiration, tremors, convulsions, salivation, piloerection, diarrhea, tail elevation, lacrimation, stereotypy. All rats had a ophthalmologic examination prior to group assignments. Rats were culled if abnormalities were observed. Except for reversibility groups, an eye examination was also done on all rats at sacrifice. The body weight for each rat was determined on the morning preceding the 1st, 2nd, 5th, 6th and 7th exposure days and again prior to sacrifice. During the post-exposure period the body weights of the reversibility group were recorded on post-exposure days 4 and 7 and prior to sacrifice, day 14. Haematological tests were performed on blood samples collected from all rats on the day prior to sacrifice. The analysis included: haemoglobin, haematocrit, erythrocyte count, white blood cell count, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Reticulocyte and white blood cell smears were made for each animal, but were only evaluated for high exposure and control animals. All rats were killed for necropsy on the day following the final exposure (except the rats of the reversibility groups which were sacrificed on the 14th day following the final exposure. Liver, lungs, kidneys and testes were weighed.

No deaths occurred during the study. After the first and second exposure, only red staining of urine was seen in animals of the high dose group. A slight increase over the control values for audible respiration and nasal discharge were observed in male rats of the 245 ppm group. Urogenital area wetness was observed in a few females of the 245 ppm exposure group. Male rats in the 245 ppm or control groups appeared normal during the modified Irwin Screen testing sessions. One female rat of the 245 ppm exposure group exhibited an abnormal righting reflex following exposure. A decrease in body weight gain was observed for animals treated with 245 ppm and for females treated with 86 ppm. During post exposure observation, animals recovered and no differences were seen with controls at post exposure day 14. Erythrocyte count, haemoglobin and MCHC values were significantly lower in males and females of the 245 ppm group compared to controls. These animals also exhibited a significant increase in MCV, nucleated red blood cells, reticulocytes and in males only, lymphocytes. Haematological values in both males and female rats exposed to 86 ppm were significant for haemoglobin (decrease) and MCV (increase). The females of the 86 ppm group also had significant decreases in MCH and MCHC and an increase in the haematocrit values. All effects seems to be dose-related. No haematological effects were seen in the 20 ppm group. In the reversibility group, animals exhibited a substantial recovery following 14 day post exposure period. However a significant decrease in erythrocyte count (male only) and a significant increase in MCV (all animals) and in MCH (male only) were still present in treated animals. The leukocyte count returned to control value during this recovery period. The mean liver weight values (relative and absolute) for the female of the 245 ppm group were higher than the controls. In addition, the relative liver weight for the females of the 86 ppm group and for the males of the 245 ppm group were higher than the controls. The mean relative testes weight for the males of the 86 ppm group was lower than controls, this can be considered to be an isolated finding not related to the treatment. After 14 day recovery period, no significant differences from controls in male or in female organ weight were observed. At necropsy, no finding could be specifically related to EGBE exposure. In this study, a NOAEC of 20 ppm can be indentified, according to the effects seen on haematological parameters at 86 ppm.

EGBE (purity 99.4 %) vapour was administered by whole body exposure to Fischer 344 rats at concentrations of 0, 5 – 25 or 77 ppm 0, (24 – 121 – 372 mg/m^3), 6 hr/day, 5 day/week for approximately 13 weeks (16 males and 16 females/group) (Bushy Run Research Center, 1981b). Animals were observed for signs of toxicity before, during and after exposures. A modified Irwin screen test was performed on 5 rats/sex/group from the high concentration and controls (see 9 day study for details, ref: Bushy Run Research Center, 1981a) and a sub-group was killed after 2 weeks (9 exposures) for interim examination. Body weight determination were performed regularly. Food consumption was determined weekly. All rats had an ophthalmologic examination prior to group assignments, male rats of the 77, 25 ppm and controls were re-examined prior to terminal sacrifice. Only the females of the 77 ppm group were examined. Haematologic tests were performed on blood samples collected from all rats on the day prior to sacrifice. Clinical examinations included urinalysis, haematology and serum clinical chemistry. Urine from rats of the high exposure group was analysed for haemoglobin and red blood cells. Urine from the same 5 rats per sex was analysed prior to exposure for the first 5 exposure days and prior to exposure each Wednesday thereafter. Urine was also collected and a more comprehensive analysis was performed one week prior to the terminal sacrifice from all rats. An interim sacrifice was performed on 6 rats per sex per exposure level. All remaining rats were necropsied at the final sacrifice on the day following the last exposure. After sacrifice, a gross dissection and an evaluation were performed on each animal. The following organs were weighed at terminal sacrifice: liver, kidneys, lungs, and testes. No organ weights were obtained for the rats at the interim sacrifice. At the time of the necropsy, histopathological examinations were performed on the usual tissues selected for such studies.

No death occurred related to treatment. No effects were seen concerning ophthalmologic examination and Irwin Screen test. For rats that were killed at the interim sacrifice time, after 9 exposures, the females of the 77 ppm group had lower body weight gain values comparing to the controls. No differences were found among the females of the lower exposure groups or males of any exposure group. For the main group, similar results were obtained for the same periods of time. However, following this time period the depression in body weight gain noted in the females was reversible and returned to values similar to the values of the control group. The decreases of food consumption are consistent with the changes noted in body weight gain. Slight signs of haemolytic anaemia were seen in females exposed to high doses of EGBE. Subtle haematological effects noted in females were characterised by minimal depression of red blood cell counts, haemoglobin and haematocrit with an attendant slight increase in MCH. These effects were noted at the interim sacrifice time and persisted throughout the study without increasing in severity. There was no indication of red blood cells or haemoglobin in the urine collected daily during the first exposure week or weekly thereafter from the male and female rats of the high exposure level. Moreover, there was no treatment related alteration in erythrocyte fragility at either the interim or terminal sacrifice times. No other haematological, urinary or clinical chemistry findings of toxicological significance were observed among the rats. Several incidental lesions were present in various organs, however no treatment-related, gross or microscopic lesions were found in either the male or female rats sacrificed at the end of the study. On organ weights, the only significant finding was lower relative kidney weight among the males of all treated groups. This finding is not believe to be related to the treatment.

The NOAEC was 25 ppm, based on the non-progressive haematological effects in females and a transient reduced body weight gain at two weeks seen in females of the 77 ppm dose group.

EGBE was administered to groups of 10 male and 10 female F344/N rats by whole-body exposure to 0, 31, 62.5, 125, 250 or 500 ppm (0 – 150 – 302 – 604 – 1208 – 2416 mg/m^3) EGBE vapour (lot no. QP-921215-26D2, Dow Chemical, Plaquemine, LA, USA, purity > 99 %) for 6 h per day, 5 days per week for 14 weeks (NTP, 2000 ; Nyska *et al.*, 1999a ; Long *et al.*, 2000).

Rats were held in the testing laboratory for 11 - 12 days and were 6 weeks old when the study began. They were housed individually, water was available *ad libitum* and feed was available *ad libitum* except during the exposure periods. All rats were observed twice daily throughout the study. Body weights were recorded at the beginning of the study and then weekly until the end of the experiment. Clinical observations also were recorded weekly throughout the experiment. Haematological examinations were performed at the end of the experiment, using an Ortho ELT-8/ds 9000 analyser. Differential leukocyte and nucleated erythrocyte counts were made by microscopic examination of blood films

Autopsies were made on all study rats. Organ weights measured were for the heart, right kidney, liver, lungs, right testis and thymus. A complete histological examination was performed on the chamber control and 500 ppm males and females and on the 250 ppm female rats.

Six female rats were found moribund and killed during the study: five in the 500 ppm group (four in week 1, one in week 5) and one in the 250 ppm group (in week 8). By the end of the study, body weight gains were significantly reduced in females of the 500 ppm group, but were unaffected in all other groups. Clinical findings were most prevalent in rats of both sexes exposed to 125, 250 or 500 ppm and included abnormal breathing, pallor, red urine stains, nasal and eye discharge, lethargy and either increased salivation or lacrimation. All females of the 500 ppm group, particularly during the first two weeks, developed alternating blue and white bands on their tails that caused them to self-mutilate and loose the distal portion of their tails.

Haematological examination showed that inhalation of EGBE resulted in the development of a persistent and exposure-related macrocytic, normochromic, responsive anaemia, as indicated by decreased haematocrit values, haemoglobin concentrations and erythrocyte counts in the 125 ppm or greater group males and in all groups of exposed females. The effects were dose related. This evidence of a sex difference in the severity of the anaemia was also seen in the 500 ppm group, in which the indicators were slightly more severe in the females than in the males. Evidence of an erythropoietic response was shown by increases in reticulocyte and nucleated erythrocyte counts in males of the 125 ppm or greater groups and females of the 62.5 ppm or greater groups. Other haematological changes were decreases in lymphocyte and monocyte counts in males of the 125 ppm or greater groups and increased platelet counts in females of the 125 or 500 ppm groups. Some organ weight changes were observed. These were: increases of the kidney of males in the 500 ppm group and females in the 125 ppm or greater groups; increases of the liver of males in the 250 or 500 ppm and females in the 125 ppm or greater groups; and decreases of the thymus of females in the 500 ppm group. Thrombosis was observed in the tail vertebrae, femur, incisors, nasal cavity, lung, heart and liver of most females in the 500 ppm group, but not at all in the 250 ppm group. Additionally, in the 500 ppm group females, there was degeneration of the hepatic centrilobular areas and renal tubules (4/5 in each case) and atrophy of the thymus (4/5) and spleen (1/5). Haematopoietic cell proliferation was observed in the spleen of 2/5 group same group. Bone marrow hyperplasia was recorded in males of the 250 and 500 ppm groups and females in all groups from 62.5 ppm. The severity of this response was dose related In the forestomach of females in the 500 ppm group, but not in lower dose groups, observations

made were of inflammation (3/5), necrosis (2/5), ulcers (2/5) and hyperplasia (1/5). Effects also observed in the 250 ppm group females were hepatic necrosis, pigmentation of Kupffer cells and renal tubule cells and bone marrow hyperplasia (NTP, 2000). No NOAEC was found for female rats. LOAEL was 31 ppm based on haematological effects seen at all doses tested.A NOAEL of 62.5 ppm was found for male rats, based onhaematotoxic effects seen at 125 ppm..

EGBE was administered to groups of 50 male and 50 female F344/N rats by whole-body exposure to 0, 31.2, 62.5 or 125 ppm (0 – 150 – 302 – 604 mg/m^3) EGBE vapour (lot no. QP-921215-26D2, Dow Chemical, Plaquemine, LA, USA, purity > 99 %)for 6 hr per day, 5 days per week for 104 weeks. For haematological and bone marrow analyses, additional groups of 27 male and 27 female F344/N rats were exposed to 0, 62.5 or 125 ppm for evaluation at 3, 6 and 12 months and 9 male and 9 female rats were exposed to 31.2 ppm for 3 months (for haematological examination only) and 6 months (NTP, 2000).

Rats were held in the testing laboratory for 18 days and were 7 – 8 weeks old when the study began. They were housed individually, water was available *ad libitum* and feed was available *ad libitum* except during the exposure periods. All rats were observed twice daily throughout the study. Body weights were recorded at the beginning of the study, after which they were recorded monthly from week 5 until week 89 and then every two weeks from week 92 until the end of the experiment. Clinical observations also were recorded monthly from week 5 until week 89 and then every two weeks until the end of the experiment. Haematological examinations were performed on an Ortho ELT-8/ds 9000 analyser at 3- and 6-months and on a Roche COBAS Helios analyser at 12-months. Differential leukocyte and nucleated erythrocyte counts were made by microscopic examination of blood films.

Autopsies and microscopic examinations were made on all of the main study animals that had not been used for blood and bone marrow sampling. Organ weights were not measured. At autopsy, all organs and tissues were examined for grossly visible lesions and all major tissues were fixed and processed for histological examination. A quality assessment pathologist evaluated the slides from all tumours and potential target organs, which included bone marrow, forestomach, kidney, liver, lung, nose and spleen of males and females and the adrenal and clitoral glands of females. The slides and the preliminary quality assessment report were then submitted to a National Toxicology Program Working Group of pathologists.

Survival of exposed male and female rats was similar to the chamber control groups, the mean numbers of survivors at the end of the experiment in each of the 0, 31.2, 62.5 and 125 ppm groups were 19, 11, 21 and 24 males and 29, 27, 23 and 21 females, respectively. No clinical signs were attributed to EGBE exposure. Body weights of male and female rats in the 31.2 and 62.5 ppm groups and male rats of the 125 ppm group were generally similar to the controls throughout the experiment. Body weights of female rats of the 125 ppm group were generally lower than those of the chamber control from week 17 until the end of the experiment.

Neoplastic effects observed in these experiments are described in section 4.1.2.8.1.3.

Haematological examination showed that inhalation of EGBE resulted in the development of a persistent and exposure-related macrocytic, normochromic, responsive anaemia, as indicated by decreased haematocrit values, haemoglobin concentrations and erythrocyte counts. These changes occurred at 3, 6 and 12 months in 62.5 ppm group females and 125 ppm group males. Some anaemia also occurred at 3 and 6 months in the 31.2 ppm group females and at 12 months in the 62.5 ppm group males. Increases in circulating reticulocyte

and nucleated erythrocyte counts are consistent with an erythropoietic response to the anaemia. In bone marrow there were approximately 15 % - 35 % decreases in the myeloid/erythroid ratio in the 125 ppm rats of both sexes, but particularly in females. Bone marrow hyperplasia was recorded in males of the 250 and 500 ppm groups and females in all groups from 62.5 ppm. The severity of this response was dose related.

Non-neoplastic effects of exposure were observed in the nose, liver and spleen.

In the nose there were significant increases in the incidences of hyaline degeneration of the olfactory epithelium in all groups of males (chamber control, 13/48; 31.2 ppm, 21/49; 62.5 ppm, 23/49; 125 ppm, 40/50) and in females exposed to the two higher concentrations of EGBE (chamber control, 13/50; 31.2 ppm, 18/48; 62.5 ppm, 28/50; 125 ppm, 40/49). The severity of the lesion was minimal and did not change with increasing exposure concentration. It is the most common age-related change of the nasal passages of rats (St. Clair and Morgan, 1992) and it has been proposed to have an adaptive or protective role (Buckley *et al*., 1985).

Incidences of Kupffer cell pigmentation of the liver increased significantly in all exposed groups of male rats (chamber control, 23/50; 31.2 ppm, 30/50; 62.5 ppm, 34/50; 125 ppm, 42/50) and in the two higher exposure groups of female rats (chamber control, 15/50; 31.2 ppm, 19/50; 62.5 ppm, 36/50; 125 ppm, 47/50). The severity of the lesion increased in the 125 ppm group of both sexes.

In the spleen, incidences of fibrosis were significantly increased in the two higher exposure groups of male rats (chamber control, 11/50; 31.2 ppm, 14/50; 62.5 ppm, 19/50; 125 ppm, 20/50), but not in females (NTP, 2000). No NOAEC could be derived for non-neoplastic effects in this study. The LOAEC for both male and female rats was 31 ppm, based on an increased incidence of minimal Kupffer cell pigmentation and on significant haematological effects in females.

Mouse

EGBE (purity unknown) was administered by whole body exposure to dogs, monkeys, guinea pigs, mice and humans (Mellon Institute of Industrial Research, 1955). Groups of 70 male C3H mice were exposed to 0, 100, 200 or 400 ppm (0 – 483 – 966 – 1932 mg/m^3) EGBE 7 hr/day for up to 90 days. Body weights were recorded weekly. Serial sacrifices were performed on 15 mice from each group after 30 and 60 exposures, on 10 mice after 90 exposures, and on 11 to 18 mice after 42 days rest following 90 days of exposure. Ten mice from each group were used for serial determination of erythrocyte fragility after various exposure periods. The blood samples were obtained by section of the cervical cord. Liver and kidney weight were measured. Only the kidneys were cut for histological examination (because repeated exposures to even fatal concentrations of EGBE produced undramatic micropathology in rats. No mortality or gross pathology or renal tissue changes were observed. Statistically significant alterations of liver weights occurred in the mice exposed to 400 ppm for 30, 60 and 90 days. A similar occurrence was noted in the mice exposed for 60 days to 200 ppm. The number of mice which had bloody urine immediately after exposure was proportional to the exposure concentration. No case of bloody urine was noted after the third exposure. Increased erythrocyte fragility was found in the mice exposed to all three concentrations. The increase in erythrocyte fragility appeared to be as great after the first exposure as it was after the 89th exposure. In all cases the erythrocyte fragility value fell to essentially normal levels when blood samples were taken from mice after 17 hr rest.

Sixty mice divided in three groups were exposed to EGBE (purity unknown) at doses of 0 and 17 ppm (84 mg/m^3). The first group was exposed 3 hr/day on 6 days per weeks and the second group was exposed to 6 hr/day on 3 consecutive days per week (Lomonova and Klimova, 1977). The third group served as control. It is noted that this study was not conducted according to GLP and was poorly reported. Body weights of animals were recorded at regular intervals. A standardised battery of general parameters and the concentrations of blood cathecholamines were checked. For specific toxicity, blood parameters and erythrocyte fragility tests were performed. Catalase activity was also measured. Some functional tests were also performed: hexenal test, "cold test" and water maze. After the 4 months of exposure, an unique dose of 800 mg/m3 of EGBE was administered to assess the adaptative effects (if any) of regular administration of small doses of EGBE.

Overall, the results of all parameters assessed have shown that the toxicity of EGBE was more severe in the first group (3 hr/d 6 days/week) than in the group 2. Moreover, the charge dose of 800 mg/kg bw administered at the end of the 4 month exposure period enhanced less effects in the second group than in the first one. Significant toxic signs were limited to haemolysis and narcosis.

EGBE was administered to groups of 10 male and 10 female B6C3F_1 mice by whole-body exposure to 0, 31, 62.5, 125, 250 or 500 ppm (0 – 150 – 302 – 604 – 1208 – 2416 mg/m^3) EGBE vapour (lot no. QP-921215-26D2, Dow Chemical, Plaquemine, LA, USA, purity > 99 %)for 6 hr per day, 5 days per week for 14 weeks (NTP, 2000).

Mice were held in the testing laboratory for 11 - 12 days and were 6 weeks old when the study began. They were housed individually, water was available *ad libitum* and feed was available *ad libitum* except during the exposure periods. All mice were observed twice daily throughout the study. Body weights were recorded at the beginning of the study and then weekly until the end of the experiment. Clinical observations also were recorded weekly throughout the experiment. Haematological examinations were performed at the end of the experiment, using an Ortho ELT-8/ds 9000 analyser. Differential leukocyte and nucleated erythrocyte counts were made by microscopic examination of blood films.

Autopsies were made on all study mice. Organ weights measured were for the heart, right kidney, liver, lungs, right testis and thymus. A complete histological examination was performed on the chamber control and 500 ppm male and female mice.

In the 500 ppm group, in week 2 of the experiment, two male and two female mice died and two male and two female mice were found moribund and killed. All other mice survived. By the end of the study, body weight gains were significantly reduced in males of the 125, 250 and 500 ppm groups, but were unaffected in all other groups. Clinical findings were found only in males and females of the 500 ppm group that died or were killed and included abnormal breathing, red urine stains and lethargy.

Haematological examination showed that inhalation of EGBE resulted in the development of a persistent and exposure-related normocytic (unlike rats), normochromic, responsive anaemia, as indicated by decreased haematocrit values, haemoglobin concentrations and erythrocyte counts in the 125 ppm or greater group males and in all groups of exposed females. Normocytic and normochromic erythrocytes were demonstrated by the lack of change in the mean cell volumes and mean cell haemoglobin concentrations, respectively. Other haematological changes were increased numbers of polychromatophilic erythrocytes in the 500 ppm group and increased platelet counts in males of the 500 ppm group and females of the 250 and 500 ppm groups. Increases were found in absolute and relative liver weights

of males of the 500 ppm group and in relative liver weights of males of the 250 ppm group and females of the 500 ppm group. Haemosiderin deposition in Kupffer cells was the only change observed in livers of the 500 ppm group males and the 250 and 500 ppm group females. Haemosiderin pigmentation was also increased in renal tubule cells of both males and females of the 500 ppm group. Extramedullary haematopoietic cell proliferation (primarily erythroid) and haemosiderin pigmentation were present in males exposed to 125 ppm or greater and females exposed to 250 or 500 ppm. In the forestomach, there were significant increases in females only in the incidence of inflammation in the 250 and 500 ppm groups and epithelial hyperplasia in the 125 ppm and greater groups. Effects observed in the males and females of the 500 ppm group that were either killed or died included forestomach inflammation, necrosis and ulceration, suppurative inflammation of the peritoneum and mediastinum, atrophy of the spleen, thymus and lymph nodes, renal tubule degeneration and, in males, testicular degeneration and epididymidal necrosis (NTP, 2000). No NOAEC for female mice could be derived for non-neoplastic effects in this study, the LOAEC for female mice being 31 ppm, the lowest dose tested, based on significant haematological effects. The NOAEC for male mice was 62.5 ppm, based on haematological effects at 125 ppm.

EGBE was administered to groups of 50 male and 50 female $B6C3F_1$ mice by whole-body exposure to 0, 62.5, 125 or 250 ppm (0 – 150 – 302 – 604 mg/m^3) EGBE vapour (lot no. QP-921215-26D2, Dow Chemical, Plaquemine, LA, USA, purity > 99 %) for 6 hr per day, 5 days per week for 104 weeks. For haematological and bone marrow analyses, additional groups of 30 male and 30 female $B6C3F_1$ mice were exposed to 0, 62.5, 125 or 250 ppm for evaluation at 3, 6 and 12 months (NTP, 2000).

Mice were held in the testing laboratory for 18 days and were 7 weeks old when the study began. They were housed individually, water was available *ad libitum* and feed was available *ad libitum* except during the exposure periods. All mice were observed twice daily throughout the study. Body weights were recorded at the beginning of the study, after which they were recorded monthly from week 5 until week 93 and then every two weeks from week 94 until the end of the experiment. Clinical observations also were recorded monthly from week 5 until week 93 and then every two weeks from week 94 until the end of the experiment. Haematological examinations were performed on an Ortho ELT-8/ds 9000 analyser at 3- and 6-months and on a Roche COBAS Helios analyser at 12-months. Differential leukocyte and nucleated erythrocyte counts were made by microscopic examination of blood films. Bone marrow cellularity was measured using a Coulter Model Z_H counter, while cytological evaluations of bone marrow cell morphology and myeloid/erythroid ratios were made microscopically.

Autopsies and microscopic examinations were made on all of the main study mice that had not been used for blood and bone marrow sampling. Organ weights were not measured. At autopsy, all organs and tissues were examined for grossly visible lesions and all major tissues were fixed and processed for histological examination. A quality assessment pathologist evaluated the slides from all tumours and potential target organs, which included bone marrow, forestomach, kidney, liver, lung, nose and spleen of males and females and the preputial gland, prostate, skin (prepuce), testis and urinary bladder of males. The slides and the preliminary quality assessment report were then submitted to a National Toxicology Program Working Group of pathologists.

Survival of male mice exposed to 125 or 250 ppm was significantly less than that of the chamber control group, whereas survival in the lower dose group was similar to the chamber control group. The mean numbers of survivors at the end of the experiment in each of the 0, 62.5, 125 and 250 ppm groups were 39, 39, 27 and 26 males and 29, 31, 33 and 36 females,

respectively. No clinical signs were attributed to exposure to EGBE. Body weights of exposed male mice were generally less than those of the chamber control during the last 25 weeks of the experiment. Body weights of female rats of the 250 ppm group were generally lower than those of the chamber control from week 30 until the end of the experiment, the difference being about 20 % for much of that time. Body weights of the 62.5 and 125 ppm group females were generally lower than the chamber controls from about week 60 until the end of the experiment.

Neoplastic effects observed in this experiments are described in section 4.1.2.8.1.3.

Haematological examination showed that inhalation of EGBE resulted in the development of a persistent and exposure-related normocytic and normochromic, responsive anaemia, as indicated by decreased haematocrit values, haemoglobin concentrations and erythrocyte counts; it lacked, in general, changes in mean cell volumes and mean cell haemoglobin concentrations. These changes occurred at 3, 6 and 12 months in 125 and 250 ppm group male and female mice. Some anaemia also occurred at 6 months in the 62.5 ppm group females and there was a minimal increase in cell volume, suggesting a macrocytosis, in females of the 250 ppm group at 12 months. Increases in circulating reticulocyte counts that were observed in 125 and 250 ppm male and female mice at 3 and 6 months and 250 ppm female mice at 12 months, are consistent with an erythropoietic response to the anaemia. In bone marrow there was no change in cell counts and no decrease in the myeloid/erythroid ratio, unlike the observations reported for rats (above). Additionally, thrombocytosis developed, as shown by increased platelet counts in the 250, 125 and 62.5 ppm exposed groups of males and females at 12 months, in the 250 ppm group males at 6 months and females at 6 and 3 months and in the 125 ppm group females at 6 months.

Incidences of haemosiderin pigmentation in Kupffer cells of the liver was significantly increased in males of the 125 and 250 ppm groups and females of all groups exposed to EGBE in a dose-dependent manner (see Table 33 in 4.1.2.8.1.3).

Haematopoietic cell proliferation in the spleen was increased in males exposed to 125 and 250 ppm and females exposed to 250 ppm, but it was not accompanied by any change in myeloid/erythroid cell ratio. Incidences of haemosiderin pigmentation in the spleen were significantly increased in all exposed groups of males and in the 125 and 250 ppm groups of females. These observations were attributed to the primary haemolytic effect of EGBE that was followed by regenerative hyperplasia of the haematopoietic tissue. Increases in the incidence of hyperplasia were also observed in the bone marrow of males exposed to 125 and 250 ppm.

In the nose there were increases in the incidences of hyaline degeneration in all groups of female mice of the olfactory epithelium (chamber control, 6/50; 62.5 ppm, 14/50; 125 ppm, 11/49; 250 ppm, 12/50) and in the respiratory epithelium (chamber control, 17/50; 62.5 ppm, 35/50; 125 ppm, 26/49; 250 ppm, 23/50). The severity of the lesion was minimal and did not change with increasing exposure concentration. There was no clear dose response relationship. In males, the incidence was similar to the chamber controls.

Non-neoplastic lesions of the forestomach consisted of ulcer (full thickness defect of the forestomach epithelium), particularly in females, epithelial hyperplasia (an increased thickness of the stratified squamous epithelium sometimes accompanied by an increased thickness of the keratinised layer) that was usually focal and, particularly in females, frequently associated with ulceration (see Table 5 in 4.1.2.8.1.3).

There were a number of inflammatory changes in the urogenital system (NTP, 2000). These are shown in detail in the table below. Effects were only seen in male mice. In the kidney, incidences of glomerulosclerosis and hydronephrosis were significantly increased at 125ppm (but not at 250ppm) whilst the incidence of chronic inflammation was increased significantly at 250ppm. The incidences of inflammation of the preputial and prostate glands in males exposed to 250ppm were significantly increased as were incidences of inflammation of the bladder. The incidence of ulcer of the transitional epithelium of the urinary bladder was significantly increased at 125ppm but not at 250ppm. The NTP authors concluded that these effects were indicative of an ascending urinary tract infection described as mouse urologic syndrome. The occurrence of this syndrome is particularly common in inhalation studies with mice, in which they are housed individually in wire mesh cages (Everitt *et al*., 1988). It is likely that the condition was exacerbated by the irritative effects of EGBE or its metabolites but is not primarily mediated by the substance.

Table 4.71 bis: Incidence of non-neoplastic lesions in the urogenital system of male mice following 2-years exposure to EGBE

	Control	62.5ppm	125ppm	250ppm
Kidney[a]	50	50	47	50
Glomerulosclerosis	4 (1.3)	4 (1.3)	11* (1.3)	9 (1.4)
Hydronephrosis	1 (2.0)	0	6* (1.8)	5 (2.2)
Inflammation	0	1 (2.0)	2 (2.5)	4* (3.)
Preputial gland[a]	49	49	49	49
Inflammation	2 (1.0)	7 (2.3)	6 (2.3)	8* (2.1)
Prostate gland[a]	49	49	48	45
Inflammation	0	2 (2.0)	3 (2.3)	4* (1.8)
Skin, prepuce[a]	50	50	49	50
Inflammation	2 (3.5)	3 (3.7)	13** (3.7)	8* (3.9)
Ulcer	0	3 (2.7)	11** (2.8)	8* (2.5)
Urinary bladder[a]	50	49	5046	45
Inflammation	0	2 (3.5)	5* (3.4)	4* (3.8)
Transitional epithelium, ulcer	0	1 (3.0)	4* (2.8)	2 (3.0)

Notes: Figures in brackets are severity of lesion. 1=minimal, 2=mild, 3=moderate, 4=marked
[a]=number of animals examined.
*=significant p<0.05. **=significant p<0.01

No NOAEC could be derived for non-neoplastic effects in female mice in this study, the LOAEC being 62.5 ppm, the lowest dose tested, based on marginal, but significant haematological effects. The NOAEC for male mice was 62.5 ppm, based on body weight gain decrement, marginal, significant haematotoxic effects and inflammation of the kidney, urinary bladder and prepuce at 125 ppm.

Guinea-pig

EGBE (purity unknown) was administered by whole body exposure to guinea pigs 7 hr/day (Mellon Institute of Industrial Research, 1955). Groups of 10 males were exposed to doses of 0,375 and 500 ppm for 30 days. In addition 6 males and 6 females were added to the above groups for the study of sex differences in susceptibility. Bw was recorded weekly. Liver and kidney weight were measured after euthanasia. Liver, lung, kidney were submitted to histological examination.

In the 500 ppm group, 2 male guinea pigs died within 12 exposure and one animal died after 7 exposures to 375 ppm. The mean body weight of both groups of animals fell slightly by the third exposure but only the weights of the guinea pigs exposed to the lower level were

significantly below the controls after 30 exposures. A significant elevation in the kidney weights of both groups was also observed. No haematuria was observed and only the 3 guinea pigs that which died during exposure showed any major tissue damage (lung haemorrhage and congestion).

Dog

Groups of 2 dogs were exposed to 415 ppm EGBE (42 % saturation) (purity unknown) 7 hours daily, five days a week for 12 weeks (Werner *et al.*, 1943c). Erythrocyte, reticulocyte, leucocyte and differential counts and haemoglobin estimations were made routinely in the morning and in the afternoon. These determinations were made twice during the pre-exposure period, 3 or 4 times during the post-exposure period and 8 times during the 12 weeks of exposure. Sedimentation rates, haematocrit determinations and fragility tests were made only during the latter part of the experiment on afternoon samples of blood. Urine was analysed for albumin, sugar and acetone. Urinary sediments were examined microscopically and average daily urine volumes were determined. Estimation of urea were made on blood obtained in late afternoon immediately before feeding. After sacrifice, histological examinations of the following organs were performed: lungs, liver, kidney, spleen, heart, urinary bladder, pancreas and large and small intestines. Additional examinations of the spleen were performed using a different treatment (acidulated ferrocyanide) in order to demonstrate the presence or absence of iron-bearing pigment.

Dogs exposed to EGBE lost 6 and 9 % of their body weights during the study. Signs of central depression and slight increased of eyes and nose secretion were the only clinical effects detected. Signs of haemolytic anaemia (significant decreases in haematocrit and haemoglobin) were seen in treated animals.

EGBE (purity unknown) was administered by whole body exposure to dogs 7 hr/day (Mellon Institute of Industrial Research, 1955). Dogs were exposed to 0, 100, 200, 385 or 617 ppm EGBE (0, 483, 966, 1860 or 2980 mg/m^3). For the 617 ppm dose, one black mongrel female was treated for 2 days. For the 385 ppm dose, one male and one female Basenji were exposed 7 hours a day for 28 and 8 days respectively (haematological tests were performed daily but biochemistry only in male and after 26 and 27 exposure). For the 200 ppm group, one male and one female Basenji were exposed 7 hours a day for 31 days. For the 100 ppm group, one male and one female wire-haired terrier were exposed 7 hr/day for 91 days, usual blood chemistry were performed before the first exposure and after the last exposure. Haematocrit values and icterus indices were observed in add to the regular haematological tests were performed weekly. Bw was recorded weekly. Erythrocyte count, leucocyte count, Hb level, erythrocyte fragility and haematocrit value were measured immediately after exposure. For biochemistry, bromosulfalein retention time, sedimentation rate, icterus index, blood urea nitrogen and plasma fibrinogen levels were recorded. Erythrocyte permeability test was made with the method of Bootman and Moses only for the 200 ppm group. Coloured photographs of the visceral organs as well as any striking abnormality were performed. Histological examinations of adrenals appendix, gall bladder, gonads, heart, intestine, kidneys, liver, lung, pancreas, spleen, stomach, thyroid, urinary bladder, uterus and tissue found abnormal at autopsy were realised.

For the 617 ppm group, the only dog exposed died after 2 days of exposure. Vomiting and weakness were seen during exposure. Autopsy revealed congestion of the kidneys and lungs. For the 385 ppm group, male and female died after 28 d and 8 d respectively. Similar symptoms were seen in both male and female: loss of weight, increase in erythrocyte fragility,

weakness, apathy, anorexia and increase leucocyte count. The erythrocyte fragility of male reached a maximum in 7 days and decrease progressively through 27 days. Analysis of urine after the 25th exposure indicated the presence of 55 mg of BAA. The only abnormality found in biochemistry was an elevated plasma fibrinogen concentration. At autopsy congestion of the lungs and of liver were observed in both animals. Congestion of kidneys were seen female only. For the 200 ppm group, slight evidence of toxicity were seen after 31 days of exposure. Erythrocyte fragility increased slightly and the erythrocyte fragility was higher than controls in both animals. Erythrocyte count and HB decrease slightly through exposure. BAA was present in urine after the 14th exposure: 100 and 42.5 mg in male and female respectively. For the 100 ppm group, only slight effects on haematocrit were seen. BAA in urine reached 100 and 94 mg in male and female respectively after 81 exposures.

Monkey

EGBE (purity unknown) was administered by whole body exposure to monkeys 7 hr/day (Mellon Institute of Industrial Research, 1955). Two Rhesus monkeys (1 male and 1 female) were treated with 100 ppm (483 mg/m^3) EGBE for 90 days (group 1). Another group of 2 Rhesus monkeys, were treated with 100 ppm, after 10 days of treatment no effects were seen and the dose was increased to 200 ppm (group 2). No control group was used. Bw was recorded weekly. Erythrocyte count, leucocyte count, HB level, erythrocyte fragility and haematocrit value were measured immediately after exposure, bromosulfalein retention time, sedimentation rate, icterus index, blood urea nitrogen and plasma fibrinogen levels were also recorded. Coloured photographs of the visceral organs as well as any striking abnormality were noted. Histological examinations of adrenals appendix, gall bladder, gonads, heart, intestine, kidneys, liver , lung, pancreas, spleen, stomach, thyroid, urinary bladder, uterus and tissue found abnormal at autopsy were performed.

In group 2, of 2 monkeys, 1 died of causes unrelated to treatment. The erythrocyte fragility values which were unchanged after 10 days exposure to 100 ppm rose after 4 days of treatment with 200 ppm. Erythrocyte fragility values were increased at the beginning of exposures. Values returned to normal until the end of the study. A difference was seen between male and female: females were more sensitive than males. For group 1, erythrocyte fragility values rose to 0.56 – 0.48 (0.46 – 0.40 for pre-exposure values) several times after the 18th exposure. A greater rise (0.62 – 0.54) was seen for the female after 7 exposures. These high values of the female returned to normal by the end of the 90-exposure period.

Summary inhalation route:

Many studies are available on rats, and mice. A few short studies using dogs, guinea-pigs and non human primates have also been conducted.

In rats and mice, common toxicity signs together with effects similar to those observed in acute administration. The main effect was haemolysis, which was consistently observed and sometimes associated with secondary hepatic effects (Kupffer cells pigmentation and absolute and relative liver weight increases). Other effects were decreases of body weight gain, hyaline degeneration of the olfactive epithelium, effects on the forestomach and effects on the WBC sub-populations (T lymphocyte). In these studies, a NOAEC of 25 ppm in rats and a LOAEC of 31 ppm in mice and rats can be established (based on haemolysis, as the only significant

primary effect). The LOAEL of 31 ppm (coming from a six month satellite group in the NTP, 2000 104-week study) is taken into account for the risk characterisation.

Table 4.72 : Summary of the studies on animals performed by inhalation route

Study	NOAEC (ppm)	Effects	Reference
Rats			
5 weeks. Doses 0, 135, 320 ppm.	135 (for haematological effects)	Poorly reported	Werner *et al.*, 1943b
Variable Number of exposures . Doses 0, 250, 500 ppm.	-	Poorly reported	Mellon Institute of Industrial Research, 1952
15 exposures to 0, 20, 50 and 100 ppm. 4 exposures to 250 ppm.	20 ppm (haematological effects).	Haematological effects	Gage, 1970
4 months, 0, 17 ppm , 6 hours a day three days a week or 3 hours a day six days a week.	-	Poorly reported	Lomonova and Klimova, 1977
9 days, 6 hours a day. Doses: 0, 20, 86, 245 ppm	20 ppm (haemotoxicity)	Haematological effects and effects on liver and body weight gain.	Bushy Run Research Center, 1981a
13 weeks. Doses: 0, 5, 25, 75 ppm	25 ppm (haematological effects only)	Haematological effects only	Bushy Run Research Center, 1981b
13 weeks. Doses: 0, 31, 62.5, 125, 250, 500 ppm	LOAEC of 31 ppm	Haematological effects. Study performed to describe the vascular and bone lesion observed in moribund female.	Nyska *et al.*, 1999a Long *et al.*, 2000 NTP, 2000
104 weeks. Doses: 0, 31, 62.5,	None identified for haematological effects; 31.2 ppm may be	Haematological effects. Effects on liver (Kupffer cell	NTP, 2000

125ppm	considered as an LOAEC.	pigmentation).	
Mice			
Doses: 0, 100, 200, 400 ppm. Duration: 30, 60 or 90 exposures	< 100 ppm	Haematological effects at all doses	Mellon Institute of Industrial Research, 1955
4 months, 0, 17 ppm, 6 hours a day three days a week or 3 hours a day six days a week.		Poorly reported	Lomonova and Klimova, 1977
14 weeks. Doses: 0, 31, 62.5, 125, 250, 500 ppm	None identified for haematological effects; LOAEC of 31 ppm	Haematological effects. Effect on body weight gain. Irritant effects on the forestomach.	NTP, 2000
104 weeks. Doses 0, 62.5, 125, 250ppm	none identified LOAEC of 62.5 ppm	Haematological effects. Effects on liver (Kupffer cell pigmentation).	NTP, 2000
Guinea pigs			
30 days Dose: 0, 375 and 500 ppm	none identified	Mortality, effects on body weight and on kidneys. No effects on blood parameters.	Mellon Institute of Industrial Research, 1955
Dogs			
12 weeks Dose: 415 ppm	< 415	CNS depression, haemolytic anaemia.	Werner *et al.*, 1943c
Duration between 2 and 90 days. Dose: 0, 100, 200, 385 and 617	-	CNS depression, haemolytic anaemia. Effects on lungs, kidneys and liver.	Mellon Institute of Industrial Research, 1955
Monkeys			
90 days, Dose: 0, 100, 200 ppm.	-	Haematological effects (haemolysis)	Mellon Institute of Industrial Research, 1955

Dermal

Mice

EGBE was administered to BALB/c mice (5-6 weeks old) to assess the effects on the immune system (Singh *et al.*, 2001). Doses of 0, 100, 500, 1000 and 1500 mg/kg/day (about 25µl in a 4:1 mixture of acetone and olive oil vehicle) were applied during 4 days to the backs of the mice. The numbers of animals per dose level is not indicated in the study report. Except for one group of mice tested for IgM plaque-forming cell (PFC) response, animals were killed 24 hours after the last dose. Body weights were recorded. Thymus and spleen were removed and weighed prior to preparation of cell suspensions. For evaluation of the PFC response, mice were immunised by Intraperitoneal injection with sheep erythrocytes on the third day of dosing and killed 72 hours after the final dose. Cell suspensions of lymphoid organ tissue were prepared for spleen and thymus, cellularity and viability were assessed. Lymphoproliferative assays (Non-specific mitogenic stimulation of either B or T lymphocytes), mixed lymphocyte response (MLR) assay, activity of natural killers (NK) cells, cytotoxic T lymphocyte (CTL) activity and plaque-forming cell response to sheep red blood cells were assessed using standard procedures.

No effects on body weight gain or thymus weight were seen at any dose tested. At the 1500 mg/kg dose, an increase in the relative spleen weight and an increase of splenic cellularity of 29 % were seen. There was no changes in the lymphoproliferation assay in the B cells population whereas the T lymphocyte sub-population exhibited a decrease of the proliferative responses significant only at doses of 500 and 1000 mg/kg (a decrease was also seen for the 1500 mg/kg dose but not statistically significant, this weaken the relevance of this finding). The same results were seen in the MLR assay (decreased responses at the high doses, significant only at 500 and 1000 mg/kg). NK, CTL activity and T-dependent PFC response was unchanged after treatment. The lack of a dose-related response in these observations throws doubt on their significance for EGBE toxicology. The NOAEL for immunological effects was 1000 mg/kg bw, based on spleen weight reduction at 1500 mg/kg bw per day.

Rabbits

EGBE was administered occlusively via dermal route to 4 groups of New Zealand (NZ) white rabbits (5 animals/sex) at concentrations of 100 - 50- 25 and 5 % (1 ml/kg of mixture corresponding to 900 – 450 – 225 and 45 mg/kg bw) during 6 hr/day for 9 days (dosed 5 days, no dosed 2 days and dosed 4 days) (Bushy Run Research Center, 1980c). Control group was treated with vehicle only. Dermal irritation score were determined after each test exposure. Body weight of animals was measured regularly: all males were submitted every day (and also after exposure period) to an ophthalmologic examination (discoloration, discharge, gross abnormality, reddening). Urine samples were collected prior to exposure on the 2^{nd}, 5^{th} and 9^{th} day of exposure and on the day of sacrifice. The following parameters were measured: colour, pH, protein, glucose, ketones, urobilinogen, bilirubin and blood. Blood samples were collected before dosing the last day of dosing and the day of sacrifice and assessed of RBC count, haematocrit, MCV, MCH, MCHC, WBC count. Reticulocytes and WBC differential smears were prepared for each animal but not evaluated. Fourteen days after the last dosing, animals were euthanised. Tissues were collected and fixed, only kidneys were examined

histopathologically. Liver and kidneys of all animals were weighted, in males testes were also weighted.

In the preliminary studies: LD50 was calculated for males: 0.707 ml/kg and for females: 0.630 ml/kg. In the main study, no male rabbits died during the study. One female rabbit was found moribund on day 5, the condition was apparently unrelated to treatment. No body changes were seen in male whereas female rabbits treated with undiluted EGBE exhibited a decrease in bw gain throughout the direction of the study. All ophthalmologic examinations were negative for the male rabbits. The females were not examined. Severe necrosis and moderate oedema were observed in all animals dosed with undiluted EGBE by the 6th day of treatment. The 50 % dilution produced necrosis of the skin of 1 out of 5 males as compared to 4 out of 5 females. Erythema preceded necrosis in every instance. Slight oedema was also observed in those rabbits exhibiting necrosis. Dosage related erythema was observed at the lower dosage levels. Very slight reddening was seen in the male rabbits in the water control group. No statistical comparisons were performed on the skin irritation readings. On day 2 of treatment there was haemoglobin in the urine of two of four male rabbits dosed with undiluted EGBE. There also was a slight increase in urinary protein in four males dosed with undiluted material on day 2 of treatment. Both of these conditions returned to normal by treatment day 5. On day 2 of treatment there was blood in the urine of three of five female rabbits dosed with undiluted EGBE. There was a corresponding increase of urinary protein in two of these animals. On day 5 of treatment, four of the five females dosed with undiluted material had blood in their urine, as well as one dosed with the 50 % dilution. By day 9 of treatment only one dosed with undiluted sample still had blood in the urine; however, at this time all five females dosed with the 50 % dilution had blood in their urine. There was no evidence of haemoglubinuria at any dosage level at the time of sacrifice (14 days post exposure). No abnormalities were seen in any of the other measures of urinary effect assessed and there was no occult found in the urine of rabbits dosed with the 25 % or the 5 % dilutions. Statistically significant depressions of mean erythrocyte count, haemoglobin and mean corpuscular haemoglobin concentration were present on treatment day 9 in females dosed with undiluted substance and the mean corpuscular haemoglobin was significantly increased. Although not statistically significant, the contemporaneous decreased haematocrit and increased mean corpuscular volume are consistent changes. The presence of larger red blood cells with more haemoglobin is indicative of response to red cell loss by early release of less mature cells into the circulation. These effects were no longer seen after the 14-day observation period. Females in the three lower dosage groups had no indication of biologically significant haematologic effects. The increased mean red blood cell counts on day 9 of treatment in the 5 % dilution group and on day 14 after exposure in the 25 % dilution group are considered to be statistical artifacts. No changes were noted in any organ weight measured in all animals. Gross findings included some thickening of the skin in male rabbits given undiluted substance and possibly dose related patchy colour change of the kidneys of the three females.

For this study, the NOAEL of 450 mg/kg can be taken into account mainly based on gross findings observed in animals dosed with pure substance. Haematological effects seen during the study were transient and recovery was complete after the 14-day observation period. .

In a GLP study, 4 groups of 10 males and 10 females NZ white rabbits were treated dermally (occlusively) with EGBE (high purity) at concentration of 0 % - 2.8 % - 14.3 % and 42.8 % in distilled water (corresponding to dose levels of 0 – 10 - 50 - 150 mg/kg) for a 6 hr period/day 5 d/w for 13 weeks (Wil Research laboratories inc., 1983). Animals were observed for clinical symptoms twice daily. Prior to treatment, the site of application of each animal were graded using the Draize skin reaction scoring system. Scoring was performed daily for the first 3

weeks of the study, the once weekly thereafter. Bw and food consumption were measured weekly. Blood was collected before the study, during week 4 and just before termination. The following parameters were determined:

Haematology: white blood cell count, mean corpuscular haemoglobin concentration, erythrocyte Count, mean corpuscular haemoglobin, haemoglobin, differential white blood cell count, haematocrit, erythrocyte fragility (termination only), mean corpuscular volume.

Clinical chemistry: glucose, direct bilirubin, total bilirubin, albumin, blood urea nitrogen, total bilirubin, alkaline phosphatase.

All rabbits were subjected to a gross necropsy examination. The following organs were weighted at sacrifice for at least 6 animals/sex/level: heart, thymus, testes, spleen, liver, ovaries and kidneys. The following tissues were also examined briefly and conserved: adrenals, aorta, bone (sternebrae), brain (3 transverse sections), epididymis (both, tail), oesophagus, eyes, gall bladder, heart, intestines (caecum, duodenum, jejunum, ileum and colon), kidneys, liver (left & median lateral lobes), lung (left), lymph node (mesenteric & thoracic), mammary gland, ovaries, pancreas, parathyroids, pituitary, prostate, sciatic nerve, seminal vesicles, skeletal muscle, skin (test site & adjacent normal skin), spleen, stomach (pylorus and fundus), submandibular salivary gland, testes, thyroids, thymus, tongue, trachea, urinary bladder, uterus (both horns), vagina, All gross lesions.

Red coloured faeces and red liquid material on cage paper (probably blood) were seen in some treated animals (each group). Some irritant effects were sometimes seen in animals of both control and treated groups. There was no increase in the severity of these effects in treated animals versus controls. There were no treatment related deaths. No significant effects were seen on body weight gain or food consumption in any group treated compared to control animals. Sporadic changes in haematology parameters and RBC fragility values were noted but values were within normal ranges for this laboratory and probably not related to the test material. Sporadic changes were also noted in WBC counts but were not related to test material administration. No changes in biochemistry were related to treatment. No test material related effects were noted on absolute or relative organ weights or final body weights.

The NOAEL for this study was equal to 150 mg/kg bw, the highest tested dose. The actual NOEL may be of a higher value.

Summary dermal dose:

Table 4.73: Summary of the studies performed by dermal route

Study (rabbits)	NOAEL (mg/kg bw/d)	Effects	Reference
Mice			
4 days. Doses: 0, 100 – 500 – 1000 – 1500 mg/kg bw/day	NOAEL=1000	Effects on splenic cellularity at 1500.	Singh *et al.*, 2001.
Rabbits			
9 days. Doses: 45 – 225 – 450 - 900 mg/kg bw/d	NOAEL = 450	Transient haematological effects	Bushy Run Research Center, 1980c
13 weeks. Doses: 10 – 50 – 150 mg/kg bw/d	NOAEL > 150	No effects	Wil Research laboratories inc., 1983

Two studies are available on rabbits to assess the toxicity of repeated doses of EGBE administered dermally. In only one study, signs of toxicity were recorded and were limited to transient signs of haemolysis. This study led to a NOAEL of 450 mg/kg bw/d due to haematological effects seen at 900 mg/kg bw/d.

Given that this study was performed only during 9 days, the NOAEL of the second study, which was performed during 13 weeks, could be more reliable for the risk characterisation. This NOAEL was 150 mg/kg bw/d.

The mouse study design for the assessment of EGBE effects on the immune system, give a NOAEL of 1000 mg/kg.

Oral

Rat

Five groups of Sherman rats (5/sex/group) were fed with diet containing 0, 0.03, 0.125, 0.5 and 2 % Butyl cellosolve (EGBE purity unknown) corresponding to doses of 0, 18, 76, 310 and 1540 mg/kg/d (Mellon Institute of Industrial Research, 1952) for 90 days.

No deaths were seen attributable to the direct action of EGBE. Appetite was not affected and no pertinent micropathology was discovered. Tests for blood in pooled urine sample from the 2 %, 0.5 % and controls groups after 3 and 6 days were negative. The mean weight gain was lower than controls for the 2 % group. Relative kidney and liver weight were increased at 2 % dose and relative liver weight only was increased at 0.5 % dose.

The NOAEL can be considered to be 0.125 % (76 mg/kg/d) but considering that the test conditions were poorly reported (no control of the administrated substance, evaporation ?) this NOAEL will not be taken into account for the risk characterisation.

Groups of young DW albino rats (10 males and 10 females/group) were given food with EGBE (purity unknown) at doses of 1.25 – 0.25 – 0.05 or 0.01 % (corresponding to 919, 188, 38 and 7 mg/kg in males and 976, 222, 41 and 9 mg/kg in females respectively) during 3

months (Mellon Institute of Industrial Research, 1963). A control group was given food without EGBE Animals were weighted 4 times during the first week and once a week thereafter. After euthanasia liver and kidneys were weighted. Organs were examined for signs of pathology, the urinary bladder was examined for concretions. Histology was performed on representative tissues.

One animal died at day 73 after the beginning of the study in the high dose group. Food consumption was decreased in both males and females at 1.25 %. In males a significant decrease of food consumption was also observed at 0.25 % dose. A significant decrease of body weight gain was seen in animals dosed with 1.25 % EGBE. It was also seen in males at doses of 0.25 %. A small decrease was seen on the 2 first days of dosing only with the 0.05 % dose. The mean liver and kidney weight of males and females were definitely increased at the 1.25 % dose. At both 1.25 % and 0.25 % testes were atrophied. The NOAEL for this study can be considered to be 0.05 %. The purity of the EGBE used in this study is not given, nor does there appear to be analytical verification of EGBE doses, homogeneity and stability. This makes this study difficult to interpret given for example that testicular atrophy is not reported in other oral, repeated-dose studies even at higher doses of EGBE greater than 0.25% (e.g., 14-week drinking water study in rats and mice from NTP, 1993; continuous breeding study reported in Morrissey *et al.*, 1989). Given the availability of numerous other more recent and robust studies, this one cannot be considered reliable to derive the critical NOAEL.

Groups of 10 Albino rats (CR, COBS, CD-BR) were treated with various glycol ethers (including EGBE - purity > 99.5 %) via oral gavage, 5 days per week for 6 weeks (Eastman Kodak, 1982). Doses administrated were: 0, 222, 443 and 885 mg/kg. Body weight were recorded twice a week during the first week of exposure and every week after until termination of the study. Animals were observed daily for clinical signs. Blood samples were taken before euthanasia for the determination of haematology indices and biochemistry: SGOT, SGPT, alcaline phosphatase (ALP), LDH, urea nitrogen, creatinine and glucose. After termination, histological examinations were performed on the following organs: lung, heart, thymus, kidneys, liver, spleen, brain, salivary glands, stomach, intestinal tract, pancreas, oesophagus, adrenal glands, pituitary, thyroid, parathyroid, trachea, mesenteric lymph node (LN), testes and annexes, bone marrow, tongue, nasal cavities and eyes. Before fixation, liver, kidneys, heart, testes, brain and spleen were weighted.

2 out of 10 animals were found dead in the high dose. No mortality was seen in any other groups. A significant decrease in body weight gain was seen from D13 and after in animals treated with the high dose of EGBE. A significant decrease of food consumption was also seen in this group but only from D3 to D20. In other groups there was a trend to a decrease in bw gain compabable to controls and a lower food consumption in the middle dose group (not significant). Clinical symptoms were limited to bloody urine in the mid and high dose groups. Only one of the ten rats given the low dose of EGBE had bloody urine. This persisted through the third week of treatment. Other clinical signs at the mid and high dose were lethargy, unkempt hair coats, piloerection, rales, slight weakness and inactivity. All doses produced significant effects on RBC parameters, showing a characteristic pattern of haemolytic anaemia. No effects were seen on WBC. For biochemistry: a significant increase of alkaline phosphatase (PAL) was seen from the middle dose. Moreover, in the high dose, a significant increase of SGPT and a decrease of glucose (GLU) were noted. These changes were slightly different compared to controls so their toxicological significance is uncertain. Absolute and relative spleen weights were increased for the middle and high dose of EGBE. A small increase of the liver weight (only significant for relative weight) was seen for the high dose group. No effects were seen on testes weight (relative or absolute) at any dose group. For

gross pathology, only enlarged, dark spleen were seen for high and intermediate dose. Histopathological lesions were hyperkeratosis and acanthosis in the stomach epithelium in all treated animals. Hepatomegaly was also seen in 4 out of 10 animals of the high dose group. Haemosiderin deposition was seen in some animals (7/10 and 6/10 in the high and mid dose group respectively). Thymus atrophy was seen in one animal of the high dose group. Congestion of the spleen was seen in all treated animals. Extramedulary haematopoiesis was reported for one animal in the high dose group and haemosiderin deposit in the majority of animals of the high and mid dose group. For this study, the NOAEL can be considered to be lower than 222 mg/kg (which is the Low Observable Adverse Effect Level: LOAEL) based on effects seen on spleens and RBC parameters.

Toxic effects on the immune system of SD rats after 21 days of exposure to EGBE and EGME were assessed. Groups of 6 rats of each sex were exposed to EGBE (purity 97 %) in drinking water at concentration of0, 2 000 and 6 000 ppm corresponding to about 0, 180 and 500 mg/kg respectively for males and 1600 – 4800 ppm for females corresponding to 200 and 444 mg/kg respectively (Exon *et al*., 1991). All rats were injected SC at the base of the tail with 1 mg aqueous KLH in a 0.2-ml volume 7 days after initiation of glycol ether treatment. A second injection of aqueous KLH was given at the base of the tail on Day 13 to initiate an IgG antibody response. All animals were injected into the right footpad with 100 µl of heat-aggregated KLH on Day 20. The left footpad was injected with 100 µl of sterile saline. At the time of termination serum samples were collected for analysis of antibody levels to KLM. Spleens were used as the cell source to assess natural killer (NK) cell cytotoxicity and interleukin 2 (IL2) and interferon (IFN) production. All assays were performed on each rat on experiment.

The 24 hr Delayed-Type Hypersensitivity reaction (DTH) response was assessed on Day 21 by measuring the thickness of the heat-aggregated KLH-injected footpad and substracting the thickness of the saline-injected footpad. The reaction was expressed as millimetre difference in swelling between the two footpads.

Serum collected by cardiac puncture at time of termination (Day 21) was analysed for anti-KLH IgG antibodies by an indirect enzyme-linked immunosorbent assay (ELISA).

Natural killer cell cytotoxicity: suspension of spleen cells were prepared. Splenic natural killer cell cytotoxicity was assessed by the 4-hr ^{51}Cr release from YAC-1 tumour target cells as previously described (Exon *et al*., 1991). Specific ^{51}Cr release was calculated by: cpm experimental release - cpm spontaneous release x 100 / cpm maximum release by 2 % SDS - cpm spontaneous release. Spontaneous release of ^{51}Cr by target cells was less than 10 % in all assays.

Interleukin 2 production by rat spleen cells was measured by the capacity to support the growth of the IL2-dependent CTLL-2 cell line *in vitro*. This assay does not distinguish between IL2 and IL4 production. A sample of spleen cells was centrifuged and the cells were resuspended in RPMI (supplemented with penicillin, streptomycin, 5 % heat-inactivated foetal bovine serum). Samples were incubated 24 hr with concanavalin A and the cell-free supernatants were harvested and tested for IL2 activity. Briefly, 4 x 10^3 IL2-dependent murine CTLL-2 cells (in RPMI, 5 % foetal bovine serum, 5 x 10^{-5} M 2-mercapto-ethanol) were cultured in replicate 200-µl volumes in flatbottomed 96-cell culture plates in the presence of a log-2 dilution series of putative IL2- containing supernatants. After 18 hr, 1 µCi[^{3}H]TdR was introduced to each well for 4 hr, after which the cells harvested onto glass fiber filters, and the radioactivity measured by scintillation countering.

Interferon concentrations in supernatants of spleen cells cultured for 48 hr with 1.0 μg/ml concavalin A were assessed by the capacity to block the cytopathic effects of vesicular stomatitis virus on rat RT fibroblasts.

For pathological examination, the thymus, spleen, liver and right kidney and testis were collected and weighted at necropsy. The tissues were examined by light microscopy.

Body weights were decreased in male rats treated with 6000 ppm EGBE and in females treated with 1600 or 4800 ppm. No effects were seen in thymus or in testes (macroscopically or histologically). Relative liver weights were increased in male rats treated with the low dose of EGBE. Relative kidney weight was decreased in female rats treated with the low dose of BE. Microscopic changes were not observed in sections of liver and kidney taken from the BE-exposed rats. Histopathologic examination of the spleen was not done since all of this tissue was used as a source of cells for the immune function assays. Immune Function assessment: Natural killer cytotoxic responses were enhanced in male and female rats treated with the low dose of BE. No effect on antibody production was observed in the BE-treated rats. No significant effects of glycol ether treatment were observed on DTH reactions of these rats. The cellular infiltrate in the KLH-injected area of the footpad consisted mainly of mononuclear phagocytes, typical of a Type IV hypersensitivity reaction. The authors concluded that the immune system is not a sensitive target for EGBE.

EGBE (lot no. BT00504LP, Aldrich Chemical Co., USA, purity ≈ 99 %) was administered in drinking water at concentrations of 0, 750, 1500, 3000, 4500 or 6000 ppm to groups of 10 male and 10 female F344/N rats for 13 weeks. These concentrations provided target dose levels of 0, 100, 150, 250, 400 or 650 mg/kg bw per day. Estimates of compound consumption based on water consumption by rats were 69, 129, 281, 367 and 452 mg/kg/day for males and 82, 151, 304, 363 and 470 mg/kg/day for females. Supplemental groups of 10 rats/sex/group/time point were included for haematology and clinical chemistry observations at weeks 1 and 3 (NTP, 1993).

Rats were held in the testing laboratory for 11 - 12 days and were 6 weeks old when the study began. They were housed 5/cage, water and feed were available *ad libitum*. All rats were observed twice daily throughout the study. Body weights were recorded at the beginning of the study and then weekly until the end of the experiment. Clinical observations also were recorded weekly throughout the experiment. Haematological examinations were performed using a Series 7000 cell counter and a Series 810 whole blood platelet analyser (Baker Instruments). Clinical chemistry variables were measured with a Cobra Fara analyser (Roche Diagnostics). Evaluations of vaginal cytology and sperm morphology were done for the three highest doses tested. Results concerning these examination are reported in the reprotoxicity section.

Complete autopsies were made on all study rats. Microscopic examinations were made on all control group and treated rats.

No rats treated with EGBE died or were killed before the scheduled end of the study and there were no clinical signs of toxicity. Body weight gains were reduced in both male and female rats of the 4500 and 6000 ppm treatment groups. In males and females there were reductions in drinking water consumption in the higher dose groups, this being clearly concentration related in females, from a mean of 18.8 ml/day in the control group to 10.7 ml/day in the 6000 ppm exposure group. A markedly macrocytic and mildly hypochromic anaemia was observed at each time point and reticulocyte counts were moderately increased in weeks 1 and 13. For males there was a decrease in erythrocyte counts at all time points in the 3000 ppm and

greater groups, while in females the decrease occurred in the 1500 ppm and greater groups. A consistent thrombocytoapenia was observed at all time points in males and females of the 4500 and 6000 ppm groups; it also occurred in females of the 3000 ppm group at week 13. The most consistent blood chemistry observation was an increase in alkaline phosphatase, particularly in the high dose groups. This observation is consistent with mild cholestasis. Thymus weights were significantly reduced in males of the 4500 ppm group and males and females of the 6000 ppm group. Other organ weight changes were found, but these appeared to be secondary to body weight gain reduction (spleen was not weighed). Histopathological lesions occurred in the liver, spleen and bone marrow and male and female rats. Hepatic changes included primarily centrilobular hepatocellular degeneration and primarily centrilobular Kupffer cell pigmentation. These changes were present in the majority of dosed rats, but they were more prevalent in the 3000 ppm and greater dose groups and were slightly more severe in females. In addition, the cytoplasm of hepatocytes of rats at all dose levels was more eosinophilic and lacked the ampholytic-to-basophilic granularity typical of the controls. In the spleen there was an increase in haematopoiesis and deposition of haemosiderin. In bone marrow there was an hyperplasia characterised by an increase of haematopoietic cells and decrease in marrow fat cells. All of these lesions were present in the majority of dosed rats, but they were more prominent in the 3000 ppm and greater groups (NTP, 1993). There was no treatment effect on testis weights but there was a reduction in the size of the uterus in females at 4500 and 6000 ppm. Changes in uterine weight were considered by the authors to be secondary to the reduction in body weight gain rather than a direct effect of EGBE. The only spermatozoal measurement that showed significant change relative to the control group was sperm concentration which was slightly decreased in all groups of treated males; however, this effect was not dose-related. There were no significant differences from the control group in oestrous cycle length for treated females, although females treated at 4500 and 6000 ppm spent more time in dioestrous than the other groups. This correlates with the smaller uterine size, which was attributed to a secondary consequence of reduced body weight gain. These effects and their no relevance for risk assessment are more developed in the section 4.1.2.9.1 “other studies”. No NOAEL was identified in this study, based on cytoplasmic alterations in hepatocytes of both male and female rats at 750 ppm, equal to 69 mg/kg bw per day and 82 mg/kg bw per day in males and females respectively.

EGBE (purity > 99 %) was administered to female Fischer 344 rat (10 – 12 w old) via oral route (Nyska *et al.*, 1999b). Two groups of 8 animals were treated with EGBE at dose of 250 mg/kg or vehicle during three days. Animals were sacrificed 24 hours after the last treatment. Complete necropsy examination were performed on all rats, the following tissues and organs were examined microscopically: sternum, femur, head, vertebral column, tail, heart, lungs, liver, spleen, kidneys, eyes and brain. Both eyes were bisected mid-sagitally and 12 sections were prepared (6 stained with HE and six stained with PAS).

Gross abnormalities were noted only in EGBE treated animals and included bluish tail discoloration, watery blood, discoloured liver, dark kidneys and multiple Petechial haemorrhages on the sclera. Some ocular changes were observed in 5/8 treated rats. Retinal changes were bilateral and occasionally located in proximity to capillaries. Lesions included multifocal haemorrhage within the retinal pigment epithelium (RPE), the choriocapillaris between the RPE and the photoreceptors leading to retinal detachment, and the photoreceptors and inner and outer nuclear layer. The haemorrhage in the RPE were associated with degeneration, exfoliation and loss of these cells. The haemorrhages within the photoreceptors layers, sometimes associated with minimal polymorphonuclear cell infiltration, caused organizational disruption of the rods and cones and, at time, resulted in their loss. In the inner and outer nuclear layers, the haemorrhages were associated with degeneration and loss of cell

resulting in thinning, disorganization and fusion of these layers In a single rat, a patent retinal vessel was still surrounded by necrotic cells of the outer and inner nuclear layers, suggesting an ischemic-infarctive process. In another case, an area of necrosis was present in the outer retinal layers, probably related to occlusion of the regional blood vessel of the ciliary processes and in the limbus.

In the organs other than the eyes, a range of histopathological changes were observed. Occlusive trombi were noted in the submucosa of the most anterior section of the nasal cavity and the small calibre pulmonary vessels. Thrombosis was also noted in the dental pulp of the incisors in association with focal stromal and odontoblast coagulative necrosis. The femur and coccygeal vertebrae at intermedullary locations also exhibited thrombosis in association with necrosis of marrow cells, bone-lining cells, and both cortical and trabecular osteocytes. In the femur, the changes were usually confined to the diaphysis (medullary cavity and inner third of the cortex), while in the coccygeal vertebrae, the metephysis and epiphysis were also involved. Usual changes seen with EGBE were also noted in other organs: multifocal non-zonal hepatocellular necrosis, splenic extramedullary haematopoiesis, bone marrow erythroid hyperplasia and haemoglobinuric nephrosis.

These effects, seen in female rats only, seem to be closely related to haemolytic toxicity. As the rats are one of the most sensitive species to haemolytic effects of EGBE and humans are resistant to these effects such symptoms could occur but at very high doses (hardly obtainable in normal use conditions). The design and objectives of this study make it unsuitable to derive a NOAEL for Repeated Dose Toxicity.

Mouse

EGBE (lot no. BT00504LP, Aldrich Chemical Co., USA, purity ≈ 99 %) was administered in drinking water at concentrations of 0, 750, 1500, 3000, 4500 or 6000 ppm to groups of 10 male and 10 female $B6C3F_1$ mice for 14 weeks. These concentrations provided target dose levels of 0, 100, 150, 250, 400 or 650 mg/kg bw per day (NTP, 1993). Estimates of compound consumption based on water consumption by mice were 118, 223, 553, 676 and 694 mg/kg/day for males and 185, 370, 676, 861 and 1306 mg/kg/day for females.

Mice were held in the testing laboratory for 11 - 12 days and were 6 weeks old when the study began. They were housed individually, and water and feed were available *ad libitum*. All mice were observed twice daily throughout the study. Body weights were recorded at the beginning of the study and then weekly until the end of the experiment. Clinical observations also were recorded weekly throughout the experiment. Complete autopsies were made on all study mice. Microscopic examinations were made on all control group and treated mice.

No mice treated with EGBE died or were killed before the scheduled end of the study and there were no significant clinical signs of toxicity. Body weight gains were slightly reduced in both male and female mice of the 3000 and 6000 ppm treatment groups. No particular pattern was evident in drinking water consumption. Organ weight changes that were found were secondary to body weight gain reduction. No treatment related gross or microscopic lesions in male or female mice were found (NTP, 1993). The NOAEL of 223/370 mg/kg is not very reliable because no haematological analysis were performed on animals during or after the study.

In a series of studies, the mechanism of EGBE (purity 99 %) accumulation in forestomach was assessed (Poet *et al*., 2003). Five experiment were conducted to examine various end-points:

- target tissue histology / forestomach irritation
- tissue dosimetry and pharmacokinetics
- fur deposition
- salivary excretion
- retention in stomach content

For target tissue histology assessment, B6C3F1 adult mice (16/sex/dose) were treated with EGBE orally at doses of 400, 800 or 1200 mg/kg for 4 days (in fact due to an excessive unexpected mortality at these dose levels, surviving animals after 2 days of dosing were administered half of their corresponding original target dose). Half the animals were targeted for forestomach toxicity evaluation, the remainder were examined for liver effects. To investigate if parenteral administration could also produce forestomach lesions, extra groups of mice were administered saline solutions of EGBE by either SC or IP injection of 400 mg/kg for 4 days (6 animals/route of administration).

Following oral administration, neat EGBE caused dose-related forestomach lesions in all animals. This lesions were mainly epithelial hyperplasia and inflammation. Minimal lesions similar to those produced in the lower dose oral gavage groups were observed in 1/6 of the IP dosed mice and 2/6 of the SC dosed mice.

Summary oral route:

Table 4.74: Summary of studies performed by oral route

Study	NOAEL (mg/kg bw/d)	Effects	Reference
Rats			
90 days in food	0, 18, 76, 310 and 1540 mg/kg/d NOAEL = 76 mg/kg/d, LOAEL = 310 mg/kg/d	Poorly reported	Mellon Institute of Industrial Research, 1952
90 days in food	1.25 – 0.25 – 0.05 or 0.01 % 919, 188, 38 and 7 mg/kg in males and 976, 222, 41 and 9 mg/kg in females NOAEL = 0.05 % (38 and 41 mg/kg in male and females respectively)	Decrease in food consumption and body weight gain in males. Testes atrophy.	Mellon Institute of Industrial Research, 1963

6 weeks gavage	0, 222, 443 and 885 mg/kg LOAEL = 222 mg/kg	Haematological effects at all doses and irritant effects on the stomach	Eastman Kodak, 1982
21 days in drinking water	0, 180 and 500 mg/kg for males and 200 and 444 mg/kg for females. Not suitable for RA	No remarkable effects on the immune system	Exon *et al.*, 1991
13 weeks in drinking water	69, 129, 281, 367 and 452 mg/kg/day for males and 82, 151, 304, 363 and 470 mg/kg/day for females LOAEL of 69 and 82 mg/kg bw/day for males and females respectively.	Slight decrease in body weight gain and haematological effects. Liver effects.	NTP, 1993
3 days	250 mg/kg Not suitable for RA	Haematological effects, trombi formation.	Nyska *et al.*, 1999b
Mice			
4 days	Not suitable for RA	Irritation of the forestomach	Poet *et al.*, 2003
14 weeks in drinking water	118, 223, 553, 676 and 694 mg/kg/day for males and 185, 370, 676, 861 and 1306 mg/kg/day for females. NOAEL = 223 and 370 mg/kg bw/d for males and females respectively LOAEL = 553 and 676 mg/kg bw/d for males and females respectively	Slight decrease in body weight gain	NTP, 1993

Six studies on rats and two on mice are available. Effects seen by oral route were body weight reduction, haemolysis, hepatic effects and local irritation effects. Irritation to the

forestomach was seen after gavage dosing and, to a far lesser extent, after subcutaneous and intraperitoneal injection. This difference is most likely due to the higher local concentration after gavage dosing. Overall, a LOAEL of 69 and 82 mg/kg bw/d (in males and females respectively) can be fixed in a three-month study.

Other routes

EGBE was administered IP to female SD rats 5 times a week during 2 weeks at doses of 55 mg/kg (Bernard *et al.*, 1989). Urine collection was carried out before, every 2 days during the test and immediately after the last injection. The urinary concentration of albumin and β_2-microglobulin (β_2-m) was determined and also the activity of N-acetyl-β-glucosaminidase.

No significant effects were seen on albumin, β_2-m or N-acetyl-β-glucosaminidase urinary excretion. In conclusion, no glomerular damages were induced by EGBE treatment.

4.1.2.6.2 Studies in humans

Inhalation

Twenty one male paint formulators with an average age of 41.3 years (27 to 53) exposed to various chemicals (IPA, acetone, EA, benzene, toluene, xylene, white spirit) including EGBE, at exposure level of 0.09 time Threshold Limit Value of index solvent mixture for 20.2 years were studied with a battry of neurobehavioural performance tests including digit span, digit symbol, Benton visual retention, finger tapping, grooved peg board and aiming test (Foo *et al*., 1994). A group of 21 male workers unexposed served as controls. Personal exposure levels to EGBE ranged from 0.41 ppm to 3.13 with a mean of 1.26 ppm (N=4). The performance of the paint formulators was worse than the controls in neurobehavioural tests. Statistically significant results were observed in aiming test error score, digit span and grooved peg board. These results are not relevant for risk assessment due to the co exposure to other solvents.

Nine floorers occupationally exposed for 8 to 35 years to mixtures of organic solvents were subjected to analysis and characterisation of their lymphocyte sub-population (Denkhaus *et al*., 1986). They were matched with 9 healthy volunteers to have control values. Ages ranged from 25 to 58 years old. As an indicator of solvent exposure, air and blood samples of exposed men were taken by personal monitoring during one shift and one week before analysis. In addition to cell typing, the following parameters were obtained: haematological status, with differentiation, SGOT, SGPT, γGT, AP, cholesterol and NF. Lymphocytes obtained were labelled with monoclonal antibodies and counted for subpopulations: T4 helpers, T8 suppressor cells, total amount of T11 (all) cells, NK and B lymphocytes.

Some chemicals were identified by monitoring: butanol, iso-butanol, EGBE, EGEE, 2 methoxyethynol, toluene, m-xylene, 2-butanone, 2-hexanone. For EGBE, measurements gave the following results:

In air: mean concentration: 24.6 mg/m^3 (maximum concentration: 350 mg/m^3)

In blood: mean concentration: 4.1 μg/dl (maximum concentration: 108,2 μg/dl)

Between the exposed and the unexposed group there were only negligible differences in Hb concentration and erythrocyte number (a very slight decrease). In the relative number of

eosinophiles and stab cells (increase), segmented cells (decrease) and lymphocytes (increase), these differences were statistically significant. Others measured parameters where within the normal ranges. Significant differences in the distribution of the lymphocytes sub-populations were seen between exposed and unexposed group. In the exposed workers, T11 cell and T4 helper were decreased, while NK and B lymphocytes were increased. There were no changes in the T8 suppressor cell sub-population. In this study, the authors concluded that the observed decrease in helper/suppressor ratio might be an indicator of early haematological and/or immunological effect due to chemical exposure, which eventually may induce long term disorders of the haematopoetic and/or lymphopoetic system. But they also concluded than these results were only quantitative and did not reflect the functional state of each subpopulation. Since the workers were exposured to a variety of chemicals, it is not possible to draw conclusions for EGBE alone.

4.1.2.6.3 Mechanistic studies of liver pathology

Associated with haemolysis in rats and mice exposed to EGBE there were depositions of haemosiderin in Kupffer cells and hepatocytes. Haemosiderin is an insoluble product derived from iron-rich ferritin. In man, the major pathological effects of chronic hepatic iron overload are fibrosis and cirrhosis, porphyria cutanea tarda and hepatocellular carcinoma. Although precise pathogenic mechanisms remain unknown, iron probably produces these and other toxic effects by increasing oxidative stress and lysosomal lability (Bonkoavsky, 1991). Therefore, the development of the oxidative stress that is possible as a result of haemosiderosis was studied in male and female mice treated with EGBE by measuring the formation of 8-hydroxydeoxyguanosine (OH8dG), as an indicator of DNA damage, and malondialdehyde (MDA), as an indicator of lipid peroxidation, and the reduction in α-tocopherol concentration, as an indicator of a reduction in antioxidant capacity (Kamendulis *et al*., 1999). It is clear that α-tocopherol is not the only antioxidant in the cells, so studies in which only α-tocopherol was measured are not assessing the full antioxidant condition of the cells. In a later study, these investigations were continued by comparing EGBE induced oxidative stress and altered cell growth in male B6C3F1 mice and male F344 rats (Siesky *et al*., 2002). In addition, the relative sensitivities of male rat and male mouse hepatocytes to oxidative damage *in vitro* were examined (Kamendulis *et al*., 1999; Park *et al*., 2002a).

Groups of male and female $B6C3F_1$ mice were treated orally by gavage with EGBE at dose levels of 0, 300, 600 or 900 mg/kg bw for males and 0, 200, 400 or 600 mg/kg bw for females then killed by cervical dislocation and the livers removed, snap-frozen in liquid nitrogen and stored at -80°C until analysed for OH8dG, MDA and α-tocopherol. The results are presented in Table 4.75 as percentages of the control values.

Table 4.75: Effects of EGBE on indicators of oxidative stress in B6C3F1 mice, mean & standard deviation & percentage of the control (100 %) (Kamendulis *et al*., 1999)

Dose	MDA	α-Tocopherol	OH8dG
Males mice			
0	10.54 ±2.74	5.59±0.89	4.74±1.17
300	122	58	97
600	143*	27*	162*

900	174*	22*	248*
Female mice			
0	11.5±2.72	7.05±1.20	3.6±1.09
200	182*	74	123
400	167*	62*	196*
600	229*	45*	303*

* $P < 0.05$

units: MDA, nmol/g liver;

α-tocopherol, nmol/g liver; OH8dG, pmol/μmol dG

The α-tocopherol concentration was higher in females and was spared to a greater extent than in male mice. This could be indicative of a greater protective effect in females than in males. The oxidative damage to DNA was, however, very similar in male and female mice (high dose results being: males 11.76, females 10.91 pmol OH8dG /μmol dG) (Kamendulis *et al*., 1999).

In the more recent study, EGBE was administered daily by gavage to male B6C3F1 mice at doses of 0, 225, 450, or 900 mg/kg bw and to male F344 rats at doses of 0, 225 or 450mg/kg bw on 5 days per week for up to 90 days. These doses correlated with those of the 2-year inhalation studies (NTP, 2000). After exposure for 7, 14, 28, or 90 days, 15 mice and 5 rats from each treatment group were killed by asphyxiation. Seven days prior to sacrifice, an osmotic mini-pump filled with BrdU was implanted in each of five animals from each group. At sampling, livers were perfused with phosphate-buffered saline, removed, and weighed. A portion of liver was fixed in formalin, embedded in paraffin, and sectioned. The remaining livers were snap frozen in liquid nitrogen and stored at -80ºC. Blood was collected in micro-capillary tubes for haematocrit measurements. Liver sections were stained for ferric iron using the Perl method (Bugelski, 1985). Positive Perl staining was localized to Kupffer cells and approximately 2000 cells were counted for each animal. A "Perl index" was determined (total number of labelled Kupffer cells/ total number of cells counted x 100). Immunohistochemical detection of BrdU in liver was performed as described (Eldridge *et al.,* 1990). Cells in 15 random fields were counted (approximately 5000-6000 cells) in each animal. The labeling index was determined in both endothelial cells and hepatocytes (total number of labeled cells/total number of cells x 100). Endothelial cell identity was confirmed by staining a serial section with anti-factor VIII (Sharifi *et al*., 2000). Apoptosis and mitosis in the liver was defined and quantified as described (Bursch *et al*., 1985). The identification of apoptotic cells was confirmed using TUNEL. Cells in 15 random fields were counted (approximately 5000-6000 cells) in each animal. Apoptotic and mitotic indicies were determined (total number of labeled cells/total number of cells x 100). DNA damage expressed as OH8dG was measured in DNA isolated by sodium iodide extraction (Wang *et al.,* 1994) and MDA was measured in liver as described by Bagchi *et al.,* (1993).

EGBE decreased haematocrit and increased spleen weights in both rats (225 and 450 mg/kg bw dose groups) and mice (450 and 900 mg/kg bw dose groups). Kupffer cell Perl staining showed dose and time related increases in the Perl index that also occurred in both rats and mice. These observations confirmed that haemolysis and hepatic deposition of iron after treatment with EGBE occurred in males of both rats and mice. There was also a reduction in antioxidant reserve as a result of this treatment, because hepatic α-tocopherol concentrations

were decreased in both mice and rats at 7 and 90 days, although they were not significantly reduced at 14 or 28 days. An important difference between mice and rats is that control levels of α-tocopherol in this experiment were only about 1.5 nmol/g liver in mice, whereas they about 4 nmol/g liver in rats. Even after treatment, the reduced α-tocopherol levels in rats at both 7 and 90 days were higher than the control levels in mice. Thus, rats remained adequately protected from oxidative damage, whereas mice did not.

Treatment of mice with EGBE doses of 450 or 900 mg/kg bw resulted in an increase in oxidative damage in both DNA (OH8dG) and lipid (MDA) at both the 7 and 90 day treatment points, but not at the intermediate times of 14 and 28 days. In contrast, neither OH8dG nor MDA concentration was increased in rats at any dose level or at any sampling time.

DNA synthesis was increased in liver endothelial cells of mice at 7 and 14 days (all dose levels) while DNA synthesis in hepatocytes of mice was only increased at 90 days (all dose levels). No changes were seen at the other observations times in mice and no changes were seen in rats in either endothelial cell or hepatocyte DNA synthesis at any dose level or at any time point. No changes in either apoptosis or mitosis were observed in the liver of either species at any dose or time point.

In summary, EGBE caused an increase in oxidative damage and DNA synthesis in mouse cell types that correspond to those from which the tumours were observed to arise, while no changes were seen in rats, which did not show an increase in either haemangiosarcomas or hepatocellular neoplasms (Siesky *et al*., 2002).

Hepatocytes were isolated from male $B6C3F_1$ mice and male F344 rats by a two-step *in situ* collagenase perfusion, after which the cells were filtered and centrifuged (Kamendulis *et al*., 1999; Park *et al*., 2002a). Viability, determined by trypan blue exclusion, was routinely greater than 90 %. The cells were plated and cultured overnight. The medium was changed and replaced with EGBE or BAA at concentrations of 1, 5 or 25 mM, or ferrous sulphate at concentrations of 1, 5 or 25 μM and incubations continued for 24 hr. Cytotoxicity was determined at that time by measurement of lactate dehydrogenase leakage into the medium and this compared with total lactate dehydrogenase in lysed cells. This experiment was performed on four independent occasions. For each of the substances, the lower two concentrations were not cytotoxic to either mouse or rat hepatocytes, whereas the highest concentration of each substance increased the concentration of lactate dehydrogenase in the medium in the range 1.6-fold to 2.6-fold.

Using the same exposure conditions as in the previous *in vitro* study of cytotoxicity, measurements were made of OH8dG, MDA and α-tocopherol in three independent experiments. The summarised data are shown in Table 4.25. Neither EGBE nor BAA induced any increase in oxidative damage expressed as OH8dG or MDA at any concentration in either rat or mouse hepatocytes. In contrast, ferrous sulphate induced significant increases in both OH8dG and MDA of mouse and rat hepatocytes. A clear species difference in sensitivity of the hepatocytes was seen, with mouse hepatocytes being much more sensitive than rat hepatocytes. In rat hepatocytes, only the highest concentration of ferrous sulphate induced oxidative stress, while in mouse hepatocytes OH8dG was elevated from the lowest concentration, 1μM, and cellular MDA was increased and α-tocopherol decreased at concentrations of 5 and 25μM ferrous sulphate. MDA in the culture medium reflects the amount of lipid oxidation products expelled from the cells during exposure, in contrast to cellular MDA that only indicates what remains at the end of exposure. Medium MDA was elevated at all ferrous sulphate concentrations in mouse hepatocytes cultures, whereas only

the highest concentration of ferrous sulphate elevated medium MDA in the rat hepatocytes cultures.

Further studies in this series demonstrated that there was no interaction between ferrous sulphate and either EGBE or BAA in the production of oxidative stress. Furthermore, when iron was presented as haemolysed erythrocytes (Table 4.76), there was a dose-dependent increase in oxidative stress in mouse hepatocytes, demonstrated as increases in OH8dG and MDA and a decrease in α-tocopherol concentration (Park *et al*., 2002b).

Table 4.76: Effects of EGBE, BAA and ferrous sulphate on indicators of oxidative stress in male B6C3F1 mouse and male F344 rat hepatocytes, mean & standard deviation & percentage of the control (100 %) (Kamendulis *et al*., 1999; Park *et al*., 2002a)

Dose	OH8dG	α-Tocopherol	MDA (cells)	MDA (medium)
Males mouse hepatocytes				
0	2.78±0.26	4.85±0.42	0.70±0.04	3.40±0.39
EGBE (mM) 1.0	102	97	107	109
5.0	147	93	116	115
25.0	142	106	118	122
BAA (mM) 1.0	106	90	98	96
5.0	149	86	104	111
25.0	132	94	109	112
$FeSO_4$ (μM) 1.0	151*	96	112	177*
5.0	228*	67*	127*	335*
25.0	360*	57*	241*	458*
Male rat hepatocytes				
0	3.63±0.63	6.58±0.59	0.80±0.04	4.04±0.19
EGBE (mM) 1.0	115	92	97	100
5.0	98	92	100	102
25.0	91	90	121	118
BAA (mM) 1.0	153	94	102	103
5.0	140	94	106	103
25.0	122	91	114	120
$FeSO_4$ (μM) 1.0	123	93	116	115
5.0	141	79	115	108
25.0	188*	75	165*	163*

* $P < 0.05$

units:MDA, nmol/10^6 cells or nmol/ml medium; α-tocopherol, ng/10^6 cells; OH8dG, pmol/μmol dG.

Table 4.77: Effects of haemolysed erythrocytes (RBCs) on oxidative stress parameters in mouse hepatocytes (Park *et al*., 2002a)

Treatment	MDA	α-Tocopherol	OH8dG
Water	0.81 ±0.21	4.35±0.37	3.33±0.52
0.1 x 10^6 RBCs	0.79±0.15	3.25±0.54	3.41±0.62
1.0 x 10^6 RBCs	1.22±0.34*	2.15±0.63*	6.78±0.43*
10 x 10^6 RBCs	2.36±0.44*	2.25±0.31*	13.40±1.02*

units:MDA, nmol/10^6 cells or nmol/ml medium; α-tocopherol, ng/10^6 cells; OH8dG, pmol/μmol dG

* Significantly different from the control ($p < 0.05$)

The effect of ferrous sulphate (to mimic the iron deposition in liver resulting from haemolysis) on morphological transformation of Syrian hamster embryo (SHE) cells was studied (Park *et al*., 2002b). Significant increases in transformation frequency were observed after exposure to concentrations of 2.5 and 5.0 μg/ml for 7 days. The greatest effect was a transformation frequency of 0.71 %, compared with a medium control frequency of 0.06 %, at 2.5 μg/ml. Co-incubation of this ferrous sulphate concentration with antioxidants [25 or 50 μM (-)-epigallocatechin-3-gallate or 150 μM α-tocopherol] significantly reduced the induced cell transformation frequency. Park *et al.* (2002b) also showed that 1.0 and 2.5 μg/ml ferrous sulphate induced DNA strand breaks in SHE cells, as demonstrated with the single-cell gel electrophoresis (Comet) assay, as well as the formation of OH8dG and that co-incubation with either 25 μM (-)-epigallocatechin-3-gallate or 200 μM α-tocopherol completely abolished both of these expressions of ferrous sulphate-induced DNA damage.

In summary, these studies demonstrate that rat hepatocytes in culture are markedly less susceptible to oxidative stress than are mouse hepatocytes. *In vivo* there appears to be a greater antioxidant reserve in rat liver than in mouse liver, so that reduction in α-tocopherol levels observed in both species after EGBE treatment resulted in levels in rats that always remained higher than even the untreated control level in mice. Also *in vivo*, male mouse liver is marginally more susceptible than female mouse liver to oxidative damage, as demonstrated by the higher background rate of hemangiosarcoma lesions. In addition, it has been shown that ferrous sulphate can induce DNA damage as well as morphological transformation in SHE cells and these effects can be reduced or abolished by antioxidants.

4.1.2.6.4 Mechanistic studies on forestomach pathology

Green *et al*., 2000a, 2001 and 2002 have shown by whole body autoradiography that the administration of radiolabelled EGBE to mice by whole body exposure to its vapour results in a significant ingestion of the condensed substance during grooming. The autoradiographs showed that the fur and skin contained high concentrations of radioactivity even 48 h after the end of exposure and there was evidence of selective accumulation of radioactivity in the forestomach, the glandular stomach containing only low, background levels of radioactivity. Ingestion can, perhaps, also result from condensation of high vapour concentrations in the nasopharynx. In this way, EGBE can reach the stomach, where there is the potential to cause

the damage observed in the lifetime inhalation exposure study (NTP, 2000). Subsequently, the capacity of the stomach to metabolise EGBE was examined and a basis for the differential response of mice and rats investigated and comparison made between rodent and human stomachs. The study was not conducted to GLP guidelines.

Although this qualitative study suggests that significant exposure of the forestomach to EGBE delivered as a vapour could occur as a result of ingestion of condensed material, this is not supported by the results of one quantitative study. In an inhalation experiment in which female mice were exposed (whole-body) to 229 ppm EGBE for 6 hr, an average of 205 ± 69 µg EGBE was recovered from the fur of the animals, i.e., < 10 mg/kg bw, while the concentration of EGBE in the blood at the end of the exposure period was 3.0 mg/L and the BAA concentration was 235 mg/L (Poet *et al*., 2003). These blood concentrations were similar to those found in the inhalation exposure carcinogenicity assay (Dill *et al*., 1998). Thus, it would appear that some, but not a great deal of EGBE could reach the forestomach as a result of grooming. The authors do acknowledge, however, that some material could have been lost by evaporation and by the hot water washing procedure, which was adapted from a similar process that had been used for ethylene glycol, which is a less volatile material.

Female B6C3F1 mice were given a single intravenous dose of 2-butoxy[1-^{14}C]ethanol and killed at 4, 24 and 48 h after dosing and the distribution of radiolabel examined in the animals. High levels of radiolabel were present in the liver, oesophagus and buccal cavity throughout the time course of the study. Similar concentrations of radioactivity were found in both the forestomach and glandular stomach. The radioactivity in the stomach tissues was derived from the systemic circulation and, in addition, there was evidence of ingestion of radiolabelled material from the buccal cavity. The origin of the latter is unknown, but it may be derived from the salivary and Harderian glands, which contained significant amounts of radioactivity (Bennett, 2001).

Other experiments of Green *et al*., 2001 and 2002 lend support to other aspects of the development of forestomach lesions. Groups of five female B6C3F$_1$ mice were administered orally by gavage either EGBE or BAA daily for 10 days at dose levels of 0, 50, 150 or 500 mg/kg bw per day (Green *et al*., 2001 and 2002). Seventeen hours after the last dose and 1 hr before they were killed, the mice received a subcutaneous injection of bromodeoxyuridine, 15 mg/kg bw. Stomachs were removed for histology and quantification of cell proliferation, and blood samples were taken for haematology. In the top dose group of mice receiving BAA, one died as a result of causes unrelated to treatment. Haematological changes typical for these compounds were observed in all dose groups administered BAA, but of those receiving EGBE, they were largely confined to the 500 mg/kg bw per group. No abnormalities were seen in the glandular part of the stomach in any of the groups. No abnormalities were found in the forestomach of mice dosed with EGBE at doses of 50 or 150 mg/kg bw per day or with BAA at the 50 mg/kg bw dose level. The only abnormality observed in the forestomach was hyperkeratosis, which was minimal in two mice given EGBE at 500 mg/kg bw per day and in three mice given BAA at 150 mg/kg bw per day. In the 500 mg/kg bw per day group given BAA, all four mice that completed the study showed hyperkeratosis, three to a slight degree and one to a moderate degree. The cell proliferation part of the study was invalid because of a high control group value. Within the groups treated with either compound, there were dose related increases in S-phase cells in the forestomach areas. Furthermore, the development of hyperkeratosis is, in itself, evidence of increased cellular proliferation.

As part of the same study, a group of 10 female mice were given oral doses of 2-(1-[^{14}C]butoxy)ethanol, 500 mg/kg bw per day for 5 days, then killed 17 hr later. Glandular stomach and forestomach areas were removed, washed, separated and homogenised for

analysis. Homogenised washed forestomachs and glandular stomachs contained very little covalently bound radioactivity and none was identified as associated with particular protein bands following SDS-PAGE analysis. Therefore, it appears that covalent binding plays little, if any, role in the forestomach toxicity of EGBE.

Untreated F344 rats and B6C3F_1 mice were killed, their stomachs removed and divided into forestomach and glandular stomach sections. Pooled samples of the same tissue were homogenised, stored at -70° until required and then alcohol and aldehyde dehydrogenase enzyme kinetics determined, using EGBE and BAL, respectively, as substrates. Generally, the activities were similar in both regions of the stomach for both enzymes and there were no major species differences in aldehyde dehydrogenase activity. In the case of alcohol dehydrogenase, however, there were major differences between rats and mice, both in affinity constants (K_m) and maximal rates (V_{max}). The K_m values were two orders of magnitude greater in both regions of the mouse stomach, compared to the rat, and V_{max} was up to one order of magnitude higher in the stomach of the mouse than in the rat. The absence of any marked regional difference in enzyme activity in the homogenates would appear to fail to explain the regional differences in toxic and neoplasic response. However, histocytochemical localisation of the enzymes showed that, in the forestomach, the dehydrogenase activities are strongly localised in the stomach lining, whereas in the glandular stomach they are more homogeneously distributed throughout the stomach wall. Estimation of the relative volumes of each stomach compartment containing enzyme activity shows an approximately 1:25 ratio between forestomach and glandular stomach. Consequently, the enzyme activity is concentrated in a much smaller volume in the forestomach, so that, in a whole tissue homogenate, the activity is diluted (Green *et al*., 2001 and 2002).

Independent experiments on the mode of action in the forestomach have been conducted elsewhere. Adult male and female B6C3F_1 mice were treated orally by gavage with undiluted EGBE at dose levels of 0, 400, 800 or 1200 mg/kg bw for 2 days, followed by 0, 200, 400 or 600 mg/kg bw for 2 days. The doses were reduced because of excessive mortality caused, apparently, but unexpectedly, by the original doses. Survival did not improve at these lower doses, so treatment was discontinued. Two other groups of mice were treated by either subcutaneous or intraperitoneal injection with EGBE in saline at a dose level of 400 mg/kg bw for 5 days. Dose related incidences of forestomach lesions occurred in all male and female mice after oral administration of undiluted EGBE. These lesions included focal hyperplasia in the low dose group and more diffuse hyperplasia at the higher doses associated with significant infiltration by neutrophils and mononuclear inflammatory cells in the submucosa and muscularis. There were increases in the proliferating cell nuclear antigen labelling in regions surrounding the areas of hyperplasia. Minimal lesions similar to those found in the low dose (200 – 400 mg/kg bw) oral administration group were found in 1/6 intraperitoneally dosed mice and in 2/6 subcutaneously dosed mice, following 4 days administration of 400 mg/kg bw (Poet *et al*., 2003).

As part of this same study, the tissue dose and pharmacokinetics of EGBE were also compared after an oral dose by gavage of 265 mg/kg bw (target dose 250 mg/kg bw) and an intraperitoneal injection of 53 mg/kg bw or 261 mg/kg bw (target doses 50 and 250 mg/kg bw). Regardless of the dose and the dose route, EGBE disappeared from the circulation within 1 hr, while BAA was detectable up to 12 hr after a target dose of 250 mg/kg bw by either route. EGBE concentrations in the forestomach and glandular stomach tissues were higher than in blood and other tissues and EGBE persisted for a longer period in the stomach tissues, so that, regardless of the dose route, the $t_{½}$ and AUC for EGBE were higher in forestomach

tissues than in any other tissues. These data and the corresponding data for BAA are shown in Table 4.78.

In a separate experiment, the concentrations of EGBE and BAA in saliva were measured after oral and intraperitoneal dosing. The results are also shown in Table 4.78. Of particular significance is the relatively high concentrations of both compounds in the saliva after intraperitoneal dosing.

Table 4.78: Halve lives and AUC in blood, saliva and tissues. (Poet *et al.*, 2003).

Route, dose in mg/kg bw	Tissue	EGBE		BAA	
		AUC (µg/g/h)	$t_{½}$ (h)	AUC (µg/g/h)	$t_{½}$ (h)
Ip (261 ± 34)	Blood	NA	NA	442.8	1.05
	Liver	4.52	0.66	317.0	1.10
	Kidney	NA	NA	507.4	1.44
	Forestomach	274.2	2.60	468.4	4.62
	Glandular stomach	34.7	1.86	289.6	1.31
Oral (265 ± 13)	Blood	NA	NA	1740.7	2.11
	Liver	16.7	0.57	604.1	2.09
	Kidney	NA	NA	847.8	2.71
	Forestomach	3063.9	3.40	539.3	3.96
	Glandular stomach	746.5	4.91	186.6	1.57
Ip (258 ± 66)	Blood	43.4	0.16	2194.0	1.42
	Saliva	66.1	0.17	143.2	0.69
Oral (264 ± 42)	Blood	27.1	0.35	900.6	1.55
	Saliva	26.3	0.27	66.8	0.85

Probably as a result of the secretion of EGBE in saliva, significant quantities of the compound can reach the forestomach contents, even after intraperitoneal dosing.

Other, general factors contribute to the preferential toxicity to the forestomach and are unrelated to the actual compound being investigated.

In rodents, including the mouse, rat, and Syrian hamster, the stomach is divided into two parts by the mucoepidermoid junction separating squamous from glandular epithelium. The proximal part, or forestomach, is non-glandular, continuous with the oesophagus, and lined by keratinised, stratified squamous epithelium. The distal part, or glandular stomach, empties into the duodenum and is lined by a specialized glandular epithelium. The forestomach is separated from the glandular stomach by a grossly visible, elevated fold, the limiting ridge. This contrasts with the anatomy of the human stomach, the lining of which is entirely

glandular, the human mucoepidermoid junction occurring where the oesophagus joins the stomach. There is thus no forestomach in human beings. There is a histological similarity between the rodent forestomach and the human oesophagus, but the physiological functions of these two organs are quite different. While the forestomach is a storage organ, where ingested material may reside for several hours before its transfer to the glandular stomach, the oesophagus is not, ingested material passing through in a matter of seconds. Exposure of the human (or rodent) oesophagus to ingested material is, therefore, brief and it is to be noted that the histology of the rodent oesophagus is not unlike that of the human organ, yet the oesophagus of the rats and mice exposed to EGBE in studies described here does not respond in the same way as the forestomach.

4.1.2.6.5 Summary of repeated dose toxicity

In rats and mice, haemolysis was consistently observed (whichever the route of administration) and was sometimes associated with hepatic effects (Kupffer cell pigmentation and absolute and relative liver weight increases), effects on body weight gain, hyaline degeneration of the olfactive epithelium (by inhalation), effects on the forestomach and effects on the WBC sub-populations (T lymphocyte). In these studies and for the inhalation route, no NOAEC was identified for mice, whereas a NOAEC value of 25 ppm (121 mg/m^3) in rats was identified. In a separate study a LOAEC value of 31 ppm (150 mg/m^3) can be established in rats, based on haemolysis and Kupffer cell pigmentation. Due to the closeness of the apparent LOAEC and NOAEC, it is considered prudent to take the more conservative LOAEC of 31 ppm forward for risk characterisation. However, the likelihood that this figure is close to the NOAEC will be taken into account in deriving appropriate assessment factors.

Slight effects on the immune system were seen in rats, mice and humans on NK cells or T lymphocyte sub-population. In the human study, co-exposure to a number of chemicals does not allow reliable conclusions to be drawn for EGBE alone. In the rodent studies, however a NOAEL of 1000 mg/kg bw in mice by dermal route can be established. The effects seen were small. A role of EGBE in the induction of immunotoxicity has not been developed to a point where it can be used in risk characterisation.

For the dermal route, a NOAEL of 150 mg/kg bw/d (the highest dose tested) has been determined from a 13-week study in rabbits.

For the oral route, a LOAEL of 69 and 82 mg/kg/day for male and female rats respectively, was found in a 13 -week drinking water study(haemolytical effects).

As humans are far less sensitive than other species (except Guinea Pig) to the haemolytical properties of EGBE, we have tried to assess separately haemolytical effects and related effects and other specific toxic effects which could be induced by EGBE. For all the studies, no specific relevant toxic effects, other that haemotoxicity, can be identified.

For the risk characterisation, haemotoxicity will be the end point chosen keeping in mind the interspecies differences (human/rodents) to calculate margin of safety. No other lesion has been identified which can be specifically attributed to treatment with EGBE.

4.1.2.7 Mutagenicity

The mutagenic properties of ethers of ethylene glycol and their metabolites in general and EGBE in particular have been reviewed (Elliott and Ashby, 1997 ; McGregor, 1984 and 1996; Tyler, 1982).

4.1.2.7.1 Studies *in vitro*

Bacterial studies

EGBE and its metabolites, BAL and BAA, have been tested for their potential to induce gene mutations in bacteria on a number of occasions; these and the results are listed in Table 4.79.

Two studies in which five different strains of *Salmonella typhimurium* were used, null results were obtained with dose levels up to 20 mg per plate, in one case, and 10 mg per plate in the other. The latter was an Ames' test conducted by the US National Toxicology Program (NTP) in which supplementary metabolic activation conditions were provided by rat liver S9 mixes at 10 % and 30 % and Syrian hamster liver S9 mixes at 10 % and 30 % (Zeiger *et al*., 1992). Nevertheless, it was concluded in a later study that EGBE was mutagenic as demonstrated by its activity in *S. typhimurium* TA97a, at a minimal dose of 2.2 mg per plate in the absence of S9 mix and 9.0 mg per plate in the presence of rat liver S9 mix at 2.5 %. Thus, the activity of EGBE was reduced by the presence of the supplementary activation system. The mechanism of this reduction was not investigated. The authors imply, however, by referring to the absence of mutagenic activity in tests with BAL and BAA, that the reduction in mutagenic activity of EGBE was due to its metabolism to nonmutagens (Hoflack *et al*., 1995). *S. typhimurium* TA97a is very similar to the strain TA97, the difference between the strains being in the length of the deletion through the *uvrB* locus. Consequently, important differences in the responsiveness of the strains should not have been expected. Both strains carry the *hisD6610* mutation that has a run of six cytosines at the mutation site and requires a frame shift mutation for its reversion to histidine independence. Furthermore, EGBE is not the type of chemical structure that would be anticipated to produce a frameshift mutation.

Gollapudi *et al*. (1996) performed a carefully designed study that covered the conditions used by Hoflack *et al*., 1995 and using *S. typhimurium* TA97a, TA100 and *Escerichia coli* WP2*uvrA*. There was no indication of an increase, significant or not, at any EGBE dose level between 0.5 and 10.0 mg per plate. The possible contribution of peroxides to the mutagenicity observed in the Hoflack *et al*., 1995 study was discussed, but dismissed. The lack of an effect in strain TA 97a in the Gollapudi *et al*., 1996 study supports the absence of a mutagenic response from the closely related strain TA 97 in the Zeiger *et al*., 1992 experiments. Nevertheless, the debate continues, because Hoflack*et al.* (1997) in a letter to the journal editor, reported that the activity in strain TA97a reported earlier was again observed upon re-testing in their laboratory.

Mammalian cell studies

EGBE was tested for gene mutagenic activity in Chinese hamster ovary (CHO) cells at the *hprt* locus (Slesinski and Weil, 1980). Null responses were obtained in this assay, in which test compound concentrations used were up to 1 % (v/v), approximately 9 mg/ml, in the absence of S9 mix and up to 0.5 % (v/v), approximately 4.5 mg/ml, in the presence of S9 mix.

Null responses were also obtained by Chiewchanwit and Au (1995), at the *gpt* locus in CHO-AS52 cells exposed to either EGBE at concentrations up to 0.9 mg/ml (relative survival 79 %) or BAL at concentrations up to 0.065 % (v/v) (relative survival 91 %), the next higher dose, 0.2 %, being too toxic to permit its evaluation for mutagenicity. These cells were derived from *hprt*-deficient CHO cells and contain a single autosomal copy of the xanthine guanine phosphoribosyl transferase (*gpt*) gene, which is the bacterial equivalent of the mammalian *hprt* gene. It had been demonstrated previously in the same laboratory (Ma *et al*., 1993) that methoxyacetaldehyde, a metabolite of EGME, is mutagenic in CHO-AS52 cells, but not in the standard CHO cells. Methoxyacetaldehyde was used as a positive control in this study. However, Elias *et al.*,. (1996) reported that both EGBE and BAL induced significant increases in 6-thioguanine-resistant phenotypes (i.e., the *hprt* locus) in Chinese hamster lung V79 cells. Unfortunately, the data are inadequately described in this paper, there being no indication of variation or of survival. Reading from a graphical presentation (log dose scale), it appears that the reported significant response EGBE occurred at a concentration of about 9 mg/ml. This description is inadequate for evaluation purposes.

Table 4.79: Test for gene mutation induction in bacteria by EGBE and its metabolites

Test system	Source & purity of chemical	Result[a] Without exogenous metabolic system	With exogenous metabolic system	Dose[b] (LED/HID)	Reference
EGBE					
Bacteriophage T4D mutation	Not stated	- (severe toxicity)	NT	111 µl/ml	Kvelland, 1988
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	Dupont de Nemours, USA (purity not stated)	-	-	20,000 µg/plate	Sippel and Krahn, 1977
Salmonella typhimurium TA100, TA1535, TA1537, TA97, TA98, reverse mutation	Fisher Scientific, USA > 99 %	-	-	10,000 µg/plate	Zeiger *et al.*, 1992
Salmonella typhimurium TA100, TA98, TA102 reverse mutation	Merck, Germany > 99 %	-	-	14,000 µg/plate	Hoflack *et al.*, 1995
Salmonella typhimurium TA97a, reverse mutation	Merck, Germany > 99 %	+	+	2,200 µg/plate	Hoflack *et al.*, 1995
Salmonella typhimurium TA97a, TA100 & *Eschichia coli* WP2*uvrA* reverse mutation	Dow Chemical Co., USA 99.04 %	-	-	10,000 µg/plate	Gollapudi *et al.*, 1996
BAL					
Salmonella typhimurium TA100, TA98, TA97a, TA102 reverse mutation	Chemical synthesis > 91 %	-	-	7,000 µg/plate	Hoflack *et al.*, 1995
BAA					
Salmonella typhimurium TA100, TA98, TA97a, TA102 reverse mutation	Janssen Chimica, Belgium > 99 %	-	-	1,000 µg/plate	Hoflack *et al.*, 1995

[a] +, positive; –, negative; NT, not tested;
[b] LED, lowest effective dose; HID, highest ineffective dose

In tests for the induction of sister-chromatid exchanges (SCEs) in CHO cells, it was concluded in a US NTP-sponsored study (NTP, 2000) that there was no significant induction by EGBE concentrations up to 3.5 mg/ml in the absence of S9 mix and 5.0 mg/ml in the presence of rat liver S9 mix, although a trend test was significant (thereby judged to be an equivocal result) in the absence of S9 mix in one of two trials, the other being clearly negative. Slesinski and Weil (1980) also did not show an increase in SCE frequency at concentrations up to 0.25 %. On the other hand, Elias *et al*. (1996) reported a small induction of SCEs in V79 cells and Villalobos-Pietrini *et al*. (1989) found a significant increase in SCEs in cultured human lymphocytes in the absence of S9 mix of approximately 2.5-fold at a concentration of 0.5 mg/ml, the lowest tested, rising to approximately 4.3-fold at 3.0 mg/ml, the highest tested. Elias *et al*. (1996) reported a much larger induction of SCEs in V79 cells treated with BAL, but no effect of BAA. The data were presented only in a graphical form with no indication of variation or survival. It is noted that the reports of significant increases in the frequency of SCEs by Villalobos-Pietrini *et al*. (1989) and Elias *et al*. (1996) were observed at lower concentrations than used in the studies of Slesinski and Weil, 1980 and NTP (2000), neither of which reported any significant change in SCE frequency.

In three independent investigations, chromosomal aberrations were not induced in cultured mammalian cells. These included an investigation with CHO cells in which EGBE concentrations up to 5.0 mg/ml did not induce aberrations in two of 3 trials in the absence of S9 mix or in a single trial in the presence of S9 mix (NTP, 2000); an experiment with V79 cells for which no experimental details or results were presented (Elias *et al*., 1996); and two experiments with human lymphocytes with concentrations up to 3.0 mg/ml (Villalobos-Pietrini *et al*., 1989) and 0.3 mM (Elias *et al*, 1996). It was reported that chromosomal aberrations (not including gaps) increased by BAL in both V79 cells and human lymphocytes by Elias *et al*. (1996). Once again, the data were presented only in a graphical form with no indication of variation or survival. A small increase in micronucleated cell frequency was observed, however, in V79 cells treated with EGBE at a concentration of least 1.0 mg/ml, where there were 1.50 % micronucleated cells compared with 0.89 % ± 0.24 % in the controls. A similar very small increase that was, nevertheless, statistically significant was observed with BAA (1.71 % at 0.66 mg/ml), while BAL treatment resulted in 1.91 % micronucleated cells at about 0.005 mg/ml and 48.13 % micronucleated cells at about 0.020 mg/ml. Many of these cells contained 3 – 4 nuclei per cell and cells with several micronuclei in place of the parental nucleus. These latter observations are suggestive of aneuploidy. Investigation of the treated V79 cells showed that the percentage of hyperdiploid cells was increased by EGBE and by BAL in particular, whereas BAA had hardly any effect (Elias *et al*., 1996). These *in vitro* mammalian cell studies are summarised in Table 4.80.

Other *in vitro* mammalian cell studies

Inhibition of gap-junctional intercellular communication was studied in a mixed culture of *hprt*$^+$ and *hprt*$^-$ V79 cells. EGBE was active in the assay at non-cytotoxic concentrations, but neither BAL nor BAA showed any activity (Elias *et al*., 1996).

In a test for morphological transformation of Syrian hamster embryo cells treated for 7 days, BAL up to 1.0 mg/ml was not active, but no details of the results were presented. It is not clear whether EGBE and BAA also were tested, but found to be inactive (Elias *et al*., 1996).The text indicates that BAL was inactive, while a figure shows EGBE to be inactive. In a similar assay, but with a 24 hr exposure time, a significant result was recorded for two doses (1.0 and 1.25 mg/ml, with relative plating efficiencies of 70 and 53 %, respectively), but not for a higher dose (1.5 mg/ml at which the relative plating efficiency was 42 %) (Kerckaert *et*

al., 1996). This result was not repeated in another Syrian hamster embryo cell transformation study, in which both EGBE and BAA were tested at concentrations up to 20 mM for 7 days. No significant increase in transformation frequency was found with either compound, although at 8.0 mM EGBE the transformation frequency was 0.52 when the relative plating efficiency was 73.1 %, compared with a control value for the transformation frequency of 0.27 (Park *et al*., 2002b). Experiments were also conducted with ferrous sulphate in this system.

Poly(ADP-ribosyl)ation is a requirement for DNA base excision repair and chromosomal stability that is synthesised by the NAD^+-dependent enzyme poly(ADP-ribose) polymerase (EC 2.4.2.30). The effect of EGBE on the activity of this enzyme was studied in Syrian hamster embryo cells treated with methyl methanesulphonate. The glycol ether potentiates the clastogenic activity of this alkylating agent, perhaps by consuming NAD^+ when it is oxidised to the aldehyde, and the alkylating agent induces poly(ADP-ribose) metabolism. The study did not show that competition for NAD^+ was responsible for potentiation by EGBE (Hoflack *et al*., 1997).

In Table 4.80 there is listed a study of unscheduled DNA synthesis (UDS) by Slesinski and Weil, 1980. This is included for completeness sake. However, the scintillation counting method used to assay for incorporation of tritiated thymidine into DNA has been considered unacceptable for many years because of the possibility of a few cells escaping S-phase block and thereby distorting the results. Such complications of the test, should they occur, can only be recognised and avoided by silver grain counting with the use of a microscope.

Table 4.80: Tests for genetic and related effects in cultured mammalian cells by EGBE and its metabolites

Test system	Source & purity of chemical	Result[a] Without exogenous metabolic system	With exogenous metabolic system	Dose[b] (LED/HID)	Reference
EGBE					
Unscheduled DNA synthesis, rat primary hepatocytes (scintillation counting)*	Union Carbide Corp., USA 99.4 %	?		0.0001 %	Slesinski and Weil, 1980
Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus	Union Carbide Corp., USA 99.4 %	-	-	1 % (v/v)	Slesinski and Weil, 1980
Gene mutation, Chinese hamster ovary CHO-AS52 cells, g*rt* locus	Aldrich, USA (purity not stated)	-		7.6 mM	Chiewchanwit and Au, 1995
Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	Merck, Germany 99 %	+		20 mM	Elias *et al*., 1996
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	Union Carbide Corp., USA 99.4 %	-	-	0.25 %	Slesinski and Weil, 1980
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	Dow Chemical, USA > 99 %	-	-	3,500 µg/ml	NTP, 2000
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	Merck, Germany 99 %	±		15 mM	Elias *et al*., 1996
Sister chromatid exchange, human lymphocytes *in vitro*	Not stated	+		500 ppm	Villalobos-Pietrini *et al*., 1989
Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	Dow Chemical, USA > 99 %	-	-	5,000 µg/ml	NTP, 2000
Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	Merck, Germany 99 %	-		0.3 mM	Elias *et al*., 1996

Table 4.80: (continued) Tests for genetic and related effects in cultured mammalian cells by 2-butoxyethanol and its metabolites

Chromosomal aberrations, human lymphocytes *in vitro*	Merck, Germany 99 %	-	NT	0.3 mM	Elias *et al*., 1996
Chromosomal aberrations, human lymphocytes *in vitro*	Not stated	-	NT	3,000 ppm	Villalobos-Pietrini *et al*., 1989
Micronuclei, Chinese hamster lung V79 cells *in vitro*	Merck, Germany 99 %	±	NT	8 mM	Elias *et al*., 1996
Aneuploidy, Chinese hamster lung V79 cells *in vitro*	Merck, Germany 99 %	±	NT	16.8 mM	Elias *et al*., 1996
Cell transformation, Syrian hamster embryo cells, focus assay	Merck, Germany 99 %	-	NT	200 mM	Elias *et al*., 1996
Cell transformation, Syrian hamster embryo cells, focus assay	Not stated	+	NT	8 mM	Kerckaert *et al*., 1996
Cell transformation, Syrian hamster embryo cells, focus assay	Sigma Chemical Co., USA >99 %	-	NT	20 mM (7 days)	Park *et al*., 2002b
Inhibition of gap-junctional intercellular communication, Chinese hamster lung V79 cells *in vitro*	Merck, Germany 99 %	+	NT	8 mM	Elias *et al*., 1996
BAL					
Gene mutation, Chinese hamster ovary CHO-AS52 cells, g*rt* locus	Aldrich, USA (purity not stated)	-	NT	7.6 mM	Chiewchanwit and Au, 1995
Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	Chemical synthesis 91.4 %	+	NT	20 mM	Elias *et al*., 1996
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	Chemical synthesis 91.4 %	±	NT	15 mM	Elias *et al*., 1996
Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	Chemical synthesis 91.4 %	+	NT	0.2 mM	Elias *et al*., 1996
Chromosomal aberrations, human lymphocytes *in vitro*	Chemical synthesis 91.4 %	+	NT	0.2 mM	Elias *et al*., 1996

Micronuclei, Chinese hamster lung V79 cells *in vitro*	Chemical synthesis 91.4 %	±	NT	8 mM	Elias *et al*., 1996

Table 4.80: (continued) Tests for genetic and related effects in cultured mammalian cells by 2-butoxyethanol and its metabolites

Aneuploidy, Chinese hamster lung V79 cells *in vitro*	Chemical synthesis 91.4 %	±	NT	0.09 mM	Elias *et al*., 1996
Cell transformation, Syrian hamster embryo cells, focus assay	Chemical synthesis 91.4 %	-	NT	NG	Elias *et al*., 1996
Inhibition of gap-junctional intercellular communication, Chinese hamster lung V79 cells *in vitro*	Chemical synthesis 91.4 %	-	NT	8 mM	Elias *et al*., 1996
BAA					
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	Janssen Chimica, Belgium >99 %	-	NT	0.9 mM	Elias *et al*., 1996
Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	Janssen Chimica, Belgium >99 %	-	NT	NG	Elias *et al*., 1996
Micronuclei, Chinese hamster lung V79 cells *in vitro*	Janssen Chimica, Belgium >99 %	+	NT	2.5 mM	Elias *et al*., 1996
Aneuploidy, Chinese hamster lung V79 cells *in vitro*	Janssen Chimica, Belgium >99 %	-	NT	0.36 mM	Elias *et al*., 1996
Cell transformation, Syrian hamster embryo cells, focus assay	Spectrum Chemicals, USA >99%	-	NT	20 mM (7 days)	Park *et al*., 2002b
Inhibition of gap-junctional intercellular communication, Chinese hamster lung V79 cells *in vitro*	Janssen Chimica, Belgium >99 %	-	NT	8 mM	Elias *et al*., 1996

[a] +, positive; ±, weak positive –, negative; ? questionable result; NT, not tested;

[b] LED, lowest effective dose; HID, highest ineffective dose

* Scintillation counting is no longer considered a reliable method for measuring unscheduled DNA synthesis.

4.1.2.7.2 Studies *in vivo* (table 4.81)

Sprague-Dawley rats (3/group, sex not specified) weighing approximately 260 g were given a single dose (route not stated) of EGBE, 0 or 120 mg/kg bw and killed 24 hr later. The brain, testes, liver, spleen and kidney were removed and DNA extracted from each organ. The detection of 5-methyldeoxycytosine and hydrophobic DNA adducts was performed using the ^{32}P-postlabelling technique. No significant differences were found between the groups in the levels of methylated deoxycytosine, expressed as percentages of total cytidylic acid found in any organs. The percentages ranged from 3.4 % to 4.0 % in the controls and from 3.6 % to 4.0 % in the treated group. Furthermore, no obvious differences were found in the tlc hydrophobic adduct maps of ^{32}P-labelled nucleotides for any of the rat organs examined (Keith *et al*., 1996).

A micronucleus induction test was performed with male F344/N rats (5/group) at dose levels selected on the basis of published toxicity information and the results of a preliminary range-finding study. The rats were injected intraperitoneally three times at 24 h intervals with EGBE dissolved in phosphate-buffered saline at dose levels of 0, 7.03, 14.06, 28.12, 56.25, 112.50, 225.00 or 450.00 mg/kg bw per day; the total dose volume was 0.4 mL. The positive control rats received injections of cyclophosphamide, 7.50 mg/kg bw per day. Rats were killed 24 hr after the final injection and smears prepared from femoral bone marrow, which were then fixed and stained. Two thousand polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each rat. Data were tabulated as the pooled mean of each exposure group and the standard error of the mean. They were analysed with a one-tailed Cochran-Armitage trend test and by pair-wise comparisons between each dosed group and the vehicle control group. Statistically significant results were recorded for $P \leq 0.025$ with the trend test and $P \leq 0.025$ divided by the number of dose groups with the paired comparison test. A final decision on the biological significance (the "final call") was made on the basis of the statistical analysis, the reproducibility and the magnitude of the effects observed. Two of five rats in the 450 mg/kg bw dose group died. The frequency of micronucleated PCEs per 1000 cells in the vehicle control group was 1.9 ± 0.2 and the range of means in the dose groups was from 1.2 ± 0.2 at 225.00 mg/kg bw to 2.2 ± 0.3 at 28.12 mg/kg bw and 2.2 ± 0.6 mg/kg bw at 450.00 mg/kg bw. The trend test was not significant ($P = 0.570$). The PCE/NCE ratios were not presented in the report (NTP, 2000).

Male and female F344 rats (treated, 5 – 8, controls 10 - 12/sex/group) were administered EGBE by gavage at a dose level of 0 or 250 mg/kg bw per day for 1, 2 or 3 days and then killed 24 or 48h after the last dose. Haemoglobinuria was observed. Progressive time-dependent macrocytic, hypochromic, regenerative haemolytic anaemia was was observed in both males and females treated with EGBE. Additionally, EGBE treatment caused significant morphological changes in erythrocytes that were first observed just 24 h after a single dose, which included stomatocytosis, macrocytosis with moderate rouleaux formation and spherocytosis. These morphological changes became progressively more severe with more EGBE doses and included the occasional occurrence of schistocytes and ghost cells, rouleaux formation in rats of both sexes and, in female rats, an increased number of reticulocytes containing micronuclei. It appeared that the progression of haemolytic anaemia was more rapid in female rats than in males. In response to the anaemia, extramedullary haematopoiesis was noted in the spleen. The general pathological effects are described in more detail in section 4.1.2.2.3.2 (Ghanayem *et al*., 2001).

A micronucleus induction test was performed with CD-1 mice (4/sex/group/observation time) at dose levels selected on the basis of published LD_{50} information. The mice were injected intraperitoneally once with EGBE dissolved in Hank's balanced salts solution at dose levels of 0, 150, 300, 600, 800 or 1000 mg/kg bw. The positive control mice received injections of cyclophosphamide, 25 mg/kg bw. Mice of the three lower dose groups were killed 24, 48 and 72 hr after the injection, while mice of all other groups were killed at 24 hr after injection, and smears prepared from femoral bone marrow, which were then fixed and stained. A minimum number of 1000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each mouse. In addition, the ratios of PCE to mature normochromatic erythrocytes (NCEs) were determined among 2000 erythrocytes per mouse. Data were tabulated as the pooled mean of each exposure group and the standard deviation. They were analysed according to the criteria of Schmid (1976). Three male and three female mice of the1000 mg/g bw dose group died, the recorded result at this dose level therefore being based on just one mouse of each sex. The frequency of micronucleated PCEs per 1000 cells in the vehicle control group was 1.41 ± 0.43 and the range of means in the dose groups was from 0.36 ± 0.19 at 300 mg/kg bw to 0.97 ± 0.39 at 800 mg/kg bw, both results being in the 24 hr samples. The mean result from the two survivors of the toxic, 1000 mg/kg bw dose was 2.46. There was no dose related change in the PCE/NCE ratio at either the 48 h or 72 h observation times from the common, 24 h control value of 1,53 ± 0. At 24 h, the ration was high at the lowest, 150 mg/kg bw dose level (2.19 ± 0.32) and progressively fell to the control level with increasing dose (300 mg/kg bw, 2.11 ± 0.25; 600 mg/kg bw, 1.79 ± 0.23; 800 mg/kg bw, 1.54 ± 0.34; and 1000 mg/kg bw, 1.71, mean of two samples). In comparison with other control group values in the same publication, none of the PCE/NCE ratios obtained with EGBE was outside of the control group range (Elias *et al*., 1996).

In this study, mice were also treated with BAA at dose levels of 0, 50, 100 or 200 mg/kg bw, but sampling was restricted to 24 hr after the single injection. The frequency of micronucleated PCEs per 1000 cells in the vehicle control group was 0.00 for this particular experiment (but ranged upwards in other experiments in the series to 1.50 ± 0.46) and the range of means in the groups dosed with BAA was from 0.45 ± 0.03 at 50 mg/kg bw to 0.94 ± 0.5 at 200 mg/kg bw. BAA was toxic to bone marrow cells, there being statistically significant reductions in the PCE/NCE ratios from 1.79 ± 0.45 in the vehicle control group to 0.78 ± 0.19 and 0.79 ± 0.13 in the 100 and 200 mg/kg bw dose groups, respectively. The authors concluded that BAA did not increase the frequency of micronucleated cells in mouse bone marrow (Elias *et al*., 1996).

A micronucleus induction test was performed with male $B6C3F_1$ mice (5/group) at dose levels selected on the basis of published toxicity information and the results of a preliminary range-finding study. The mice were injected intraperitoneally three times at 24 hr intervals with EGBE dissolved in a total dose volume of 0.4 mL phosphate-buffered saline at dose levels of 0, 17.19, 34.38, 68.78, 137.50, 275.00, 550.00 or 1100.00 mg/kg bw per day. The positive control mice received injections of cyclophosphamide, 10.00 mg/kg bw per day. Mice were killed 24 hr after the final injection and smears prepared from femoral bone marrow, which were then fixed and stained. Two thousand polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each mouse. Data were tabulated as the pooled mean of each exposure group and the standard error of the mean. They were analysed with a one-tailed Cochran-Armitage trend test and by pair-wise comparisons between each dosed group and the vehicle control group. Statistically significant results were recorded for $P \leq 0.025$ with the trend test and $P \leq 0.025$ divided by the number of dose groups with the paired comparison test. A final decision on the biological significance (the "final call") was made on

the basis of the statistical analysis, the reproducibility and the magnitude of the effects observed. All mice in the 1100.00 mg/kg bw dose group died, whereas all mice in the 550 mg/kg bw dose group survived. The frequency of micronucleated PCEs per 1000 cells in the vehicle control group was 2.5 ± 0.2 and the range of means in the dose groups was from 2.3 ± 0.3 at 34.38 mg/kg bw to 3.8 ± 0.8 at 137.50 mg/kg bw. The trend test was not significant (P = 0.236) and the paired comparison test gave P = 0.05, whereas $P \leq 0.008$ was required for statistical significance (NTP, 2000).

4.1.2.7.3 Summary of mutagenicity

EGBE is not mutagenic in bacteria, not withstanding a significant response according to one report in *S. typhimurium* TA97a. This was not substantiated by another study specifically designed to investigate this finding. Neither BAL nor BAA were mutagenic in bacteria. Two of three mammalian cell mutation assays did not indicate any mutagenic activity for EGBE and a significant result was obtained in an assay using a very high concentration (20 mM) that was poorly reported. The same publication reported a significant result at 20 mM with BAL, whereas another study found no effect at concentrations up to 7.6 mM. There have been no mammalian cell mutation studies with BAA.

There have been reports of significant activity of EGBE in tests for SCE induction and cell transformation, but, again, the results have been inconsistent. Furthermore, the significant SCE results could be artefacts due to cell cycle delay. There is also some indication of inhibition of gap-junctional intercellular communication in a single study with EGBE and its two major metabolites. A single assay for UDS induction used a technique that is now considered to be invalid, if a significant response is obtained.

No evidence for chromosomal aberration induction has been found in a number of mammalian cell culture studies with EGBE, or in one with BAL or BAA, whereas weak aneugenic effects were obtained in the only available study with EGBE and BAL, but not with BAA. Micronuclei found in long exposure *in vitro* studies with BAL and, to a much lesser extent with EGBE itself, but not with BAA appear to be due to aneuploidy, rather than chromosomal breakage.

In vivo, there is no evidence for micronucleus induction in bone marrow cells or interaction with DNA in several organs of rats. The possibility of non-disjunction occurring and not being detected in these assays appears to be remote, because BAA produced no evidence of aneugenicity *in vitro*. BAA is rapidly formed *in vivo* and is by far the most prevalent blood metabolite of EGBE, so exposure of possible target cells to either EGBE or BAL at high concentrations is brief. The balance of the evidence suggests that EGBE do not pose a significant mutagenic potential *in vivo*.

Table 4.81: *In vivo* tests in mammals for the genotoxicity of EGBE and its metabolites

Test system	Source & purity of chemical	Result[a]	Dose[b] (LED/HID)	Reference
EGBE				
DNA adducts, Sprague-Dawley rat brain, kidney, liver, spleen & testis, *in vivo* (^{32}P-post-labelling)	Merck, Germany 99 %	- (at 24 h)	120 mg/kg bw orally x 1	Keith *et al*., 1996
Methylation level of DNA, Sprague-Dawley rat brain, kidney, liver, spleen & testis *in vivo*	Merck, Germany 99 %	-		Keith *et al*., 1996
Methylation level of DNA, FVB/N transgenic mouse brain, kidney, liver, spleen & testis *in vivo*	Merck, Germany 99 %	-		Keith *et al*., 1996
Micronucleus test, CD-1 mouse bone-marrow cells *in vivo*	Merck, Germany 99 %	-	800 mg/kg bw i.p. x 1	Elias *et al*., 1996
Micronucleus test, B6C3F$_1$ mouse bone-marrow cells *in vivo*	Dow Chemical, USA >99 %	-	550 mg/kg bw i.p. x 3	NTP, 2000
Micronucleus test, male F344/N rat bone-marrow cells *in vivo*	Dow Chemical, USA >99 %	-	450 mg/kg bw i.p. x 3	NTP, 2000
BAL				
	No data available			
BAA				
Micronucleus test, CD-1 mouse bone-marrow cells *in vivo*	Janssen Chimica, Belgium >99 %	-	200 mg/kg bw i.p. x 1	Elias *et al*., 1996

[a] +, positive; –, negative; NT, not tested;
[b] LED, lowest effective dose; HID, highest ineffective dose

4.1.2.8 Carcinogenicity

There appear to have been no epidemiological studies of a relationship between exposure to EGBE and human cancer, although there have case reports of testicular cancer and leukaemia in occupational circumstances that have involved co-exposure with other chemicals. There are only two experiments in rodents available, one in rats and one in mice, in which EGBE was administered by the inhalation route.

4.1.2.8.1 Studies in animals

Inhalation

Rat

The carcinogenic potential of EGBE was studied in an experiment in which it was administered by whole-body exposure of groups of 50 male and 50 female F344/N rats to 0, 31.2, 62.5 or 125 ppm (0, 151, 302 or 604 mg/m^3) EGBE vapour (lot no. QP-921215-26D2, Dow Chemical, Plaquemine, LA, USA, purity > 99 %) for 6 hr per day, 5 days per week for 104 weeks. For haematological and bone marrow analyses, additional groups of 27 male and 27 female F344/N rats were exposed to 0, 62.5 or 125 ppm for evaluation at 3, 6 and 12 months and 9 male and 9 female rats were exposed to 31.2 ppm for 3 months (for haematological examination only) and 6 months (NTP, 2000).

Rats were held in the testing laboratory for 18 days and were 7 – 8 weeks old when the study began. They were housed individually, water was available *ad libitum* and feed was available *ad libitum* except during the exposure periods. All rats were observed twice daily throughout the study. Body weights were recorded at the beginning of the study, after which they were recorded monthly from week 5 until week 89 and then every two weeks from week 92 until the end of the experiment. Clinical observations also were recorded monthly from week 5 until week 89 and then every two weeks until the end of the experiment. Haematological examinations were performed on an Ortho ELT-8/ds 9000 analyser at 3- and 6-months and on a Roche COBAS Helios analyser at 12-months. Differential leukocyte and nucleated erythrocyte counts were made by microscopic examination of blood films. Bone marrow cellularity was made using a Coulter Model Z_H counter, while cytological evaluations of bone marrow cell morphology and myeloid/erythroid ratios were made microscopically.

Autopsies and microscopic examinations were made on all of the main study animals that had not been used for blood and bone marrow sampling. Organ weights were not measured. At autopsy, all organs and tissues were examined for grossly visible lesions and all major tissues were fixed and processed for histological examination. A quality assessment pathologist evaluated the slides from all tumours and potential target organs, which included bone marrow, forestomach, kidney, liver, lung, nose and spleen of males and females and the adrenal and clitoral glands of females. The slides and the preliminary quality assessment report were then submitted to a National Toxicology Program Working Group of pathologists.

Survival of exposed male and female rats was similar to the chamber control groups, the mean numbers of survivors at the end of the experiment in each of the 0, 31.2, 62.5 and 125 ppm groups were 19, 11, 21 and 24 males and 29, 27, 23 and 21 females, respectively. No clinical

signs were attributed to EGBE exposure. Body weights of male and female rats in the 31.2 and 62.5 ppm groups and male rats of the 125 ppm group were generally similar to the controls throughout the experiment. Body weights of female rats of the 125 ppm group were generally lower than those of the chamber control from week 17 until the end of the experiment.

Non-neoplastic findings are described in section 4.1.2.6.1.3.

The incidence of benign or malignant phaeochromocytoma (combined) of the adrenal medulla in females exposed to 125 ppm EGBE was not significantly increased compared to the chamber controls, but it did exceed the historical control range. There was only one malignant phaeochromocytoma, which occurred in the 125 ppm group. The data are summarised in Table 4.82.

Table 4.82: Neoplastic and non-neoplastic lesions of the adrenal medulla in female F344/N rats

	Chamber control	31.2 ppm	62.5 ppm	125 ppm
No. examined	50	50	49	49
Hyperplasia (severity)	11 (1.9)	11 (2.3)	8 (2.1)	17 (2.5)
Benign phaeochromocytoma	3/50 (6 %)	4/50 (8 %)	1/49 (2 %)	7/49 (14 %)
Benign or malignant phaeochromocytoma	3/50 (6 %)	4/50 (8 %)	1/49 (2 %)	8/49 (16 %)

It was on the basis of these data that the authors of the report conclude that there was *no evidence* for carcinogenicity in male rats and *equivocal evidence* for carcinogenicity in female rats. The primary criterion used to distinguish phaeochromocytomas from medullary hyperplasia was the presence of mild-to-moderate compression of the adjacent tissue. Most of the phaeochromocytomas were small and not substantially larger than the more severe grades of adrenal medullary hyperplasia. Indeed, there was only one phaeochromocytoma that was graded as malignant. Therefore, it is considered that these data from female rats provide only the weakest of marginal evidence for carcinogenicity.

Two nasal tumours were discovered: a chondroma in a 31.2 ppm male and an adenoma in a 62.5 ppm male. In the absence of any preneoplastic changes, these are considered incidental findings. Although the incidence of hyaline degeneration was significantly increased in all exposed groups of male rats, the severity of the lesion was minimal and was not affected by exposure.

Mouse

The carcinogenic potential of EGBE was studied in an experiment in which it was administered by whole-body exposure of groups of 50 male and 50 female $B6C3F_1$ mice to 0, 62.5, 125 or 250 ppm (0, 302, 604 or 1208 mg/m^3) EGBE vapour (lot no. QP-921215-26D2, Dow Chemical, Plaquemine, LA, USA, purity > 99 %) for 6 hr per day, 5 days per week for 104 weeks. For haematological and bone marrow analyses, additional groups of 30 male and 30 female $B6C3F_1$ mice were exposed to 0, 62.5, 125 or 250 ppm for evaluation at 3, 6 and 12 months (NTP, 2000).

Mice were held in the testing laboratory for 18 days and were 7 weeks old when the study began. They were housed individually, water was available *ad libitum* and feed was available *ad libitum* except during the exposure periods. All mice were observed twice daily throughout the study. Body weights were recorded at the beginning of the study, after which they were recorded monthly from week 5 until week 93 and then every two weeks from week 94 until the end of the experiment. Clinical observations also were recorded monthly from week 5 until week 93 and then every two weeks from week 94 until the end of the experiment. Haematological examinations were performed on an Ortho ELT-8/ds 9000 analyser at 3- and 6-months and on a Roche COBAS Helios analyser at 12-months. Differential leukocyte and nucleated erythrocyte counts were made by microscopic examination of blood films. Bone marrow cellularity was made using a Coulter Model Z_H counter, while cytological evaluations of bone marrow cell morphology and myeloid/erythroid ratios were made microscopically.

Autopsies and microscopic examinations were made on all of the main study mice that had not been used for blood and bone marrow sampling. Organ weights were not measured. At autopsy, all organs and tissues were examined for grossly visible lesions and all major tissues were fixed and processed for histological examination. A quality assessment pathologist evaluated the slides from all tumours and potential target organs, which included bone marrow, forestomach, kidney, liver, lung, nose and spleen of males and females and the preputial gland, prostate, skin (prepuce), testis and urinary bladder of males. The slides and the preliminary quality assessment report were then submitted to a National Toxicology Program Working Group of pathologists.

Survival of male mice exposed to 125 or 250 ppm was significantly less than that of the chamber control group, whereas survival in all other treated groups was similar to the chamber control group. The mean numbers of survivors at the end of the experiment in each of the 0, 62.5, 125 and 250 ppm groups were 39, 39, 27 and 26 males and 29, 31, 33 and 36 females, respectively. No clinical signs were attributed to exposure to EGBE. Body weights of exposed male mice were generally less than those of the chamber control during the last 25 weeks of the experiment. Body weights of female rats of the 250 ppm group were generally lower than those of the chamber control from week 30 until the end of the experiment, the difference being about 20 % for much of that time. Body weights of the 62.5 and 125 ppm group females were generally lower than the chamber controls from about week 60 until the end of the experiment.

Non-neoplastic findings are described in section 4.1.2.6.1.

There was a positive trend in the incidences of forestomach squamous cell papilloma and squamous cell papilloma or carcinoma combined in female mice. The incidences were significantly increased in the 250 ppm group, in which the only squamous cell carcinoma occurred (Table 4.83). These incidences exceeded the historical control range for female mice (9/973 = 0.9 % ± 1.1 %; range 0 % - 3 %). There was no significant increase in the incidence of these neoplasms in male mice, but they did exceed the historical control range for male mice (7/973 = 0.7 % ± 1.0 %; range, 0 % - 2 %). Again, there was one squamous cell carcinoma, this time in the 125 ppm group (Table 4.83).

Table 4.83: Neoplastic and non-neoplastic lesions of the forestomach in male and female B6C3F$_1$ mice

	Chamber control	62.5 ppm	125 ppm	250 ppm
Males				
No. examined	50	50	50	50
Ulcer (severity)	1 (1.0)	2 (1.5)	9** (1.6)	3 (1.7)
Epithelial hyperplasia (severity)	1 (3.0)	7* (2.3)	16** (1.8)	21** (2.3)
Squamous cell papilloma or carcinoma (percentage)	1 (2 %)	1 (2 %)	2 (4 %)	2 (4 %)
Females				
No. examined	50	50	50	50
Ulcer (severity)	1 (3.0)	7* (1.3)	13** (1.5)	22** (1.4)
Epithelial hyperplasia (severity)	6 (1.8)	27** (2.0)	43** (2.4)	44** (2.9)
Squamous cell papilloma or carcinoma (percentage)	0 (0 %)	1 (2 %)	2 (4 %)	6* (12 %)

* Significantly different from the chamber controls $P \leq 0.05$; ** $P \leq 0.01$

In the liver of male mice there was a positive trend in the incidence of haemangiosarcomas that led to a significant difference between the 250 ppm group and the chamber controls (Table 4.84). The incidence at 250 ppm also exceeded the historical control range for this tumour in male mice (14/968 = 1.5 % ± 1.5 %; range, 0 % - 4 %). There was also a positive trend in the incidence of hepatocellular carcinomas that led to a significant difference between males of the 250 ppm group and the chamber controls. There was, however, no change in the incidence of hepatocellular adenomas and carcinomas combined, because of a reduced incidence of hepatocellular adenomas in the treated groups (Table 4.84). In female mice there was a single haemangiosarcoma, in the 62.5 ppm group, which was considered to be an incidental finding. Furthermore, the incidences of hepatocellular adenomas were reduced in the 125 and 250 ppm groups of female mice and there was no change in the incidence of hepatocellular carcinomas (Table 4.84).

One possible explanation for the liver neoplasms is infection with *Helicobacter hepaticus*, which had been previously implicated in significant liver disease of mice, including hepatocellular neoplasms and haemangiosarcomas (Hailey *et al*., 1998). Investigation of liver samples from this experiment failed to demonstrate the presence of this bacterium, therefore the experiment was not confounded by *H. hepaticus* infection.

The NOAEC for tumourigenicity in mice is 125 ppm, based on an increased incidence of haemangiosarcomas in males and squamous cell papillomas or carcinomas in females at 250 ppm.

Table 4.84: Neoplastic and non-neoplastic lesions of the liver in male and female B6C3F$_1$ mice

	Chamber control	62.5 ppm	125 ppm	250 ppm
Males				
No. examined	50	50	49	50
Kupffer cell, pigmentation, haemsiderin (severity)	0	0	8** (1.0)	30** (1.2)
Haemangiosarcoma	0/50 (0 %)	1/50 (2 %)	2/49 (4 %)	4/49* (8 %)
Hepatocellular adenoma	22	18	18	17
Hepatocellular carcinoma	10	11	16	21**
Hepatocellular adenoma or carcinoma	30	24	31	30
Females				
No. examined	50	50	49	50
Kupffer cell, pigmentation, haemsiderin (severity)	0	5* (1.0)	25** (1.0)	44** (1.0)
Haemangiosarcoma	0	1	0	0
Hepatocellular adenoma	16	8*	7*	8*
Hepatocellular carcinoma	10	12	13	10
Hepatocellular adenoma or carcinoma	22	16	18	18

* Significantly different from the chamber controls $P \leq 0.05$; ** $P \leq 0.01$

Transgenic mouse models

A study for increased tumour incidence or decreased tumour latency was conducted in FVB/N trangenic mice (Oncomice Neo 01™), which carry the viral Harvey *ras* (v-Ha-*ras*) oncogene controlled by the mouse mammary tumour virus (MMTV) promoter. These are mice that present a stable, heritable mutation in the Ha-*ras* gene. The endpoints of the assay can be achieved, in principle, by both genotoxic and non-genotoxic agents, but the limitations of the assay have not been defined. Two groups of seven-week old mice (25/sex/group) were dorsally implanted with subcutaneous minipumps (2002 Alzet™, Alza Corp.) delivering either saline or EGBE continuously at a rate of approximately 120 mg/kg bw perday for two weeks. Sub-groups of mice were killed for histological examination on days 5, 10, 15, 60 and 120 after implantation of the minipumps. Special attention was given to the development of mammary gland masses. There was no adverse effect upon either food or water consumption. At the 120-day sampling time, the mammary glands of six control female mice and five treated mice showed cellular hyperplasia. In addition, a control female mouse had a papillary cystic mammary adenoma and a treated mouse had an anaplastic carcinoma of the bladder (Keith *et al*., 1996). The authors conclude that EGBE does not promote initiated cells carrying

the v-Ha-*ras* oncogene. There is some uncertainty about the numbers of mice used in the experiment and each sub-group.

Dermal

No data were available

Oral

No data were available

4.1.2.8.2 Studies in humans

No data.

Mechanism of haemangiosarcomas formation in male mice and its significance for human health

Haemangiosarcomas, which arise from the endothelial cell component of the liver (Frith and Ward, 1979), have been observed to increase in incidence in male mice treated with EGBE, but not in female mice or rats of either sex at high exposure concentrations of EGBE. The apparent species and sex specificity of this response may impact upon the human risk assessment process so possible mechanisms for the induction of these tumours are addressed in this section. The compound-specific experimental data are described in preceding sections of this document.

While some *in vitro* studies for genotoxicity have reported significant responses to exposure to EGBE or its metabolites, others have not and there is no evidence from *in vivo* studies for clastogenic activity or for covalent interactions with DNA. It is considered, therefore, that there is a lack of evidence for a role for genotoxicity induced by EGBE or its metabolites in the neoplastic process.

A possible alternative mechanism of haemangiosarcomas induction can be based on the haematotoxicity of the major urinary metabolite, BAA. It has been established that BAA is the metabolite that induces haemolytic anaemia in mice of both sexes. However, it also induces anaemia in rats, this species being slightly more sensitive than mice. For the haemolysis hypothesis to be sustained, there should be, therefore, other species and sex differences amongst the rodents that are important components of this mechanism.

Following the haemolysis produced by exposure to EGBE, haemosiderin is deposited in several cell types in liver, including Kupffer cells and hepatocytes of mice and rats. In addition, it has been recognised that murine endothelial cells have a significant phagocytic activity (Steffan *et al*., 1986), which may, therefore, serve as a mechanism by which insoluble iron complexes or senescent erythrocytes can enter these cells. Ferrous iron in haemosiderin can undergo redox cycling, the oxidative portion of which produces ferric iron and the highly reactive and damaging hydroxyl radical according to the Fenton reaction:

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\bullet}OH + OH^-$$

The possible development of oxidative stress as a result of these iron deposits was studied in hepatocytes of male rats and mice (see Mechanistic studies of liver pathology, above), with the conclusion that rat hepatocytes in culture are markedly less susceptible to oxidative stress than are mouse hepatocytes. The antioxidant capacity of the endothelial cells of the liver of rats is much less than that found in either the hepatocytes or the Kupffer cells (DeLeve, 1998; Sporalics, 1999). Therefore, if there should be similar haemosiderin deposition in endothelial cells, Kupffer cells and hepatocytes, the endothelial cells would be the least well protected and thereby suffer the greatest oxidative damage. An *in vivo* study (Siesky *et al*., 2002) has demonstrated that treatment of male mice with EGBE at dose levels (orally administered by gavage) equivalent to those that were associated with increased incidences of haemangiosarcomas in a 2-year inhalation experiment leads to oxidative damage to hepatic DNA and lipid and increased DNA synthesis, particularly in endothelial cells, but also in hepatocytes. None of these effects occurred in rats. The *in vivo* and *in vitro* demonstrations of species differences in susceptibility to oxidative stress are, therefore, consistent with each other and the hypothesis that oxidative stress is an essential component of the process leading to haemangiosarcomas development in male mice treated with EGBE.

In addition, an *in vivo* study of possible differences between male and female mice does suggest that there is a marginally greater susceptibility of male mouse liver to oxidative damage than of female mouse liver. This sex difference does not seem to be large in terms of measurements of either acute oxidative DNA damage or lipid peroxidation, but there did remain a larger reserve of antioxidant potential in the females than in the males. Accordingly, it would not be possible to predict with any assurance that haemangiosarcomas would develop in males, but not in females. Indeed, the neoplastic response was not large even in the male mice, so it could be that a particular critical level of oxidative damage had been surpassed in the males, but had not been attained in the females. Deguchi *et al*. (1995) showed that the presence of female sex hormones imparts higher antioxidant properties than do male sex hormones. This same group (Okada, 1996) also found much lower levels of lipid peroxidation in female mouse kidney than in male mouse kidney. This result demonstrates that there is a higher level of protection in female than in male mouse kidney. It also demonstrates that sex-related differences in susceptibility to oxidative damage are not restricted to the liver and, therefore, the consequences of such damage may show a sex-related incidence pattern in other organs of the body.

In response to BAA, human erythrocytes in culture are clearly much less sensitive than the rodent cells by at least an order of magnitude. Furthermore, haemotoxicity was not observed in the two available occupationally exposed people or in volunteers under controlled conditions to EGBE (see 4.1.1.1). Exceptionally high exposure to EGBE, as encountered in a single case of attempted suicide in an adult (estimated dose 4500 mg/kg bw), did show that it is possible for human erythrocytes to be damaged as a result of exposure to EGBE, but the effects require doses that are close to lethal (they were not seen in other attempted suicide cases where the estimated doses were at least 1000 mg/kg bw) and are not encountered in occupational circumstances or in normal consumer use (see 4.1.1.2). Consequently, they should not be considered in an assessment of risk. In cases of accidental poisonings of children, no evidence of haemolytic effects has been found. Therefore, if haemolysis is an essential requirement for the induction of haemangiosarcomas by EGBE then man is not a susceptible species.

The data available are consistent with the proposal that haemangiosarcomas observed in male mice could arise in mice of both sexes as a result of haemolysis leading to haemosiderin deposition. These deposits form nuclei for oxygen radical production that can damage many

cellular components, including DNA, unless there is sufficient antioxidant protection. When this deposition in the sinusoidal cells of the liver reaches a certain level, the oxidative defence mechanisms available to the cells are overwhelmed, creating the conditions for neoplastic responses in the endothelial cells of the hepatic blood vessels. Since man is much less sensitive to the haemolytic effects of EGBE, damage to blood cells not having been observed except in cases of very high exposure found in attempted suicides, the low level of haemangiosarcomas induced in male mice, but not in either female mice or in rats of either sex might have no significance for human risk assessment. The weakness in this argument is that haemosiderin deposition was reported to occur in Kupffer cells, whereas its occurrence or otherwise in sinusoidal endothelial cells has not been reported. However, it has been demonstrated that active oxygen species can migrate from Kupffer cells to the adjacent endothelial cells (Klaunig, 2004), and an endothelial cell response, in the form of proliferation, has been demonstrated in male mice, but not male rats (Siesky *et al*., 2002), at dose levels that are associated with an increased incidence of haemangiosarcomas in male mouse liver.

Relevant information from studies not involving EGBE

The process by which haemangiosarcomas incidence is increased in male $B6C3F_1$ mice treated with EGBE has been presented as a compound-specific mechanism. Elements of this mechanism may be applicable to other chemicals that induce haemangiosarcomas, but it is extremely unlikely that generalisations can be made that include all of the steps described. This is because of:

- differences in metabolism and kinetics between species, sexes and routes of administration for specific compounds;
- the interplay of other toxicological processes (e.g., genetic toxicity) that may modify fundamentally, or to a lesser degree, the pathway leading to haemangiosarcomas development;
- chance differences in incidence between sexes (particularly when the incidence is low);
- missing information, such as the lack of haematological data (particularly in older studies).

Results have been summarised (Table 4.85) on chemicals tested for carcinogenicity in $B6C3F_1$ mice and/or F344/N rats for which evidence of haemosiderin deposition is available in relatively complete reports that are in the public domain (specifically, NCI and NTP reports). In compiling this list, it was evident that some chemicals did not cause haemosiderin deposition in the liver, but did so in other organs. These have not been included. Also, in the columns listing the haemangiosarcoma induction response, it is stressed that the incidences refer only to the liver. Thus, the emphasis is on the relationships between haematotoxicity, haemosiderin deposition and haemangiosarcoma in the liver. In this organ, it was expected on the basis of the current hypothesis that, while the reasons for haematotoxicity might be different after exposure to different chemicals, the hepatic response to hepatic deposition of haemosiderin should be the same for any chemical, including EGBE. In other organs, modifying factors may prevail. The spatial relationships between the phagocytic cells and the cell populations in which the neoplastic response arises might be different from those found between Kupffer cells and hepatic sinusoidal endothelial cells; there may also be differences

in protective capacity, e.g., splenic phagocytes have a higher antioxidant capacity than do sinusoidal endotheliam cells (DeLeve, 1998).

The data show that mice are more susceptible to haemangiosarcoma development than are rats, and male mice are more sensitive than female mice. None of the chemicals that are listed in section C (male mice) of table 4.85 induced haemangiosarcomas in section D (female mice) and only those chemicals in section C that demonstrably induced a life-long exposure of the liver to Kupffer cell pigmentation also induced increases in hepatic haemangiosarcoma incidence. At first glance, the main conclusion to be reached is that most of the data remain consistent with the suggested mechanism, in that significant haemosiderin deposition does not occur in cases where there is an absence of an increased incidence of haemangiosarcomas. However, some results require further investigation. It is not suggested that haemosiderin deposition is the only mode of action that can result in haemangiosarcomas. The highest incidence of haemangiosarcomas occurred in male mice gavaged with pentachloroanisole. In this series of experiments, the Kupffer cell pigmentation observed at 13 weeks did not contain iron, bile or PAS-positive material. It appears that special staining techniques were not applied to the pigment found in the 2-year study, therefore it is not known if it was haemosiderin or the non-iron pigment found in the 13 week study. It is also noted that material was more prevalent in hepatocytes than in Kupffer cells. If it were not haemosiderin then pentachloroanisole would cease to be of use in looking for support for the mechanism.

Another case requiring discussion is C.I.Pigment Red 3, which did not induce haemangiosarcomas in either mice or rats, but haemosiderin deposition was observed in spleen and a "green-brown pigment" occurred in Kupffer cells. The latter was not identified as haemosiderin in the report and, if it were not haemosiderin, then C.I.Pigment Red 3 would cease to be of use in looking for support for the proposed mechanism. Furthermore, if haemolytic anaemia was produced by C.I.Pigment Red 3 in mice, it was transient – unlike in rats – as suggested by the haematological changes observed at the end of the preliminary 2-week study, but not at the end of the 13-week study (p.62 of NTP TR 407). This contrasts with the haematotoxic activity of EGBE, which persisted for at least 12 months (the longest period examined). This does lend some support to the hypothesis that life-long exposure of the liver to Kupffer cell pigmentation is required as a pre-requisite for an increase in liver haemangiosarcoma rates in male mice.

In the case of methyleugenol, haemosiderin deposits were found in the livers of female mice, but not of male mice or in rats of either sex. Haemangiosarcomas were not induced, whereas there were increases in hepatocellular neoplasms. Although the mice in this study were infected with *Helicobacter hepaticus*, it was argued that this did not compromise the outcome. Even in the EGBE study, the increase in haemangiosarcomas incidence in male mice was modest and did not occur in female mice; therefore, the absence of these neoplasms in female mice of the methyleugenol study does not conflict with their proposed mechanism of induction by EGBE.

The conclusion that can be reached is that, in addition to EGBE, only two compounds support the pattern of chronic haemosiderin deposition and increased incidence of haemangiosarcomas in male mice but not female mice, these being *p*-chloroaniline and *p*-nitroaniline. Another group has recently studied the possible association liver hepatic haemangiosarcomas and chemically induced haemosiderosis in mice in the US NTP database (Nyska *et al*., 2004) and reached essentially the same conclusion. In addition to the qualitative association described here, however, Nyska *et al*. (2004) also showed that there was a very high statistically significant association between hepatic haemangiosarcomas and

Kupffer cell pigmentation.($p < 0.001$). In all cases, the cause of the haemosiderosis was haemolysis due to the chemical.

There are very few rodent studies with other compounds that are capable of providing support for the hypothesis proposed to explain the low level increased incidence of haemangiosarcomas in male mice exposed to EGBE. The available evidence is, however, consistent with the hypothesis. Currently, the most likely mechanism is that proposed, based on metabolism to BAA, followed by rodent erythrocyte-sensitive haemolysis, deposition of iron-protein complexes in the liver and the sustained generation of toxic (cytotoxic or DNA damaging) radicals from this source.

Human studies have demonstrated an enormously increased risk for hepatocellular cancer in hereditary haemochromatosis (e.g., Niederau *et al*., 1985 found an increased risk of 219, based on 16 cases) that has been attributed to the toxic effects of iron, but there also seems to be slightly increased risks for extra-hepatic cancers. Some studies have found genetic associations between several cancers and the most common mutation, C282Y, of the HFE gene that causes hereditary haemochromatosis. Such associations only occur, however, in the presence of a particular allele of the transferrin receptor gene. This suggests that the increased cancer risk is due to the effects of iron (Dorak *et al*., 2002). Additionally and independently of the genetic factors, dietary iron appears to be a risk factor for some cancers, e.g., colorectal cancer (Nelson, 2001), and dietary iron overload has been associated with about a 10-foldgreater risk of hepatocellular carcinoma among black Africans (Mandishona *et al*., 1998). Focussing specifically upon liver angiosarcoma, however, the frequently quoted risk factors for this rare human cancer (0.5 – 2.5 cases per 10^7 people), which include hereditary haemochromatosis, contribute to explain no more than about 20 % of the published cases. Indeed, apart from occupational exposure to vinyl chloride, the aetiology of this cancer remains largely unknown (Zocchetti, 2001). Therefore, it is not possible to conclude whether iron plays any role in human angiosarcoma.

Table 4.85: Summary of relationship between chemicals inducing haematoxicity and haemangiosarcomas in B6C3F1 mice and F344 rats Incidences are for control, low, middle and high dose groups, respectively.

A. Male Rats

Chemical	Haematology indicating toxicity to erythrocytes	Incidence of Kupffer cell pigmentation (KCP) or hepatic haemosiderin (HS) at 2 years	Kupffer cell pigmentation (KCP) or hepatic haemosiderin (HS) observed at 3 months	Incidence of hepatic haemangiosarcoma	Reference **
EGBE	Yes, at 14 wks	KCP: 23/50, 30/50, 34/50, 42/50	KCP	0/50, 0/50, 1/50, 0/50	TR484
o-Nitroanisole	Yes, at 2 & 13 wks	KCP: 0/20, 1/20, 18/20	KCP	None	TR416
p-Chloroaniline	Yes, at 13 wks	HS: 1/49, 0/50, 0/50, 26/50	KCP	None	TR351
Pentachloroanisole	Normal at 13 wks.	KCP: 0/50, 1/50, 4/50	None (hypertrophy of Kupffer cells)	None	TR414
Pyridine	Yes, at 13 wks	KCP: 4/50, 11/50, 20/50, 25/50	HS, but cell-type not identified	None	TR470
Titanocene dichloride	Yes, at 2 wks	KCP: 1/60, 39/60, 41/60	None	None	TR399 (rats only)
D&C Yellow No. 11	No haematology	KCP: 7/50, 15/51, 23/51, 26/54	KCP	None	TR463 (rats only)
Butyl benzyl phthalate		KCP, 0/59, 1/58, 6/59, 6/58		None	TR458
Cupferron (N-hydroxy-N-nitroso-benzenamine)	No haematology.	HS: 0/49, 15/48, 28/43	No data	None	TR100
No significant haemosiderin/pigmentation deposition was observed with: Methyleugenol, 2,4-diaminophenol.2HCl, CI Pigment Red 3.					

B. Female Rats

Chemical	**Haematology indicating toxicity to erythrocytes**	**Incidence of Kupffer cell pigmentation (KCP) or hepatic haemosiderin (HS) at 2 years**	**Kupffer cell pigmentation (KCP) or hepatic haemosiderin (HS) observed at 3 months**	**Incidence of hepatic haemangiosarcoma**	**Reference**
EGBE	Yes, at 14 wks	KCP: 15/50, 19/50, 36/50, 47/50	KCP	None	TR484
o-Nitroanisole	Yes, at 2 & 13 wks	KCP: 8/20, 2/20, 20/20	KCP	None	TR416
Pentachloroanisole	Normal at 13 wks.	Pigmentation in hepatocytes	None (hypertrophy of Kupffer cells)	None	TR414
Pyridine	Yes, at 13 wks	KCP: 6/50, 2/50, 6/50, 17/50	HS, but cell-type not identified	None	TR470
Titanocene dichloride	Yes, at 2 wks	KCP : 3/60, 45/61, 50/60	None	None	TR399 (rats only)
CI Pigment Red 3	Yes, at 2 & 13 wks.	KCP : 0/50, 3/50, 14/50, 41/50*	Pigment (type & cell-type not identified)	None	TR407
D&C Yellow No.11	No haematology	KCP: 9/50, 11/51, 16/50, 32/51	KCP	None	TR463 (rats only)
Butyl benzyl phthalate		KCP: 4/60, 1/60, 6/60, 10/60		None	TR458
Cupferron (N-hydroxy-N-nitroso-benzenamine)	No haematology	HS: 0/48, 9/44, 33/44	No data	None	TR100
No significant haemosiderin/pigmentation deposition was observed with: Methyleugenol (anaemia at 14 wks), 2,4-diaminophenol.2HCl, butylbenzyl phthalate, *p*-chloroaniline					

C. Male Mice

Chemical	**Haematology indicating toxicity to erythrocytes**	**Incidence of Kupffer cell pigmentation (KPC) or hepatic haemosiderin (HS) at 2 years**	**Kupffer cell pigmentation (KPC) or hepatic haemosiderin (HS) observed at 3 months**	**Incidence of hepatic haemangiosarcoma**	**Reference**
EGBE	Yes, at 14 wks	KCP: 0/50, 0/50, 8/50, 30/50	KCP	0/50, 1/50, 2/50, 4/50	TR484
p-Chloroaniline	Yes, at 14 wks.	KCP/HS: 0/50, 0/49, 0/50, 50/50	KCP	2/50, 2/49, 1/50, 6/50	TR351
Pentachloroanisole	No haematology	KCP: 1/50, 50/50, 50/50	KCP (but not containing iron)	2/50, 8/50, 10/50	TR414
p-Nitroaniline	Yes, at 2 & 13 wks	KCP: 1/50, 1/50, 8/50, 50/50	KCP	0/50, 1/50, 2/50, 4/50	TR418
o-Nitroanisole	Yes, at 13 wks	KCP: 0/50, 0/50, 3/50, 16/50	No	2/50, 2/50, 1/50, 0/50	TR416
CI Pigment Red 3	Normal at 13 wks	KCP : 0/50, 5/50, 30/50, 41/50*	No	0/50, 1/50, 1/50, 0/50	TR407
2,4-Diaminophenol.2HCl	No haematology at 13 wks. Yes, at 15 months	KCP: 0/50, 44/50, 47/50	No	1/50, 0/50, 1/50	TR401
No significant haemosiderin/pigmentation deposition was observed with: Methyleugenol, Pyridine, Cupferron					

D. Female Mice

Chemical	**Haematology indicating toxicity to erythrocytes**	**Incidence of Kupffer cell pigmentation (KPC) or hepatic haemosiderin (HS) at 2 years**	**Kupffer cell pigmentation (KPC) or hepatic haemosiderin (HS) observed at 3 months**	**Incidence of hepatic haemangiosarcoma**	**Reference**
EGBE	Yes, at 14 wks	KCP: 0/50, 5/50, 25/50, 44/50	KCP	0/50, 0/50, 1/50, 0/50	TR484
p-Chloroaniline	Yes, at 14 wks	KCP: 0/50, 0/50, 1/50, 46/50		1/50, 0/50, 0/50, 1/50	TR351
Pentachloroanisole	No haematology	KCP: 0/50, 37/50, 48/50		0/50 0/50, 1/50	TR414
p-Nitroaniline	Yes, at 2 & 13 wks	KPC: 1/50, 1/50, 4/50, 39/50		1/50, 1/50, 0/50, 0/50	TR418
Methyleugenol	No haematology	HS: 0/50, 11/50, 24/50, 19/50*		0/50, 1/50, 0/50, 0/50	TR491
CI Pigment Red 3	Normal at 13 wks	KCP: 2/50, 1/50, 1/50, 29/50*		0/50, 0/50, 1/50, 0/50	TR407
2,4-Diaminophenol.2HCl	No haematology at 13 wks. Yes, at 15 months	KCP, 0/50, 31/50, 50/50		None	TR401
No significant haemosiderin/pigmentation deposition was observed with: Pyridine, Cupferron, *o*-Nitroanisole					

*See discussion in the text.

**NTP Technical Report Numbers

Mechanism of forestomach tumour formation in mice and its significance for human health.

Squamous cell papilloma and carcinoma of the forestomach have been induced in female mice, with some evidence (that does not reach a level of significance) for an increase in male mice. In contrast, there is no evidence for the induction of forestomach tumours or even preneoplastic effects, such as hyperplasia, in rats of either sex in the 2 year study, although hyperplasia was observed in female rats in a 14 week study in which higher exposure concentrations were used.

EGBE is a liquid with low vapour pressure that can therefore deposit on the fur of animals in whole body exposure experiments. The inhaled vapour may also condense in the nasopharynx. By both this mechanism and as a result of grooming contaminated fur, EGBE can achieve a significant exposure by the oral route, even in inhalation experiments. Although this is a plausible explanation for the toxic and tumourigenic effects of EGBE on the forestomach, only small amounts of EGBE (< 10 mg/kg) were found on the fur of mice at the end of 6 hr, whole body exposures to 250 ppm (the highest concentration used in the NTP carcinogenicity experiment). In addition, dosage of mice by either intraperitoneal or subcutaneous injection also resulted in forestomach lesions. It is clear, therefore, that EGBE accumulates in the forestomach as a result of a combination of processes. In addition to grooming of EGBE present on the fur of the mice and, possibly, mice licking the walls of the exposure chamber, it is likely that salivary excretion as well as mucociliary transport of material deposited in the bronchi, followed by ingestion, are other means by which exposure of the stomach could have occurred during the study.

The stomach of rats and mice consists of a forestomach (not found in man) and a glandular portion. The forestomach acts as a storage organ, where ingested material can remain for several hours, before it is transferred to the glandular portion in which there is a rapid transit and the first stages of digestion occur. Again, this is not the only mechanism that results in prolonged exposure of this organ. EGBE and, to a lesser extent, BAA is also eliminated more slowly from forestomach tissue than from either blood or other tissues following either oral gavage or intraperitoneal injection. Consequently, there is greater potential for damage to be induced by toxic substances in the forestomach than in the glandular stomach.

Study of the stomachs of mice orally administered either EGBE or BAA shows that, at the doses used, there was no damage to the glandular stomach by either compound and that damage in the form of hyperkeratosis occurred in the forestomach at lower doses of BAA than of EGBE. There is significant alcohol and aldehyde dehydrogenase activity in both parts of the stomach in rats and mice. While there was little difference in the activity of aldehyde dehydrogenase in rats and mice, there were major species differences in alcohol dehydrogenase activity, the mouse enzyme having very much higher affinity constants and maximal rates than the rat enzyme, when using EGBE as the substrate. Therefore, there is greater potential for EGBE to be metabolised to BAA in the forestomach of mice than in the forestomach of rats.

Hyperplasia and hyperkeratosis are histological responses to exposure to a wide range of chemicals that also produce papilloma and squamous cell carcinoma of the forestomach. Many of these chemicals are devoid of genotoxicity, the neoplasms apparently developing as a result of persistent cellular damage and sustained hyperplasia (Kroes and Webster, 1986). While there is nothing unique to rodents about this process, the fundamental differences in physiology and function between the rodent forestomach, on the one hand, and the human

stomach and the rodent glandular stomach, on the other hand, point to the low probability that the latter would be targets for neoplasia by this mechanism. This proposal is substantiated by the lack of any neoplastic response in the glandular stomach of mice exposed to EGBE under conditions that produce forestomach tumours.

PROPOSED MECHANISMS OF ACTION ASSESSED WITHIN THE IPCS FRAMEWORK

Introduction

The experiments that form the bases for these evaluations of mechanism of carcinogenic action are summarised in the preceding sections of this document.

EGBE, when delivered as a vapour, induces tumours of the forestomach in female mice and haemangiosarcomas of the liver in male mice. There was also some evidence (which did not reach statistical significance) for an increase in forestomach tumours in male mice. Neither of these tumours do not occur in the other sex in mice and no significant elevation of tumour incidence is found in rats. These findings have not been verified in independent experiments.

Postulated Mode of Action for the Induction of Tumours of the Forestomach in Female Mice

The proposed mechanism of action for production of tumours in the forestomach is the local generation, as well as accumulation of cytoxic metabolite(s) that induce a sustained, compensatory cell proliferation, neoplasia arising out of this proliferating cell population. The neoplasia was mainly papillomas, a single squamous cell carcinoma arising in the highest dose group of female mice.

Key Events

The following important steps are involved in the generation of forestomach tumours:

Enhanced exposure of the forestomach to EGBE and its metabolites from multiple external and internal sources. EGBE delivered to female mice by inhalation was distributed throughout the internal organs and was present in the stomach contents within 5 min after exposure. It was present in the mucosa of the forestomach, the buccal cavity and the oesophagus 24 and 48 h after exposure, but the high levels observed in the forestomach was in contrast to the much lower levels found in the glandular stomach and the duodenum. EGBE was also found on fur and in the buccal cavity, oesophagus and stomach contents. EGBE delivered to female mice by intravenous injection was selectively concentrated in several tissues, including liver, bone, Harderian glands and buccal cavity. The mucosa of the forestomach and glandular stomach were also labelled, but to similar extents. After EGBE administration by this route or subcutaneous injection, both EGBE and butoxyacetic acid were excreted in saliva and found in the stomach periods for prolonged periods.

Metabolism to cytotoxic metabolite(s), most probably butoxyacetic acid, if not already presented to the forestomach in this form. EGBE is metabolised by preparations from the forestomach and glandular stomach of rats as well as mice. The enzymes principally responsible for EGBE metabolism are alcohol and aldehyde dehydrogenase (a minor oxidative pathway is mediated by cytochromeP450 enzymes). These enzymes are found in both the glandular stomach and forestomach of rats and mice, but whereas they are concentrated in the stratified squamous epithelium of the forestomach, their distribution is diffuse in the glandular stomach of both species. There are no major species differences in the tissue average activity of aldehyde dehydrogenase, but the maximum rate of alcohol dehydrogenase activity is up to an order of magnitude higher in mice than in rats. The forestomach is a target tissue because EGBE entering the stomach is held there and because the metabolising enzymes are concentrated in the superficial layers of the forestomach. BAA has shown to be a more potent in producing adverse effects on the forestomach than EGBE. It may be that there is a species difference in part because the rate of metabolism of EGBE to 2-butoxyacetaldehyde is slower in rats than in mice, hence it is probable that either the generation of the more cytotoxic butoxyacetic acid is slower, or its maximum concentration is lower in rats than in mice.

Cytotoxicity and *cell proliferation*, as indicated by epithelial hyperplasia, was often accompanied by ulceration in a two-year inhalation experiment in mice, especially in females, but these responses were not reported in rats. Administration of undiluted EGBE to male and female mice by gavage induced dose-related irritation of the forestomach; the compensatory cell proliferation was confirmed by immunochemical staining. Similar forestomach lesions could be induced by EGBE administered by intraperitoneal or subcutaneous injection and by butoxyacetic acid given orally. This metabolite was more potent than EGBE itself.

Squamous cell papillomas or carcinomas were significantly increased only at the highest concentration in female mice. It also appears that mice may be more susceptible to the induction of forestomach tumours than rats, the background incidence being higher in mice.

- **Dose-Response Relationship**. In female mice, the incidence and severity of epithelial hyperplasia of the forestomach increased with rising dose in female mice, as did the incidence of ulceration of the forestomach. Ulceration occurred in male mice also, but the incidences were lower and not clearly concentration related.

- **Temporal Relationship.** In a 14 week inhalation study in mice, epithelial hyperplasia of the forestomach was observed at 125 ppm and above and had progressed at 250 ppm or 500 ppm (the highest concentration) to inflammation, necrosis and ulceration. Thus, preneoplastic events preceded neoplasia, which was only observed in the two-year study.

- **Strength, Consistency and Specificity of Association of the Tumour Response with Key Events.** There has not been any investigation of the association of key events with a neoplastic outcome at the level of individual female mice. In broad terms, however, it is clear from the dose-response and the temporal relationships that there is consistency in these events. The induction of forestomach tumours has been observed only in a single experiment. The entire chain of events has not, therefore, been verified by independent study. Such verification should be more readily achieved by oral administration of EGBE directly into the forestomach, by gavage;

however, such an experiment would have little applicability to occupational or consumer exposures.

- **Biological Plausibility and Coherence.** As generalisations, chemicals that do not induce carcinogenesis through mutagenesis do so by modes of action that include an increase in cell proliferation, either by mitogenesis or by stimulating reparative cell proliferation provoked by cytotoxicity. Based on the evidence available, the latter mode of action is biologically plausible for the induction of forestomach tumours in female mice by EGBE.

- **Other Modes of Action.** While no other mode of action has been proposed, genetic toxicity has been considered and rejected. Other possibilities could include aberrant control of gene expression because of alkylation of regulatory proteins (e.g., in chromatin), with ensuing loss of genetic stability. No studies have been conducted to investigate such a mechanism.

- **Assessment of the Postulated Mode of Action.** The data available are fully consistent with the proposed mechanism for the induction of forestomach tumours in female mice. The apparent female sex specificity is likely to be due to chance.

- **Uncertainties, Inconsistencies and Data Gaps.** The experimental observations are consistent with the general hypothesis proposed; however, there are two areas that require more specific reasoning. These are: the species difference in response and the progression from hyperplasia to neoplasia. The proposed reason for the difference in response between rats and mice is the differences in kinetic properties of the rat and mouse alcohol dehydrogenase. Whether this actually leads to a difference in concentration or quantity of butoxyacetic acid in the forestomach is unknown. As an alternative hypothesis, mice may be more susceptible than rats to the induction of forestomach tumours. This suggestion is based on the observed higher incidence of forestomach tumours in untreated mice. It is not known how proliferation of apparently normal cells becomes transformed into neoplasia. This criticism can be levelled at any non-genotoxic mechanism of carcinogenesis (but see 1.3.5). There is consistency within the database as currently known. It would be expected, however, that exposure of male mice by inhalation to somewhat higher concentrations of EGBE should also lead to forestomach neoplasia. Similarly, exposure of female and male rats to higher concentrations could also lead to neoplasia if such concentrations could be tolerated. Since the lower concentrations used in the two-year inhalation experiment with rats were selected on the basis of carefully executed shorter term studies to identify the maximum tolerated dose, this hypothetical experiment would be unlikely to be successful.

Postulated Mode of Action for the Induction of Hamangiosarcomas of the Liver in Male Mice

The proposed mechanism of action for production of haemangiosarcomas of the liver is the deposition of haemosiderin in relevant cell-types, possibly including endothelial cells from which haemangiosarcomas arise, and the generation of cytotoxic reactive oxygen species that either induce genetic changes by this secondary mechanism or sustained cell proliferation within the endothelial target tissue, neoplasia arising out of this proliferating cell population.

Key Events

The following important steps are involved in the generation of haemangiosarcomas:

Metabolism to haemolytic metabolite(s), most probably butoxyacetic acid. EGBE is principally metabolised by alcohol dehydrogenase and aldehyde dehydrogenase to 2-butoxyacetaldehyde and butoxyacetic, respectively. Butoxyacetic acid is a more potent haemolytic agent than either of its precursors; furthermore, these precursors appear not to be haemolytic unless they are metabolised. Inhibition of aldehyde dehydrogenase reduced the haemolytic activity of 2-butoxyacetaldehyde. At least *in vitro*, butoxyacetic acid causes haemolysis at much lower concentrations in rat erythrocytes than in human erythrocytes.

Haemolysis caused by butoxyacetic acid and followed by persistent, dose related anaemia results in haemosiderin deposition in the liver. This material has been observed in Kupffer cells and hepatocytes of both mice and rats, but there is currently no evidence for haemosiderin deposition in endothelial cells. Since haemolysis occurs in mice and rats of both sexes, it is necessary to provide a rationale for the specificity of the neoplastic response to male mice; however, this could be difficult in view of the low (although statistically significant) tumourigenic response in male mice.

Cell toxicity resulting from the iron-mediated generation of reactive oxygen species from haemosiderin. Compared to rats and female mice, male mice have a reduced antioxidant capacity in the liver, causing them to be more susceptible to oxidative damage; however, it is not clear that this difference is important, given the greater inherent susceptibility of male mice to the neoplasm of interest. Oral administration of EGBE to mice at doses up to 600 mg/kg bw/day for up to 90 days produced increased DNA synthesis in endothelial cells during the first 14 days of exposure and in hepatocytes after 90 days. Increased oxidative damage was also observed mice in this experiment, while no change was observed in rats.

Haemangiosarcomas arise from the endothelial target cells and are significantly increased in male mice to 8% in the 250 ppm group. It also appears that male $B6C3F_1$ mice may be more susceptible to haemangiosarcomas than are female mice (control incidence rates in NTP experiments being about 2.5% in males and 0.9% in females) and mice are more susceptible than F344 rats (none having been reported in control groups in NTP experiments). Thus, the apparent sex and species specificity could merely be a reflection of inherent sensitivity.

- **Dose-Response Relationship.** Haemolysis and persistent anaemia have been shown to occur in a dose related manner in a number of experiments. In two-year inhalation experiments, Kupffer cell pigmentation was significantly increased over that seen in control animals for male and female rats exposed at 62.5 or 125 ppm, male mice at 125 or 250 ppm and female mice at 62.5, 125 or 250 ppm. The increases observed in the male and female mice were dose related, but the incidence of Kupffer cell pigmentation was greater in female mice than in male mice. A significant increase in the incidence of haemangiosarcomas occurred only in male mice and only at the highest exposure concentration of EGBE.

- **Temporal Relationship.** The experiments show that haemolysis is an acute response and anaemia is a sustained response to repeated exposure to EGBE. Haemosiderin deposition is observed in short-term experiments and therefore it is present for a

considerable period before the emergence of the haemangiosarcomas, which occurred at increased incidence only in a two-year inhalation exposure experiment.

- **Strength, Consistency and Specificity of Association of the Tumour Response with Key Events.** Data from experiments with other chemicals show that mice are more susceptible to haemangiosarcoma development than are rats, and male mice are more sensitive than female mice, but the database in support of the hypothesis that life-long exposure of the liver to Kupffer cell pigmentation is a pre-requisite for an increase in liver haemangiosarcoma rates in male mice is small, after exclusion of doubtful examples.

- **Biological Plausibility and Coherence.** It has long been proposed (e.g., for peroxisome proliferators) that increased, persistent generation of reactive oxygen species can result in neoplasia by an indirect genotoxic mechanism. It has been shown that EGBE does increase oxidative damage in cells, as demonstrated by the generation of malondialdehyde, a deposition product of lipids, and 8-hydroxydeoxyguanosine, an oxygen adduct of DNA, and that differences in reduced antioxidant concentrations within cells may be responsible for sex and species differences in response. Nevertheless, no substance has been shown unequivocally to induce tumours through this mechanism.

- **Other Modes of Action.** While no other mode of action has been proposed, genetic toxicity has been considered and rejected. Other possibilities could include aberrant control of gene expression because of alkylation of regulatory proteins, as suggested for forestomach tumours, but there is no evidence to support this suggestion either for the forestomach mucosa or endothelial cells of the hepatic sinusoids. Kinetic studies with liver slices suggest that concentrations of 2-butoxyacetaldehyde that might induce genetic damage are unlikely to be reached under the conditions of the long-term inhalation experiments.

- **Assessment of the Postulated Mode of Action.** Haemangiosarcomas are uncommon and their incidence in this experiment was low (4/49) although out with the historical control range, at the highest dose. Because this is a low increase in incidence, and because it has not been confirmed, it is difficult to assign any mechanism. With the rejection of genotoxicity as a possible mechanism and the strong evidence for a potential source of reactive oxygen species within the liver, it is reasonable to presume that these may play a definitive role in neoplasia.

Uncertainties, Inconsistencies and Data Gaps. By their nature, reactive oxygen species are difficult to localise in the target tissue. In addition, the generator of these cytotoxic species, haemosiderin, has not been demonstrated in endothelial cells, from which haemangiosarcomas arise. Haemosiderin was demonstrated in Kupffer cells, so reactive oxygen species could arise in these cells, and the recent demonstration in one study of the migration of reactive oxygen species from Kupffer cells to endothelial cells does suggest a manner in which the latter might be damage. The involvement of cytokines secreted by Kupffer cells has been suggested in the case of peroxisome proliferators, but the target in those cases is the hepatocytes. A similar interplay of cytokines between Kupffer cells and endothelial cells may be involved in the case of haemosiderin deposition, but this remains to be demonstrated and may not be necessary in view of the alternative suggestion. A fundamental major uncertainty is that it is unknown if the observation in a single experiment

of a low, barely significant incidence of haemangiosarcomas in male mice is reproducible or whether it might also be observable in female mice, should the experiment be repeated. However, there is reason to expect that female mice would be less susceptible to this lesion.Summary of carcinogenicity

EGBE is carcinogenic in male mice, where it causes a low incidence of haemangiosarcomas, and female mice, where is causes an increased incidence of forestomach tumours. It is not carcinogenic in rats. Genotoxicity is not an important toxicological property of this chemical and it is unlikely that the low, variable and uncertainly defined genotoxic activity can be the cause of the carcinogenic responses. Hypotheses have been proposed and supported by experiment data in an attempt to explain the carcinogenic responses. These have been described above. In the case of forestomach tumours, the argument that they arise in a tissue subject to sustained abuse and consequent repair is clear. It is likely that this finding is in reality not sex specific but merely due to chance that the low level incidence in females rose above the level of statistical significance but it did not do so in males. In the case of the haemangiosarcomas, data from experiments with other chemicals show that mice are more susceptible to haemangiosarcoma development than are rats, and male mice are more sensitive than female mice, but the database in support of the hypothesis that life-long exposure of the liver to Kupffer cell pigmentation is a pre-requisite for an increase in liver haemangiosarcoma rates in male mice is small, after exclusion of doubtful examples. With the rejection of genotoxicity as a possible mechanism, strong evidence for a potential source of reactive oxygen species within the liver, and a mode of action where each step has at least some supporting data, it is reasonable to presume that these may play a role in the neoplasia.

With regard to human relevance, the mechanism proposed for the induction of haemangiosarcomas strongly suggests that EGBE is not likely to be a carcinogenic hazard under conditions of normal handling and use, because human erythrocytes are demonstrably more resistant to haemolysis than are rodent erythrocytes. The mechanism proposed for the induction of forestomach tumours would also point to a lack of human relevance under conditions of normal handling and use. As stated recently (IARC, 2003), while people do not possess forestomachs, they do have comparable squamous epithelial tissues in the oral cavity and the upper two-thirds of the oesophagus. Thus, in principle, carcinogens targeting the forestomach squamous epithelium in rodents are relevant for man. However, the relevance for man is probably low for agents that have no demonstrable genotoxicity and that are solely carcinogenic for the forestomach squamous epithelium in rodents after oral administration. Consequently, for these agents, the mode of carcinogenic action could be specific to the experimental animals (IARC, 2003). EGBE satisfies only some of these conditions. On the other hand, there are proposed mechanisms that are supported by experimental evidence to show how this chemical, even when inhaled, can accumulate in the forestomach contents, where it can remain for many hours to cause damage directly or after its metabolism to BAA.

In conclusion, given the species and sex specificity of the neoplastic responses and the current evidence supporting the hypothesis that the more likely mechanism of action is based on haematotoxicity, then EGBE is unlikely to be a human carcinogen.Therefore, an appropriate classification for *"No classification"* is proposed for *carcinogenicity*. This classification proposal was agreed by the C&L working group. Moreover, the last IARC evaluation (2004 and 2006) has classified EGBE in the list 3 of carcinogens : *not classifiable as to their carcinogenicity to humans (Group 3)* on the basis of *limited evidence* in experimental animals and *inadequate evidence* in humans.

Since the only carcinogenic effects can be considered secondary to haemolysis, and haemolysis is the key end point for repeat dose toxicity, no separate risk characterisation is necessary for the cancer end point. If there are no concerns for repeat dose toxicity, it can be considered that there will be no concerns for cancer either.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Effects on fertility

Oral route

Repeat Dose Toxicity Studies

Two of the repeated dose toxicity studies reported effects of EGBE on the testis in male rodents. The first of these studies was from the Mellon Institute of Industrial Research (1963), wherein rats were given diets containing 0, 0.01, 0.05, 0.25 or 1.25% EGBE for 93 days. These levels of EGBE were approximately equivalent to 0, 7, 38, 188 and 919 mg/kg/day. At 1.25% EGBE, male rats had decreased appetite, decreased body weight gain (50% less than control animals), increased liver and kidney weights and testicular atrophy. Rats given 0.25% EGBE also exhibited decreased appetite and body weight gain, as well as testicular atrophy. There were no apparent effects at 0.05% EGBE. Note that the purity of the EGBE used in this study is not given, nor does there appear to be analytical verification of EGBE doses, homogeneity and stability. The results of this study are difficult to interpret given that testicular atrophy is not reported in other oral, repeated-dose studies even at higher doses of EGBE greater than 0.25% (e.g., 14-week drinking water study in rats and mice from NTP, 1993; continuous breeding study reported in Morrissey *et al.*, 1989). The weight of evidence indicates that EGBE does not affect the testis at doses below those causing significant systemic toxicity.

In the 14-week inhalation study, B6C3F1 mice (10/sex/group) were exposed to 0, 31, 62.5, 125, 250 and 500 ppm EGBE vapor (6 hr/day, 5 days per week). During the first 2 weeks of study, 2 male and 2 female mice died and an additional 2 males and 2 females were killed moribund. These mice had numerous clinical signs of toxicity, including abnormal breathing, red urine stains, and lethargy. Histopathologically, male mice had ulcerations and necrosis of the forestomach and female mice had forestomach necrosis. Forestomach ulcerations and necrosis were accompanied by inflammation, which in some cases included suppurative inflammation. These animals also exhibited atrophy of the spleen, thymus, testis and lymph nodes, liver and epididymal necrosis, and renal tubule degeneration. With 8 of 20 animals succumbing to toxicity at 500 ppm, it is apparent that the maximum tolerated dose MTD) had been exceeded. Mice exposed to 500 ppm that survived to scheduled necropsy had effects on the spleen liver, forestomach and kidney, but did not exhibit testicular or epidiymal lesions. There were no effects on the testis or epididymis at lower concentrations, nor were there effects on the testis/epididymis in the 14-week rat study, the 2-year mouse chronic study, or the 2-year rat chronic study. Thus, effects on the testis and epididymis at 500 ppm EGBE were not specific to the reproductive organs as numerous tissues were affected and systemic toxicity was lethal.

As mentioned previously, the most sensitive endpoint in the 14-week study was haematology changes that were seen in males at ≥125 ppm and in females at all concentrations of EGBE.

Continuous breeding

Male and female Swiss CD-1 mice received 0 0.5, 1.0 or 2.0 % EGBE (purity > 99 %), equivalent to daily intakes of 0, 720, 1340 and 2050 mg/kg bw, in their drinking water during a continuous breeding phase (CBP) with a 7-day pre-mating period and a 98-day cohabitation period (Morrissey *et al*., 1988 and 1989 ; Heindel *et al.*, 1990). The study was performed according to GLP. During the cohabitation period, deaths occurred in the female mice: 13/20 in the 2 % group, 6/20 in the 1 % dose group, 1/20 in the 0.5 % dose group and 1/40 in the control group. The average body weights in the female 2 % dose group were consistently lower than the controls. In the male mice, no deaths occurred but weight loss (1-2 % of initial body weight) in the two highest doses and reduced weight gain were noted. Reduced fluid consumption was observed at all dose levels in both sexes (22 %, 18 % and 36 % reduction relative to controls at 0.5 %, 1.0 % and 2.0 %, respectively after 14 weeks of dosing). The numbers of fertile pairs from the surviving pairs were 38/39, 19/19, 13/14 and 5/7 at 0, 0.5, 1.0 and 2.0 % dose levels, respectively. Significant reduction in reproductive performance occurred at 1 and 2 % dose levels as indicated by dose-related decrease in number of litters per fertile pair, litter sizes, pup viability and live pup weight. A small but significant reduction (by 5 %) of live pup weight was also observed in the 0.5 % dose group without other significant reproductive effects.

At the completion of the continuous breeding phase, the F_0 breeding pairs were separated and housed individually and exposure to EGBE continued. When the last litter was weaned F0 males and females from the 1 % dose group were mated with male and female control animals in a one-week crossover mating study to determine any sex-related reproductive effects of EGBE. Exposure to EGBE was discontinued during the one-week mating period and then reintroduced at 1 % dose level (estimated daily intake 1830 mg/kg bw). Control males and females were also mated for comparative purposes. The proportion of successful copulations from the breeding pairs was similar in all groups. However, the number of fertile females was significantly reduced in the group where treated females were mated with control males. When evaluated over the 7 day period prior to necropsy, proportionally more females (7/13) in this 1 % treated group than controls (9/38) had oestrus cycles longer than 7 days. At necropsy, male and female mice from this 1 % dose group had significantly lower body weights and increased relative kidney weights,and female also had significant increases in relative liver weight. No significant differences were observed between the control and treated animals for the weights of reproductive organs, sperm motility, morphology or averageoestrous cycle length and frequency. In the only histopathological examination carried out on the treated females, no treatment related kidneys lesions were observed. The cross-breeding results suggest that the fertility effects were only due to effects on the female mice. Furthermore, these effects may have been an indirect consequence of the severe general systemic toxicity rather than a direct effect of EGBE on the reproductive organs.

A final phase was conducted to assess the fertility and reproductive effects of EGBE in second generation (F_1) pups. The pups selected were those born after the CBP and when the maternal animals were individually housed. As there were insufficient pups in the 1 and 2 % dose groups, only the pups from the 0.5 % dose group were used. The F_1 generation pups were nursed, weaned and reared to sexual maturity. After weaning, the mice received 0.5 % 2-butoxyethanol in their drinking water (estimated daily intake 950 mg/kg bw). At 74 ± 10 days of age, the F_1 animals from different litters were mated. The animals were necropsied after delivery. No significant fertility and reproductive effects were observed in the F_1 animals as indicated by the proportions of successful copulation and fertile females, litter size, pup viability and live pup weights. Similarly, no treatment-related changes in the weights of

reproductive organs, sperm motility, morphology and the oestrous cycle length and frequency were noted. However, a significant increase in relative kidney weight in the females and a significant increase in relative liver weight in both the males and females were observed.

In summary, significant adverse reproductive effects were observed in the females at very high dose levels (1340 mg/kg and above) which also caused severe toxicity, including death. Under the conditions of the study, the NOAEL for reproductive toxicity of EGBE (fertility) can be set as 720 mg/kg/day. For developmental toxicity, no NOAEL can be derived. A conservative LOAEL of 720 mg/kg/day can be taken as only a very slight decrease in pup weight was observed at this dose. No NOAEL or LOAEL can be determined for systemic parental toxicity because this kind of study is not designed to assess systemic toxicity although it is of note that there were effects (reduced fluid consumption) even at the lowest dosage of 720 mg/kg/day.

Other studies:

Groups of five male JCL-ICR mice received doses of 500, 1000, or 2000 mg/kg bw/d EGBE (purity and GLP status unknown) by gavage for 5 days/week for 5 weeks (Nagano *et al.*, 1984). 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 level all the mice died and dose-related haematotoxicity (reduced red blood cell count) was observed at the other doses. There were no significant effects on the absolute or relative weights of the testis or seminal vesicle and coagulating gland at any dose tested. An earlier publication (Nagano, 1979) mentioned testicular atrophy in one of five mice dosed with EGBE (1000 mg/kg). This observation was not considered significant by Nagano and his co-authors in their later publication (Nagano *et al.*, 1984).

In 13 week duration toxicity studies in F344/N rats and B6C3F1 mice conducted by the National Toxicology Program (1993), EGBE (purity 99 %) was administered in drinking water at concentrations of 0, 750, 1500, 3000, 4500 or 6000 ppm to groups of 10 male and 10 female rats and at 0, 750, 1500, 3000, 4500 or 6000 ppm to groups of 10 male and 10 female mice. These concentrations provided target dose levels of 0, 100, 150, 250, 400 or 650 mg/kg bw per day (NTP, 1993). In addition to conventional toxicological endpoints, vaginal cytology and sperm morphology evaluations were performed. Vaginal cytology was assessed over the 7 days prior to sacrifice and sperm morphology and motility were estimated from epididymal sperm samples taken at necropsy. In the rat, haematotoxicity was evident in males at doses of and above 3000 ppm and in all female groups. Terminal body weight was decreased by 9 and 14% in males given 4500 and 6000 ppm EGBE. In females, body weights were decreased by 14 and 22% at these concentrations. There was no treatment effect on testis weights but there was a reduction in the size of the uterus in females at 4500 and 6000 ppm. Changes in uterine weight were considered by the authors to be secondary to the reduction in body weight gain rather than a direct effect of EGBE. The only spermatozoal measurement that showed significant change relative to the control group was sperm concentration which was slightly decreased in all groups of treated males; however, this effect was not dose-related. There were no significant differences from the control group in oestrous cycle length for treated females, although females treated at 4500 and 6000 ppm spent more time in dioestrous than the other groups. This correlates with the smaller uterine size, which was attributed to a secondary consequence of reduced body weight gain. In mice there were no chemical-related gross or microscopic lesions and no biological significant changes in any of the reproductive parameters evaluated. The small differences in the rat reproductive

endpoints with EGBE were in marked contrast to the significant effects of EGME and EGEE in both species, where concurrent studies demonstrated treatment-related testicular degeneration/ atrophy and, for EGEE, extended oestrous cycle length.

Table 4.85 bis: Data on estrous cycling for rats exposed to EGBE for 13 weeks in drinking water.

Study parameters	0 ppm	3000 ppm	4500 ppm	6000 ppm
n	7	6	7	6
Necropsy body weight (g)[1]	186 ± 4	172 ± 2	160 ± 2	145 ± 2
Estrous cycle length (days)	6.50 ± 0.70	6.83 ± 0.95	7.57 ± 0.53	5.83 ± 0.70
Estrous stages (% of cycle)				
Diestrus	28.9	45.6	52.8	67.5
Proestrus	8.8	11.4	13.9	7.0
Estrus	57.9	38.6	20.4	17.5
Metestrus	4.4	4.4	13.0	7.9

[1] Based on n = 10.

With respect to oestrous cycle evaluations, normally cycling Fischer 344 females have 5-6 day oestrous cycles. While there were 10 females per dose group, 3-4 females per group had estrous cycles longer than 12 days or cycles that were unclear, including the control group. These animals were excluded from the analysis, meaning that only 6-7 females per group were evaluated. Furthermore, given the approximate times in the various stages of estrous (see table below from Zarrow *et al*., 1964), it appears that the control animals spent an excessive amount of time in estrus. Typically, estrus would comprise approximately 30% of the estrous cycle, rather than the 57.9% reported for the control females. Using Zarrow's table, if one assumes 2 days for estrus and minimal durations for the other estrous stages, estrus would only comprise 44% of the cycle (2 days out of 4.5 days total estrous cycle). This value is well below the 57.9% reported in the control animals in the NTP study. Vaginal smears were collected only once per day. With only 6-7 rats per group and stages of the cycle that are less than one day in duration, some phases could have gone undetected.

Table 4.85 ter: Vaginal cytology during the estrous cycle of the rat[1]

Time (days)	Stage	Vaginal smear
0.5-1	Proestrus (P)	Many rounded epithelial cells, few leukocytes
1-2	Estrus (E)	Cornified epithelial cells, no leukocytes
0.5-1	Metestrus (M)	Maximum number of leukocytes, often in clumps surrounding epithelial cells. For our purposes this will be classified as part of Diestrus.
1.5-2	Diestrus (D)	Few epithelial cells; few to many leukocytes. Sometimes divided into Diestrus I and II

[1] Modified from Zarrow *et al.* (1964).

These points, coupled with the lack of effect on overall estrous cycle length and absence of histopathologic lesions, suggest that the effects on estrous cycle length are unlikely to indicate a specific treatment-related reproductive effect.

Thus, the effects on male and female rat reproductive endpoints are not indications of selective reproductive system toxicity. These effects were minor in nature, only present in one species and only occurred in the presence of other systemic toxicity (i.e., decreased body weights, haematotoxicity). There were no histopathological lesions in reproductive organ

Inhalation route

In an inhalation study (Doe, 1984), male Alpk/Ap Wistar-derived rats were exposed whole body to 800 ppm (4 mg/l – saturated vapour) EGBE (purity unknown) for 3 hours (no GLP). This study was performed to compare the effects of EGBE and EGME in rats. The rats were then observed throughout the next 14 days. The animals were sacrificed on day 15 and subject to gross macroscopic post-mortem examination, which included the weighing of the testes. Clinical observation revealed haematuria in treated animals. EGBE had no effect on testicular weights.

In the 90 day toxicity study of EGBE (purity 99.4 %) administered by the inhalation route (whole body) to Fisher 344 rats at doses of 5, 25 or 75 ppm for 6 hours/day, 5 days/week for approximately 13 weeks (16 males and 16 females/group) (Bushy Run Research Center, 1981b), there were no adverse effects on testicular weights and no reported pathological changes in the reproductive organs.

Studies on metabolites :

***In vitro* studies**

Primary testicular cell cultures were treated with a series of GE and Alkoxyacetic acids (Gray *et al.*, 1985). In contrast to the action of methoxy-acetic acid (MAA) and ethoxy-acetic acid (EAA), neither n-propoxy-acetic acid (PAA) nor n-butoxyacetic acid (BAA) was overtly toxic at a concentration of 10 mM for 72 hours. Under these conditions BAA produced no discernible effects. At 20 mM, BAA was still without appreciable effects.

To explore the development of quantitative indices of toxicity in the cell cultures, measurements were made of Carnitine AcetylTransferase (CAT) and lactate dehydrogenase-X (LDH-X), two enzymes whose activity is higher in the pachytene spermatocytes than in the other cell types present. When the 4 alkoxyacetic acids were compared, there was a parallel between their effects on CAT and LDH-X activity and the severity of the morphological changes. BAA produced a decrease in CAT activity (10 mM) in the attached germ cell fraction less marked than those of others alkoxyacetic acids, although statistically significant (88 nmol/mim/culture vs 121.7 nmol/mim/culture in controls). When expressed as specific activities, the result was still significant (54.8 nmol/mim/mg cell protein vs 68 nmol/mim/mg cell protein). No effect was seen with BAA treatment in LDH-X activity.

Mixed cultures of Sertoli and germ cells were incubated during 24 hours in the presence of BAA at doses ranging from 0 to 10 mM (Foster *et al.*, 1987). Cultures were also made with MAA and EAA. BAA did not produce any changes when tested in Sertoli-germ cell cultures at 5mM in contrast with effects seen with MAA and EAA (to a lesser extent) for which an

enhancement of germ cell loss (pachytene spermatocytes) were seen at this dose level. Some non specific cell loss was observed at 10 mM..

***In vivo* studies**

BAA was administered orally by gavage to young SD rats (4 weeks old) for 4 days concurrently with 3 other alkoxyacetic acids at equimolar doses to characterise their relative testicular toxicity (Gray *et al.*, 1985). The rats were killed 24 hr after the last dose. With PAA (776 mg/kg) and BAA (868 mg/kg) no morphological changes in the testis were observed at the dose studied. Only MAA produced testicular toxicity at the dose of 592 mg/kg.

In male Wistar rats (Foster *et al.*, 1987), the administration by gavage of a single dose of BAA did not result in any testicular damages at the highest dose tested of 868 mg/kg (the animals were terminated 14 days after dosage). At the dose of 868 mg/kg, animals showed evidence of haematuria. No other systemic nor specific effects on testes were seen at doses of 174 and 434 mg/kg. This study was concurrently performed with MAA and EAA and confirmed the testicular toxicity of these other alkoxyacids.

In all sub-acute, sub-chronic and chronic studies no direct effects were seen on male or female reproductive organs at all doses tested (even sub-lethal doses). Metabolism of EGBE leads to the formation of a majority of BAA, which is not a male reproductive toxic substance, unlike MAA or EAA (main metabolites of EGME and EGEE respectively).

In the continuous breeding study, effects were seen on fertility only at doses which were severly toxic to the animals (1340 and 2050 mg/kg bw/d). A NOAEL of 720 mg/kg bw/day can be fixed for fertility effects by oral route in mice.

4.1.2.9.2 Developmental toxicity

Studies in animals

Oral route

Studies in rats

The teratogenicity of EGBE (> 99.0 % purity) was evaluated using Fisher 344 rats (Sleet *et al.*, 1991; Schwetz and Harris, 1993 ; Research triangle institute, 1988 as part of the National Toxicology Programme (NTP)). The purpose of the study was to determine whether the exposure of pregnant rats to EGBE during critical periods of cardiovascular development adversely affected the structure of the foetal heart and great vessels. Other developmental toxicity was also recorded. EGBE (in distilled water) was administered via gavage to a total of 298 rats; 104 rats served as controls. The distribution of doses for the 184 animals dosed on gestation days (GD) 9 through 11 was as follows: 46 rats (0 mg/kg/day), 46 rats (30 mg/kg/day), 47 rats (100 mg/kg/day), and 45 rats (200 mg/kg/day). A total of 93 rats (all dose groups included) were killed on GD 12 of gestation, and the remaining 91 rats, on GD 20. For the 220 rats dosed on GD 11 through 13, the distribution of doses was as follows: 58 rats (0 mg/kg/day), 52 rats (30 mg/kg/day), 59 rats (100 mg/kg/day), and 51 rats (300 mg/kg/day). A total of 104 rats (all dose groups included) were killed on GD 14, and the remaining 116 rats, on GD 20. Also, on GD 20, foetuses were killed and examined.

The maternal effects of EGBE given from GD 9 - 11 or from GD11 - 13 at doses of and greater than 100 mg/kg/day included marked reductions in body weight and/or weight gain, increases in organ weights (kidney and spleen) and severe haematotoxicity. In particular, dramatic reductions in circulating red blood cells, haematocrit and haemoglobin resulted by 24 hours after treatment. By GD20 the haematotoxic effects were nearly reversed. The changes observed in haematological parameters and organ weights in this study are typical of haemolytic anaemia and the compensatory haematopoietic response associated with recovery. The maternal NOAEL is 30 mg/kg/day based on the effects describes before.

Embryo/foetal effects were: increased resorptions, nonlive implants, and adversely affected implants per litter in the 200 mg/kg/day group dosed on GD 9 - 11, and decreased platelet count but no embryolethality in the 300 mg/kg/day group dosed on GD 11 - 13.

EGBE exposure during gestation did not increase the incidence of foetal malformations; particularly, no cardiovascular malformations were observed. When 200 mg/kg were given from day 9 to 11, an increased foetal lethality without malformations was noted. When 300 mg/kg were given from day 11 to 13, a decrease platelet count was seen in foetuses. For developmental toxicity the NOAEL is 100 mg/kg when EGBE is administered from GD 9 - 11 and the conservative NOAEL is 100 mg/kg when EGBE is administered GD11 – 13 (although the NTP conclusion was for a NOAEL of equal to or greater than 300 mg/kg/day).

Studies in mice

In a gavage probe study in pregnant CD-mice (Exxon, 1985 ; Wier *et al.*, 1987), EGBE (purity 97 %)was administered in distilled water at 0 – 350 – 650 – 1000 – 1500 and 2000 mg/kg bw/day (6 animals per group) during gestational days (GD) 8-14(Note GD0 = vaginal sperm plug and sacrificed GD18). Haemolytic effects in the dams were observed from 650 mg/kg/day. At 1500 mg/kg/day the maternal mortality rate was 3/6 and at 2000 mg/kg/day 6/6. Increased resorption rates and numerically reduced number of viable foetuses were observed at 1000 and 1500 mg/kg/day. 4/43 foetus (in one litter) at 1000 mg/kg/day and 1/25 at 1500 mg/kg/day had cleft palates. For this study, the NOAEL for maternal toxicity is 350 mg/kg/day and the NOAEL for developmental toxicity is 650 mg/kg/day.

In a subsequent GLP reproduction study (following a Chernoff modification study design with treatment of the pregnant mice (20 per group) at 650 or 1000 mg/kg/day between GD 8-14, the animals were allowed to give birth and the offspring were observed till Post natal Day (PND)22). No significant effects on pup growth or survival resulted. No adverse developmental effects were reported. Signs of haemolytic effects and bw gain reduction were seen at 1000 mg/kg in dams. The NOAEL for maternal toxicity is 650 mg/kg/day in this study, and as no effects were seen in pups, the NOAEL for developmental toxicity can be considered to be greater than 1000 mg/kg/day.

In another Chernoff assay, fifty mated CD1 mice were orally administered EGBE (99 % purity) by gavage at 1180 mg/kg/day (calculated LD_{10} based on a non-pregnant mouse pilot study) in corn oil from GD7-14 (GD1=vaginal sperm plug), then allowed to litter and to rear pups to PND3. 20 % of the dams died, maternal weight gain was reduced and, of 31 surviving pregnant females, there were only 24 viable litters (77 %) compared with 97 % control litter viability. No external malformations were seen, pup survival to PND was unaffected and no other indication of specific developmental toxicity was found (Schuler *et al.*, 1984). A maternal NOAEL could not be calculated and, although pup development was unaffected, the

observation of reduced numbers of live litters precludes a calculation of the developmental NOAEL.

Summary oral route :

One study is available in rats and 2 studies in mice. The lowest foetal NOAEL is 100 mg/kg/day in the Research triangle institute (1988) rat study. It is based on effects seen at 200 mg/kg/day: increase foetal lethality without malformations. These effects were seen with maternal toxicity (haemolytic anaemia) and retarded body weight gain evidenced from 100 mg/kg/day. The maternal NOAEL is 30 mg/kg/day in this study.

Inhalation route

Studies in rats

In an initial GLP study, mated female Fischer 344 rats (30 per group) were exposed whole body to 0, 100, 200 or 300 ppm (0, 483, 966 or 1449mg/m^3) EGBE vapour for 6 hours/day on GD6-15 (GD0 = sperm plug positive) (Tyl *et al.*, 1984 ; Bushy Run Research Center, 1984). Maternal weight loss was observed at 300 ppm and 200 ppm and retarded weight gain at 100 ppm. During exposure, females were hypoactive, with a dose-related increase in the frequency of signs indicative of haematuria and with dose dependent reductions in food consumption at all levels and in water consumption at 200 ppm and 300 ppm. At 300 ppm one animal died. In blood samples taken from females at necropsy (six days after the last exposure), red blood counts were reduced at 300 ppm and 200 ppm. At 300 ppm, 16 of 23 pregnant dams showed 100 % resorptions. There was no increase in resorptions at the lower doses, but low pregnancy incidences in all groups, including controls, resulted in only 7, 15, 21 and 21 litters being evaluated at 0, 100, 200 and 300 ppm, respectively. The authors considered this number of litters too few for definitive interpretation of results at higher doses. Interpretation of the results was complicated by maternal toxicity, which included decreased body weight gain at 100 ppm, and body weight losses at 200 and 300 ppm, a condition that causes marked maternal stress. Contributing to this stress was decreased food consumption at all concentrations, and decreased water consumption at 200 and 300 ppm. Dams were hypoactive with signs of haematuria, indicating maternal haemolysis. Red blood cell counts were reduced at necropsy in dams exposed to 200 and 300 ppm. 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, although it did trigger the conduct of a developmental phase staged oral study described above (Sleet *et al.*, 1991; Schwetz and Harris, 1993 ; Research Triangle Institute, 1988 as part of the National Toxicology Program (NTP)) which confirmed the lack of teratogenicity. In increased incidences of foetuses at 300 ppm in the inhalation study, the development of the inominate artery was affected (shortened or missing), the lateral brain ventricles were enlarged, ossification was retarded and extra ribs were present. Foetal ossification was also retarded at 200 ppm and enlargement of the lateral brain ventricles was observed at increased incidences at 200 ppm and 100 ppm. These variations were considered indicative of embryo/foetal toxicity at maternally toxic dose levels. To investigate these potential effects further, a second inhalation study was performed in rats together with a study in rabbits. It should be noted that the effects seen on lateral ventricle size were not reproduced in the subsequent rat and rabbit studies, despite exposure over the same sensitive period with previously effective concentrations of EGBE (100 and 200 ppm). In contrast, other effects

(e.g., delayed ossification) were reproducible across studies. Thus, the finding of increased incidence of enlarged lateral ventricles cannot be considered significant.

In a GLP study, mated female Fischer 344 rats (36 per group) were exposed whole body to 0, 25, 50, 100 or 200 ppm (0, 121, 242, 483 or 966 mg/m^3) EGBE vapour (purity 99.6 %) for 6hr/day on GD 6-15 (Tyl *et al.*, 1984 ; Bushy Run Research Center, 1984)(GD0 = sperm plug positive). No adverse reproductive or developmental effects were observed in animals exposed to 25 ppm or 50 ppm. Maternal toxicity was observed in a dose-related incidence during the exposure period, included evidence of haematuria from 100 ppm and pale, cold extremities with necrosis of the tail tip at 200 ppm. Dose-dependent maternal weight loss was observed at 200 ppm and 100 ppm, associated with significant reductions of food consumption (for both groups) and water consumption (for the 200 ppm group). Haematological findings from blood samples taken at necropsy on GD21 (six days after the last exposure) evidenced signs of haemolytic anaemia for treatment with 100 ppm EGBE and higher.

At sacrifice, maternal gravide uterine weight was significantly reduced and the absolute and relative spleen and relative kidney weight were elevated compared to controls at 200 ppm.

Exposure to 200 ppm was associated with a statistically significant increase in the number of totally resorbed litters and a reduction in the number of viable implants and in percentage of live foetuses per litter. However, there were no statistical significant increases in incidences of external, visceral, skeletal, or total malformations associated with exposure to EGBE. Exposure to 200 ppm was also associated with a significant increase in the number of litters with one or more foetuses with unossified skeletal elements and poorly ossified skeletal elements (anterior arch of atlas, cervical centra 5 and 6 and forelimb proximal phalanges) and poorly ossified skeletal elements (cervical arches and sternebrae 1, 3, 4 and 6). Primarily because the skeletal elements were poorly ossified there was a decreased incidence of bilobed cervical centra 5 and 6 and bilobed thoracic centra 9 and 13. There was also a decreased incidence of poorly ossified hindlimb proximal phalanges. At 100 ppm, the number of litters with one or more foetuses with unossified cervical centrum 6 was significantly increased and, because fewer also showed ossification of cervical centrum 5, the number bilobed was significantly decreased. No significant differences were observed for other aspects of skeletal ossification. The occurrence of unossified skeletal elements was considered by the authors to be an indication of delayed development in animals exposed to EGBE under maternally toxic concentrations. For this study the NOAEL is 50 ppm for maternal toxicity based on haemotoxicity and bw and food consumption retardation at 100 ppm. A LOAEL of 100 ppm for developmental toxicity is due to the small increase in skeletal ossification retardation, but without any other foetal effects, variant, observed at this dose level, this leads to a NOAEL of 50 ppm for developmental toxicity.

In another inhalation study, the teratogenicity of EGBE (98 to 99.5 % purity) was evaluated using female SD rats (Nelson *et al.*, 1984). The animals were exposed whole body to EGBE vapour on GD 7 – 15 (GD0 = sperm positive vaginal smear) for 7hr/day (GLP status unknown). Sixteen and fifteen rats were exposed to 150 ppm and 200 ppm, respectively. The untreated control group consisted of 34 dams. Higher dose levels (250 - 500 ppm) in a preliminary study were associated with dose-related maternal deaths, haematuria and tail necrosis.

On the first day of exposure, haematuria was observed in the dams in 150 and 200 ppm groups. This was the only effect observed in dams. There were no significant differences in

the number of resorptions foetal weights and the incidence of malformations or variations between the group exposed to 200 ppm EGBE and the control group. A statistically significant decrease in foetal weights was observed in the 150 ppm exposure group. Since differences in foetal weights between experimental and control groups were slight, and, also, that significant differences were not observed in the 200 ppm exposure group, these differences were not thought to have been of biological significance. For this study a very conservative LOAEL of 150 ppm can be determined for dams for haematuria seen in the first day of treatment. For developmental toxicity, the NOAEL is 200 ppm.

In a briefly reported study (Barilyak, 1989 in Russian), mated female rats were exposed to EGBE at concentrations of 77, 12 and 2 mg/m^3, 4 hours a day during GD1 - GD19. At GD20, animals were killed and pups removed by laparoscopy. Number of Corpora Lutea and, normal and abnormal pups were determined. A complete identification of teratogenic effects was performed in abnormal animals. In this study, EGME was also tested concurrently with Glycols.

For EGBE, at the two high dose tested, a significant increase of total losses was seen compared to controls. These increases were due to an increase in pre-implantation loss for the high dose group (77 mg/m^3) and due to a post-implantation loss for the mid-dose group (12 mg/m^3). No abnormalities were seen in any treated group but 32.5 % of the pups of the high dose group exhibited a delayed development.

This publication is not detailed enough to consider it usefull for risk assessment.

Studies in rabbits

In a GLP study, mated New Zealand white rabbits (24 per group) were exposed whole body to 0, 25, 50, 100 or 200 ppm (0, 121, 242, 483 or 966 mg/m^3) EGBE vapour (purity 99.6 %) for 6hr/day on GD 6-18 (GD0 = day of copulation) (Tyl *et al.*, 1984 ; Bushy Run Research Center, 1984). No adverse reproductive or developmental effects were observed in animals exposed to 25 ppm or 50 ppm. At 200 ppm four females died or were sacrificed by the third day after the onset of dosing and four aborted. The blood samples taken at necropsy on GD29 (eleven days after the last exposure) showed increased haemoglobin concentration and haematocrit at 100 and 200 ppm, but statistical significance was only evident at 100 ppm.. Maternal body weight loss was observed in all groups including controls during exposure, but the difference was greatest at 200 ppm and, by GD15, the actual body weight at 200 ppm was significantly lower.. Exposure to 200 ppm produced a significant reduction in maternal body weight, gravide uterine weight and number of total implants and viable implants. There were no significant increases in the number of foetuses or litters with one or more affected foetuses with pooled external, visceral, skeletal (total) malformations in any treatment group. For individual malformations, there was a significant increase relative to controls in the number of litters at 100 ppm with one or more foetuses exhibiting fusion of papillary muscles in the left ventricle. Five foetuses from 4/19 litters were affected. However, since this malformation was not observed at the higher or other concentrations it was considered by the authors, the reviewers and the rapporteur as coincidental and not treatment-related. The incidences of skeletal variations did not indicate an adverse treatment effect on ossification. There were significant decreases in the incidences of litters at 200 ppm with unossified sternebra 6 and with rudimentary ribs on lumbar vertebra 1. However the former observation was indicative of better ossification and the latter observation was because more ribs at 200 ppm were full (extra) rather than rudimentary and there were actually no intergroup differences in supernumerary rib numbers. For this study, the NOAEL for maternal toxicity is 50 ppm and

the NOAEL for developmental toxicity is 100 ppm based on fewer viable implants seen at 200 ppm.

Summary inhalation route:

In both Tyl studies (1984), the developmental NOAEL of 50 ppm can be taken into account for the risk assessment (rats). This value is based on variations seen at 100 ppm. These effects were seen in presence of maternal toxicity (haemolytic anaemia) which was seen at 100 ppm and higher. The maternal NOAEL for this effect was 50 ppm. In one study performed on rats, no effects were seen at the highest tested dose (200 ppm).

Dermal route:

EGBE was applied to the skin (unoccluded) of 9 pregnant SD rats to investigate its potential for developmental toxicity (Hardin *et al.*, 1984). Four doses each 2.5 hours apart of 106 mg EGBE (total daily dose of 424 mg, 1760 mg/kg/day) were applied daily to shaved interscapular skin of rats on GD 7 – 16 (GD0 = sperm positive). A previous replicate of females dosed at a concentration three times higher had been terminated because of marked toxic effects (haematuria, tail necrosis, death after 3-7 days treatment). No maternal, embryotoxic, fetotoxic, or teratogenic effects were detected with EGBE treatment at the lower concentration of approximately 1760 mg/kg/day.

No clear conclusion can be drawn from the findings of this study since EGBE was applied to the skin without occlusion which would potentially enable evaporative loss from the site of application.

Other routes :

Studies in rats

Groups of rats were injected subcutaneously with aqueous solutions of EGBE to determine the teratogenicity of the chemical (Unilever, 1976). In the first of two preliminary studies to set the dose range, non-pregnant rats received a single injection with 225-1350 mg/kg; 80% of the high dose animals died, haemoglobinuria was observed and general poor condition of the rats resulted. In the second preliminary study, no effects were observed when a similar group of rats received repeated injections at 23-90 mg/kg/day. Experimental details for the preliminary studies were not available.

In the main study, conducted in accordance with OECD test guideline 414, groups of 20 pregnant CD rats received sub-cutaneous injections of 0. 45, 90 or 180 mg/kg/day as an aqueous solution on GD6 - 15. No mortality resulted but haemoglobinuria and body weight loss were observed in the medium and high dose dams after the first two injections only. No significant pathological findings were noted at necropsy. Pre-implantation loss increased in the medium (13.8 %) and high dose (11.9 %) dams compared with the controls (6.9 %), but the values were within the laboratory's normal range. Other parameters were normal. In the foetuses, a slight increase in rib effects (wavy/thickened) and a dose-dependent increase in incomplete ossification of cranial bones were observed, but such changes are considered as variations and not malformations.

Under the conditions of the study, pre-implantation loss occurred in dams at maternally toxic doses and there were no treatment related signs of teratogenicity. In this study, the NOAEL

for dams is 45 mg/kg/day and the NOAEL for developmental toxicity is 90 mg/kg/day based on the variations observed at 180 mg/kg/day.

In vitro studies

EGBE and other glycol ethers were tested in 2 series of Hydra Regeneration Assays (Bowden *et al.*, 1995 ; Johnson *et al.*, 1984 and 1988). The Hydra Regeneration Assay compares the toxicity of a test compound to an adult hydra polyp with its ability to inhibit the regeneration of an isolated digestive region (Bowden *et al.*, 1995) or the regeneration of pellets prepared from non-specific dissociated adult cells (Johnson *et al.*, 1984 and 1988). In the Bowden study (1995), EGBE was administered at doses ranging from 0.09 mg/ml to 0.92 mg/ml to groups of 10 polyps and 10 regenerates. The mean morphological scores for polyps were 10, 8.6, 9.8, 9.0, 4.4 and 4.1 and for regenerates were 8.1, 7.2, 6.6, 6.6, 3.0 and 1.9 for the control and concentrations of 0.09, 0.18, 0.37, 0.74 and 0.92mg/ml, respectively. Comparing the two polyp versus regenerate scores, the estimated T_{50} (corresponding to the concentration 50 % toxic to the polyps) was 0.69 mg/ml and the I_{50} (corresponding to the concentration which cause an inhibition of 50 % of the regeneration) was 0.54 mg/ml. However, if the treated concentrations versus their respective controls are compared, this gives equivalent T_{50}/I_{50} concentrations of approximately 0.6mg/ml . The T_{50}/I_{50} ratio is considered equivalent to the mammalian A/D ratio (adult to developmental toxicity) and is used as a predictor for developmental hazard. In this study the T_{50}/ I_{50} ratio was 1.3 for the first calculation and approximately 1 for the second, indicating that the adult and developmental stages were similarly sensitive. If this ratio is compared to other glycol ethers, the developmental hazard was less than with EGEE and EGME and higher or approximately equivalent to DGEE.

These results were not consistent with the Johnson study in which the "A/D" ratio was 4.4 for EGBE, 5 for EGEE and 1.3 for EGME, indicating that EGBE could be more toxic to the "developmental" stage than EGME. However, the developmental stages were different between the studies so a direct comparison is inappropriate. In any event, and assaid by the authors, these *in vitro* studies cannot be use for quantitative risk assessment.

EGBE and other glycol ethers were tested in the Rat Whole Embryo Culture (WEC) assay (Bowden *et al.*, 1995). The WEC system used mated Sprague-Dawley CD rats (GD1 = sperm positive). Animals were paired on a one-to-one basis. The morning following pairing was designated day 1 of gestation. On GD 10, dams were sacrificed and embryos dissected out. Embryos were cultured in a special medium with S9 metabolic activating system. EGBE in DMSO solution was diluted to 10 % (v/v) with sterile water and added to the culture medium. EGBE was tested at concentrations of 0, 0.3, 0.5, 0.75 and 1 mg/ml. Culture were terminated after approximately 48 hours. To estimate growth, the yolk sac diameter, crown-rump length and head length were measured and the number of somites counted. Each embryo was examined for dysmorphogeneses and the morphological score was assessed. The rate of dysmorphogeneses was calculated as: Malformation index = n° of observed malformations / n° of embryos in the group. Results were compared with other glycol ethers: EGME – EGEE – DGEE – EGTA – EGS.

EGBE produced marginal reductions in growth and developmental parameters at 0.3 mg/ml; these effects being greatly increased at 0.5 mg/ml and above. Increased incidences of malformations were observed at 0.3 mg/ml and above, including poor yolk sac circulation, thin allantois, twisted flexions, incomplete fusion and/or irregular formation of the caudal neural tube and brains, irregular posterior neuropore and growth-retarded forelimb buds. At 1 mg/ml, 5/10 embryos were dead. For this test, results obtained with glycol ethers are not

suitable for effect assessment because, *in vivo*, they have a very low half life in the circulating blood (< 10 min for EGBE) and the high doses at which glycol ethers produced effects *in vitro* are totally unrealistic *in vivo*. Although a S9 metabolic activation system was included in the embyo culture medium, the results might therefore have been expected to show the toxicity profile of the metabolite. However, the alcohol / aldehyde dehydrogenase system is not present in the S9, this may explain the discrepancy between embryo culture results and *in vivo* data. For these reasons in addition to the general difficulties in extrapolating *in vitro* data, the results obtained in this study cannot be taken into account for the risk characterisation.

In another WEC assay (Jakobsen, 1995), rats embryos were cultured in a medium containing Glycol ethers (EGME, EGEE, EGIPE and EGBE) and their alkoxy acids (MAA, EAA, IPAA and BAA). The doses tested were 2, 4, 8 and 16 mM for alkoxy acids and 10 20, 40 and 80 mM for the glycol ethers. After 48 hours of incubation, effects on development were assessed.

The results obtained are summarized in the following tables:

Table 4.86: effects of EGBE on the development of rat embryos explanted on day of gestation and cultured for 48h.

Concentration	Control	EGBE 5 mM	EGBE 10 mM	EGBE 20 mM	EGBE 40 mM
Nb of embryos evaluated	123	20	19	10	16
Development					
Yolk sac ∅ (mm)	3.5 ± 0.4	3.5 ± 0.3	2.9 ± 0.3 ***	1.6 ± 0.5 ***	1.0 ± 0.3 ***
Crown rump lengh (mm)	2.8 ± 0.5	2.4 ± 0.3 ***	1.9 ± 0.2 ***	1.3 ± 0.2 ***	-
Nb of somites	29.2 ± 2.2	29.6 ± 2.3	25.0 ± 4.4	-	-
Morphological score	36.3 ± 2.1	35.5 ± 1.6	29.0 ± 5.2 ***	11.7 ± 10.1 ***	0.0 ***
% abnormalities					
Grossly abnormal embryos	0	0	36.8 ***	100.0 ***	100.0 ***
Cranial neuroporus, open	0	0	57.9 ***	100.0 ***	100.0 ***
Caudal neuroporus, open	9.8	15.0	10.5	20.0	-
Brancial arches, abnormal	0	0	0	16.7 ***	-
Vesicles	0	15.0 ***	5.3 *	-	-
Tail, abnormal	0.8	0	5.3	-	-
Flexion, ventrally convex	2.4	5.0	10.5	60.0 ***	-
Somites irregular	0	0	0	-	-
Yolk sac, abnormal	0	0	0	0	0
Yolk sac, none circulation	30.9	100.0 ***	100.0 ***	100.0 ***	100.0 ***

*** $p < 0.001$ ** $p < 0.01$ * $p < 0.05$

Table 4.87: Effects of BAA on the development of rat embryos explanted on day 9 of gestation and cultured for 48 hr.

Concentration	Control	BAA 2 mM	BAA 4 mM	BAA 8 mM	BAA 16 mM
Nb of embryos evaluated	123	15	19	20	18
Development					
Yolk sac ∅ (mm)	3.5 ± 0.4	3.6 ± 0.4	3.5 ± 0.3	3.4 ± 0.4	2.7 ± 0.2 ***
Crown rump lengh (mm)	2.8 ± 0.5	2.8 ± 0.5	2.7 ± 0.3	2.2 ± 0.3 ***	1.4 ± 0.4 ***
Nb of somites	29.2 ± 2.2	29.4 ± 2.1	30.5 ± 1.6 *	25.8 ± 4.3 ***	21.8 ± 3.0 ***
Morphological score	36.3 ± 2.1	33.3 ± 9.5	35.3 ± 2.3	30.9 ± 3.1 ***	19.6 ± 5.3 ***
% abnormalities					
Grossly abnormal embryos	0	6.7 **	0	0	16.7 ***
Cranial neuroporus, open	0	6.7 **	0	25.0 ***	100.0 ***
Caudal neuroporus, open	9.8	40.0 ***	68.4 ***	45.0 ***	0
Brancial arches, abnormal	0	0	0	10.0 ***	0
Vesicles	0	0	0	0	0
Tail, abnormal	0.8	0	0	15.0 ***	50.0 ***
Flexion, ventrally convex	2.4	0	0	0	22.2 ***
Somites irregular	0	0	0	0	0
Yolk sac, abnormal	0	0	0	0	0
Yolk sac, none circulation	30.9	46.7	57.9 *	90.0 ***	100.0 ***

*** $p < 0.001$ ** $p < 0.01$ * $p < 0.05$

With EGME and EGEE, specific embryotoxic effects are found following exposure to very low levels of their alkoxyacetic acids whereas no specific effects were found when embryos were exposed to comparable concentrations of EGBE and BAA. The reproductive effects observed were due to non specific toxic damage to the embryos at comparable concentration.

This study demonstrates that embryotoxicity of glycol ethers increases with the increasing chain lengh of the alkyl group and also that the developmental toxicity of the alkoxyacetic acids decrease with the increasing chain lengh of the alkyl group.

In another Rat Whole Embryo Culture assay (Giavini *et al.*, 1993) EGBE, Butoxy acetic acid (BAA) and 2 other glycol ethers and their corresponding alkoxyacids were tested. EGBE was tested at 3.12, 6.25, 12.5 and 25mM and BAA (adjusted to pH6 with NaOH solution) at 0.4, 0.8, 1.6 and 3.2mM. General embryotoxic effects, as shown by a reduction of the somite number and of total protein/embryo, were seen with EGBE concentrations of and greater than 6.25 mM. Embryonic development was blocked at the EGBE concentrations of 25 mM. Exposure of embryos to 12.5 mM resulted in severe dysmorphogenic effects (inhibition of rotation, severe reduction of telecephalic vesicles). A dose dependant increasing percentage of abnormal development was seen with BAA at concentrations of 0.8, 1.6 or 3.2 mM. For this test, results obtained with glycol ethers are not suitable for effect assessment because, *in vivo*, they have a very low half life in the circulating blood (< 10 min for EGBE) and the high doses at which glycol ethers produced effects *in vitro* are totally unrealistic compared to the *in vivo* situation.

For BAA, effects were seen from 0.8 mM (40 % for 0.8 mM, 44.4 % for 1.6 mM and 60 % for 3.2 mM vs 22.2 % for controls and 20 % for 0.4 mM). Total proteins and somites numbers were also decreased for the 0.8 mM dose. This could suggest effects due to general toxicity rather than specific toxicity but as usual developmental parameters were not assessed, this could not be fully confirmed (Saillenfait, pers. com.). The malformative effects affecting prosencephale in this study are common effects seen with such a test.

In Whole Embryo Cultures of rat BAA (in the form of sodium salt) and 6 other alkoxy acids were investigated (Rawlings *et al.*, 1985). At a concentration of 5 mM, BAA adversely affected the development of conceptuses both in growth and the incidence of abnormal embryos. The most frequent abnormality produced was an irregularity of the neural suture line. No effect was observed at 2mM. In comparison with other alkoxy acids (MAA, EAA, nPAA, 3MPA and 4MBA), it was apparent that the potency of the effects decreased with increasing length of the alkoxy chain. Dysmorphogenesis was least affected with BAA, nPAA, 3MPA and 4MBA. The authors state that the lack of teratogenicity of butoxyethanol *in vivo* is a reflection of the limited teratogenic potential of its alkoxy acid metabolite.

EGBE was examined for its ability to block junction-mediated intercellular communication (Welsch and Stedman, 1984 ; Loch-Caruso *et al.*, 1984). The blockage of a specific type of intercellular communication, that is mediated by gap junctions, could be a possible mechanism of action of some teratogens. Interruption of intercellular communication was measured *in vitro* by an assay that depends on the transfer of metabolites via gap junctions, i.e., metabolic cooperation (V79 cell assay). EGBE was an effective blocker of intercellular communication at non cytotoxic doses in this assay. Results obtained with other glycol ethers demonstrated that as the length of the aliphatic chain increased so did the cytotoxicity.

Summary *in vitro* studies:

In rat whole embryo culture assays EGBE and BAA seems to cause developmental abnormalities in conjuction with effects on growth. The NOAEL for these effects are 3.12 mM for EGBE and 0.4 mM for BAA (Giavini *et al.,* 1993). These studies have demonstrated that the glycol ether embryotoxicity increased with increasing chain length (EGBE>EGEE>EGME). In contrast, the alkoxyacid embryotoxicity decreased with

increasing alkoxy chain length (MAA>EAA>BAA). The *in vitro* studies performed with EGBE (and glycol ethers in general) exibit effects which are not relevant *in vivo* (according to the kinetic of glycol ethers *in vivo* and the great doses needed to produce effects) and therefore are not directly usable in the risk assessment.

Studies in humans

For developmental toxicity, no studies are available for assessing the risk of exposure to EGBE alone.

In an case-control study, the risk of congenital malformations related to glycol ether exposure during pregnancy have been evaluated (Ha *et al.*, 1996). Cases were any infant, born alive or stillborn, and foetuses of any gestational age aborted because of a malformation. Malformations of known origin were excluded. Control were normal neonates born within one week of the case in the same place (maternity or locality). This study involved 991 cases and 1144 controls. Mothers were submitted to a standard questionnaire including occupational history, socio-demographic characteristics, pregnancy history and hobbies. Father's work was also determined. Occupational exposure to glycol ethers was assessed. Glycol ethers were classified in four group:

- group 1: EGME, EGEE and their acetates and some polyethylenic compounds
- group 2: EGBE, EGPE, and their acetates
- group 3: PGME, Propylene Glycol Methyl Ether Acetate (PGMA) and some polyethylenic and propylenic compounds
- group 4: other glycol ethers

In this study, there was a significant excess of mothers occupationally exposed to glycol ethers among the cases (27 % versus 20 % of the controls). When types of malformations were studied, a significant excess of oral clefts and central nervous system malformations was found in glycol ether exposed mothers compared to controls (OR = 2, 95 % CI = 1.1 – 4.1 and OR = 1.8, 95 % CI = 1.1 – 3.3 respectively). An excess of musculoskeletal malformations in exposed mothers was also seen although it was on the borderline of significance.

In an case-control study (same data and same study than the study described before ?), the risk of congenital malformations related to glycol ether exposure during pregnancy have been evaluated (Cordier *et al.*, 1997). The cases were women with any product of conception with a major congenital malformation diagnosed prenatally of during the perinatal period. The study period was between 1989 and 1992 and was conducted on 6 European registries (2 in France, one in UK, 2 in Italie and one in The Netherlands. Each case was match with a control chosen with the same birth date and born in the same town. Mothers was submitted a standardized questionnaire to assess their socio-economic status, medical and obstetric history, consumption of alcohol – tobacco – drugs, hobbies and occupation, both before and during each trimester of the pregnancy. Concerning oral clefts, a distinction was made between cleft lip with or without cleft palate [CL(P)] and cleft palate only (CP) due to their etiological difference.

Between 1989 and 1992, a total of 984 cases of malformation were reported. The overall odds ratio (OD) of congenital malformation associated with glycol ether exposure was 1.44. after adjustment for several potential confounders. The association with exposure to glycol ethers

appeared particularly strong in 3 malformation subgroups: neural tube defects (OR=1.94, 95 % CI = 1.16 – 3.24), multiples anomalies (OR=2.00, 95 % CI = 1.24 – 3.23) and cleft lip (with or without cleft palate) (OR=2.03, 95 % CI = 1.11- - 3.73). In this last subgroup, risk, especially of an isolated defect, tended to increase with level of exposure.

In the same study, the risk of oral cleft formation related to maternal occupation was studied in depth (Lorente *et al*., 2000).

Upon the including 984 cases, 161 cases were oral clefts. Differences between working mothers concerned only the place of residence (mothers of CL(P) cases came from less urban background than their referent) and mother's age (older for CL(P) cases).

The analysis of occupational exposures was restricted to women who worked during pregnancy (100 cases and 75 referents). For occupational, the odds ratios were estimated for each type of cleft and were highest for service occupation. Within this group the ORs were higher for CP than for CL(P) and significant for CL(P) for only 2 occupations: housekeepers and hairdressers. For production workers, the elevated ORs concerned CL(P). For chemical exposure, Ors adjusted were estimated. For glycol ethers Ors significantly different for 1 were observed:

Exposure	CL(P) n=64	CP n=36	Referents n=751	OR CL(P)	95 % CI CL(P)	OR CP	95 % CI CP
Glycol ethers	23	11	137	2.1	1.14-3.88	1.82	0.82-4.03

For CL(P) exposure to glycol ethers was associated with an excess risk. A more detailed analysis of the exposure variables of the level, frequency, and reliability of exposure could be performed only when enough subjects were exposed to each product. This analysis showed that, for glycol ethers among isolated CL(P) cases, the risk increased with the level and frequency.

In a case-control study, the risk of Neural Tube Defects related to maternal occupation al and hobby chemical exposure was assessed (Shaw *et al.*, 1999). 653 Neural Tube Defects cases (anencephaly, spina bifida cystica, craniorachischisis or iniencephaly) were extracted from a cohort of 708129 births and foetal death between June 1989 and May 1991. An interview of 538 cases and 539 controls was performed. Information about paternal occupation were also checked. Using information about occupation and hobbies, exposures to 74 chemical agents were classified as likely, maybe or not exposed. A classification was also made regarding end-use of chemicals in 9 group.

Compared to control mothers, case mothers were more likely to be Hispanic, less than 25 years old and to have a household income under 10000 dollars. They also were less likely to have graduated from college, to have used multivitamins in the 3 months before conception, to have smoked cigarettes in the first trimester or to have been employed in the periconceptional period.

Exposure to house keeping cleaner give an OD of 0.97. Overall there was no increase risk for solvent exposure based on combined occupational and hobby/household exposure or for occupational exposure along among employed women.

For glycol ethers and derivatives:

NTD cases	Controls	Odds ratio	95 % confidence interval
75	80	0.93	0.66-1.3

The risk of NTD was not increased for glycol ethers exposures.

A case control study similar than those already performed was made in Slovakia with data collection ranging from 1995 to 1996 (Cordier *et al.*, 2001). 196 case of live or stillborn babies with a major malformation or foetuses from therapeutic abortion were included in the study. Assessment of various chemical exposure (including glycol ethers) was performed.

The distribution of congenital malformations in each broad group of anomalies was very similar to those observed in the previous study. Odds ratio for glycol ethers exposure during the first trimester of pregnancy were estimated among working mothers for each subgroup of abnormalities, adjusted for maternal age, rural residence and mother's socio-economic status. The overall risk of congenital abnormalities was elevated (OR = 2.3; 95 % CI = 0.7-7). Among 15 potentially exposed women, 10 (7 cases, 3 controls) were exposed to the highest exposure category; in this group, the OR for all malformations was slightly higher (OR = 2.7; 95 % CI = 0.7-11). Malformation-specific Ors were increased with high exposure for all except cardiac anomalies (unchanged) and urinary anomalies (decreased). Caution should be given to this study due to the very small size of the studied population.

In a case-control study, the risk of Neural Tube Defect related to parental occupation was assessed among Mexican Americans (Brender *et al*, 2002). Case women were Mexican Americans whom had a product of conception (live or stillborn infant or spontaneous or induced abortion) with an NTD during the period of June 1995 to May 2000. NTD included were: anencephaly, spina bifida and encephalocele. A complete questionnaire was submitted to each case and control woman to determine their maternal health and reproductive history, family demographic data and medical history, and the use of medications, nutritional supplement, tobacco, alcohol, and drugs. Environmental and occupational exposures were also assessed.

Of the 225 cases, 184 completed the interview (82 %). For the controls, only 225 of the 378 selected (60 %). Case women tended to be slightly younger, less educated and significantly more case than controls where obese. For occupation, case women are more likely to work as cleaners and in health case. They are also more likely to have work exposures to glycol ethers (OR ∞, 95 % CI 1.8 - ∞). Women who reported activities associated with solvent exposure outside the workplace also were likely to have NTD-affected pregnancies. For paternal work: risk estimates were close to the null for exposure to glycol ethers.

Based on these findings, maternal work exposure to solvents or glycol ether and maternal occupations in health care or cleaning may be risk factors for NTDs in the studied population.

Through a review of the epidemiological studies available, Maldonado *et al.* (2003) demonstrated that the results available all suffers from methodological deficiencies which weaken the conclusions of these studies.

Other relevant information

In a study by Wilson (1953), dams were bled to induce haemorrhagic anaemia at different stages of pregnancy. Dams were bled at 3 critical periods during development: (1) on gd 7-9 to cause anaemia when the mesoderm is forming and organogenesis is beginning on gd 9-10; (2) on gd 9-11 or gd 11-13 to produce anaemia during the most active period of organogenesis on gd 11-14; and (3) on gd 13-15 during late organogenesis and early histogenesis, which occurs on gd 15-16. Blood loss by dams was 5 cc or more per 100 g body weight, which caused a marked drop in haematocrit values. For dams bled on gd 7- 9, removal of 5 to 5.9 cc blood per 100 g body weight resulted in a 52 % decrease in haematocrit (relative to controls) and totally resorbed litters for 33 % of pregnancies (3 of 9). Haemorrhages in excess of 6 cc caused a 63 % decrease in haematocrit and early termination of 13 out of 15 pregnancies due to either maternal death (8 dams) and total resorbed litters (5 dams). For dams bled on gd 9-11, 25 % of dams losing 5-5.9 cc per 100 g body weight exhibited a 54 % decrease in haematocrit and totally resorbed litters with 1 of 8 term litters (gd 20) exhibiting retarded development. For dams with more severe anaemia (in excess of 6 cc with a 59 % decrease in haematocrit), one third exhibited total resorbed litters. Of the 13 pregnancies with fetuses surviving to term (gd 20), the mean rate of prenatal survival was decreased (18 % of fetuses resorbed). Four litters exhibited growth retardation (delay). In general, the authors concluded that "although an occasional abnormality may be produced at this time, maternal anaemia usually causes either early termination of pregnancy or permits continuation of pregnancy without detriment to the young." Bleeding the dams on the 11-13 or 13-15 days of pregnancy caused a higher rate of maternal death (45 % of dams). In some cases, death of the fetuses preceded death of the dam. Total litter loss due to resorption was not seen at these time points. Some delayed development was seen. Bleeding that was initiated on gd 9 had the greatest likelihood of resulting in malformation, growth retardation (4 of 13 litters), or increased rate of resorption.

Similar results have been reported for pregnant rabbits in the presence of maternal anaemia. In studies with diflunisal, an antiinflammatory drug, pregnant does given a single oral dose of diflunisal on gd 5 exhibited anaemia that persisted through gd 15, despite clearance of the drug from maternal blood by gd 9 (Clark *et al.*, 1984). Signs of anaemia in pregnant does included haemoglobinuria, and decreased erythrocyte counts (a 74 to 46 % decrease relative to controls on gd 9 to 15) and decreased haemoglobin concentrations (a 63 to 21 % decrease relative to controls on gd 9 to 15). Haematotoxicity was the most severe on gd 9, the first day of data collection, with some recovery by gd 15. These does showed an increase in preimplantation loss (10 of 25 inseminated does showed no evidence of implantations and there was an increase in preimplantation loss in other does), as well as an increase in totally resorbed litters (11 of 25 litters). The percentage of resorptions and dead fetuses (mean of litter values) was 10.6 % in control does compared with 74.3 % in diflunisal-treated does, despite clearance of the test material from maternal blood by gd 9. These effects were attributed to maternal anaemia rather than a direct effect of diflunisal, because diflunisal is highly protein bound in maternal blood (greater than 98 %); thus, there are only low concentrations of diflunisal in rabbit embryos. Furthermore, rats, which are resistant to diflunisal-induced maternal anaemia, do not demonstrate embryo/fetal effects, despite reaching approximately equivalent doses of diflunisal in rat embryos. Furthermore, there is little accumulation of diflunisal in embryos, which is likely a reflection of its low placental transfer. This study provides strong evidence that the embryo/fetal effects (similar to those seen with EGBE) were secondary to maternal anaemia.

4.1.2.9.3 Summary of toxicity for reproduction

Unlike EGME and EGEE, EGBE seems to have no specific effects on fertility (no effects were seen in the continuous breeding study and neither macroscopic nor microscopic effects on reproductive organs in the repeated dose toxicity studies at doses which does not exhibit severe general toxicity.) A NOAEL of 720 mg/kg was derived from the continuous breeding study for fertility effects (it should be noted that effects seen at the higher dose tested are certainly due to general toxicity)

For developmental toxicity, studies performed on animals via various administration routes did not demonstrate any teratogenic potential, but foetotoxicity and embryotoxicity (lethality and resorptions) were often observed in relation with maternal toxicity (regenerative haemolytic anaemia). Other effects seen on foetuses were an increase in the incidence of skeletal variations which are generally described as ossification delays. *In vitro* studies showed some adverse effects on development with EGBE and its metabolite BAA, but only in conjuction with growth effects. Effects seen in foetuses are certainly related to maternal toxicity. Some studies have previously shown a relationship between maternal haemotoxicity and effets seen with EGBE (resorption, growth retardation and variations).

Haemotoxicity described in § 4.1.2.2.3 generally occured at low doses of EGBE whatever the route of administration used. In theses studies, data on haemolysis were often observed with acute dosing. Developmental toxicity studies would require daily dosing with test material, which may produce more marked effects on haematopoietic parameters. In addition, female mice and rats were more affected by EGBE haemolysis than males. Thus, these data demonstrate that the concentrations of EGBE used in these developmental toxicity studies were adequate to produce severe maternal anaemia of the magnitude sufficient to cause effects on embryo/fetal survival. These data give plausible support to the hypothesis that the effects seen in developmental toxicity studies with EGBE were due to haemolysis and subsequent maternal anaemia.

In human, all the epidemiological studies, except one, studying glycol ethers, showed an increased risk of malformation (cleft lip, neural tube defect). For EGBE, these studies did not allow to draw any conclusion about its potential effects on human because no studies are able to distinguish clearly an unique source of glycol ether, usually studies described co-exposure to various glycol ethers, including known developmental toxins such as EGME and other chemicals as well.

Overall, it is not possible to obtain a suitable NOAEL for developmental toxicity relevant for humans and based on animals studies. Regarding kinetic properties and SAR with other glycol ethers, it can be assumed that developmental toxicity due to EGBE in humans could not be expected without maternal toxicity. Consequently, there is no concern for this end-point and no need for risk characterisation.

4.1.3 Risk characterisation [5]

4.1.3.1 General aspects

The human population may be exposed to EGBE at the workplace, both from use of consumer products and indirectly via the environment (see 4.1.1.1, 4.1.1.2 and 4.1.1.3)

From the oral absorption studies it is concluded that oral absorption is complete. For risk characterisation 100 % oral absorption should be assumed.

From dermal absorption studies, a wide range of absorption values were observed depending on the species (rats having a greater dermal penetration than humans), the dilution of EGBE (40 % or 80 % water solutions of EGBE being absorbed at twice the rate compared to lower dilutions or undiluted EGBE), physical state of EGBE and occlusion status of administration. In general, dermal absorption of liquid EGBE varies between 20 to 30 % of applied dose in rats. Variations are seen in the reported values for individuals humans for the rate of absorption (ranging from 0.064 mg/cm^2/hr to 1.66 mg/cm^2/hr *in vitro* and from 0.826 to 11.3 µg/cm^2/hr *in vivo*). For dermal absorption of vapour EGBE, studies on volunteers have shown a percentage of internal dose due to dermal absorption of 11 to 39 % (depending on the conditions of exposure). According to the PbPk model, for a worst case exposure (100 % of the body exposure with no cloths), internal dose of EGBE due to the percutaneous uptake when a subject is exposed to EGBE as vapour would be 15 to 27 % (this range being not negligible compared to the dose due to inhalation). For risk characterisation, dermal absorption of liquid EGBE can be assumed to be 30 % of applied dose. For dermal absorption of vapour a value of 39 % of the internal dose due to dermal absorption can be taken into account but keeping in mind that this value has been demonstrated only during extreme exposure conditions (high temperature, high humidity and overalls clothing) which can be considered to be the worst case of exposure.

From human volunteers inhalation studies, an absorption of 55 % to 60 % has been measured. These values varies from the theoretical absorption value of 80 %, calculated from various studies, due to a wash in / wash out mechanism on the surface of the respiratory tract. For risk characterisation, 60 % inhalation exposure should be assumed (highest measured value).

Table 4.88: absorption coefficients taken into account for the calculations of internal doses

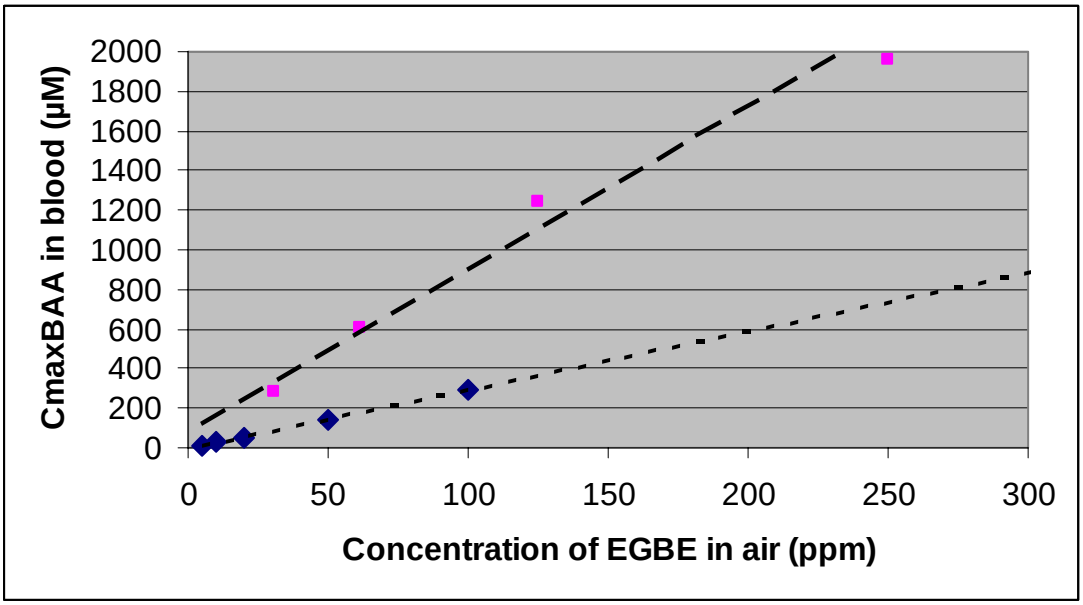
	Oral	Inhalation	Dermal route	
% of absorption	100 %	60 %	EGBE liquid	EGBE vapour
			30 % [1]	39 % of the internal dose [1]

1 Maximum worst case percentage, can be reassessed for each scenario in a case by case basis if needed .

[5] Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

For interspecies extrapolation, PBPK models exist for the rat, mouse and human. These enable the internal dose of EGBE to be estimated with some precision. Of the models available, that from Corley *et al*. 1994 and 1997 is considered the most complete and appropriate for potential use in the derivation of an interspecies extrapolation factor for oral exposure because it has been experimentally validated, covers relevant routes of exposure, and addresses both the distribution and excretion of the metabolite BAA which is invariably the key substance of interest. For the inhalation route, the model of Lee *et al*. (1998) was used to estimate BAA blood concentrations in female rats following inhalation exposure to EGBE because it is a recent extension of the Corley *et al*. (1994 and 1997) model for inhalation exposures and includes added parameters for female rats.

The PBPK model allows the concentration of the proximate toxicant (BAA) to be predicted following either inhalation or oral exposure to EGBE. The following four steps are carried out to obtain a human equivalent concentration (HEC), following a similar approach to that adopted by the US EPA (US EPA, 1999): (1) calculate the internal dose metric (C_{max} BAA in blood) corresponding to the female rat LOAEL, using the actual experimental exposure pattern (6hrs/day, 5 days/week) in the model simulation; (2) verify that steady state was achieved (e.g., no change in BAA C_{max} as a result of extending the exposure; (3) simulate the internal dose metric (C_{max} BAA in blood) for humans inhaling EGBE and 4) calculate the HEC (mg/m^3) that results in this internal dose C_{max} BAA. The relationship between EGBE exposure and resultant BAA for humans and female rats as used in step 3 is shown below:



Note: Data for rats is the upper line, data for humans the lower line

For acute toxicity, animal studies have given a LD50 of 1000 mg/kg by oral route in reliable mice and rats studies. By dermal route, depending on the application (occlusive or not) the LD50 was 500 mg/kg or > 2000 mg/kg respectively. By inhalation route, mortality was evidenced in reliable studies from about 500 ppm concentration (the LC50 being of about the same level). The effects seen in acute toxicity studies in animals were mainly acute haemolysis accompanied with non specific signs of toxicity (laboured breathing, lethargy, ataxia). Some hepatic and renal pathology was also seen in these studies.

In humans cases of massive ingestions the main toxic effect was a metabolic acidosis with sometimes haematotoxicity. For haematotoxicity, humans are much less sensitive than rodents and according to human studies, it appears that there is not a great intraspecies sensitivity (no influence of age or haematological status). Moreover, this end point was not the most sensitive end point for acute toxicity in human because in case of massive ingestions haemotoxicity was not observed in all cases. But metabolic acidosis was observed in all case. The lowest dose leading to metabolic acidosis in human was 400 mg/kg (LOAEL).

As human data is preferred if exists, this LOAEL will be taken into account for the risk characterisation. As no data exists in human by dermal or inhalation route, an extrapolation of this LOAEL will be done for risk characterisation for these routes using appropriate route to route extrapolation factors.

EGBE liquid is classified as irritating to the skin and to the eyes. For risk characterisation of EGBE vapour, a human NOEL of 50 ppm is used based on recent volunteer studies. No sensitisation properties liked directly to EGBE were seen from the available data on humans and animals.

For repeated dose toxicity, it has been shown that rodents are much more sensitive than human to the haemolytical effects of EGBE, therefore if a NOAEL based on these effects is used for risk characterisation, the MOS used should reflect the sensitivity of rodents. Kupffer cell pigmentation is secondary to the haemolytic effects. Effects on spleen (including spleen fibrosis) can also be related also to haemolysis. This effect usually occur following administration of substances which is able to cause iron accumulation in the splenic red pulp (Goodman *et al.*, 1984 ; Weinberger *et al.*, 1985). Effects on the forestomach of rodents do not appear to be relevant for humans. With regard to the increased incidence of hyaline degeneration of the olfactory epithelium observed in rodents, this appears to be an adaptive response, the severity of the lesion being unaffected by increasing exposure concentrations.

In other species than rodents, old studies are available. In dogs and monkeys, haemolysis was also described at relatively low doses. In guinea pigs, a species which is resistant to EGBE induced haemolysis, mortality was seen from 375 ppm and higher. Due to the limitations of these old studies these results cannot be taken into account for the risk assessment. The most reliable inhalation data is the LOAEL of 31 ppm derived from a a 6 month satellite group in a two-year study in rats. For oral route, a LOAEL of 69 mg/kg bw/d in male rats was derived from a 14 week oral study in rats. For dermal exposure, a NOAEL of 150mg/kg/day can be used.

Since all key effects are induced by haemolysis in rodents, a NOAEL based on haemotoxicity will be used in the risk characterisation. The selection of an appropriate interspecies chemical safety assessment factor (CSAF) must take into account the lower sensitivity of humans to BAA than rats (or mice). IPCS have proposed splitting the CSAF two components representing the toxokinetic and toxodynamic adjustment factors (AK_{AF} and AD_{AF} respectively). The toxicokinetic factor is taken account of by use of the PBPK model described above. The AD_{AF} factor needs to be set to an appropriate value to reflect the lower sensitivity of humans to haemolysis. The data available on the most sensitive measure (pre-hemolytic changes) suggests that a value of 0.01 would be realistic. However, a more cautious and conservative initial approach would be to propose a value of 0.1.

For mutagenicity, it has been demonstrated that EGBE is not a mutagenic substance *in vivo.*

For reproductive toxicity, no specific effects were seen for fertility. In the continuous breeding study a NOAEL of 720 mg/kg was set based on non specific effects observed at the higher doses tested. For developmental studies, embryonic and foetal effects seen in animals were related to maternal toxicity (haemolysis).

For carcinogenicity, some effects were seen in mice in the 2-year studies. As the mechanism of haemangiosarcomas in male mice is related to haemotoxicity, the risk characterisation made for repeated dose toxicity is considered sufficient to also assess for carcinogenicity. The other tumours (mouse forestomach) are considered not relevant to humans; no risk characterisation is needed for them.

4.1.3.2 Workers

Assuming that oral exposure is prevented by personal hygienic measures, the risk characterisation for workers is limited to the dermal and the inhalation routes of exposure.

Three sets of values have been utilised for dermal exposure: the EASE estimates, the data measured on DEGBE, and biomonitoring data from EGBE use in actual situations. As previously discussed in 4.1.1.1.2.2 and in 4.1.1.2.4, it is proposed to take the available RISKOFDERM data as a basis and to adapt them using EGBE's biomonitoring data when applicable. Summary of proposed reasonable worst case exposure is indicated below. MOS are calculated and presented in the relevant part of the risk characterisation.

Table 4.89: Summary of proposed reasonable worst case exposures (see also table 4.29 in & 4.1.1.2.4)

Scenario	Inhalation (mg/m^3)	External dermal exposure (mg/day)
1 - Manufacture	12	42
2 - Formulation	15.7	2,000
3 - Use of products		
3.1 Coating/Painting[a]		
- industrial		
- Spraying	58.1	2000
- Other works	30.4	430
- decorative	30.4	70
3.2 Printing		
- silk screening	20	23
- general printing	5	168
3.3 Cleaning		
- spraying	49	250
- wiping	49	1,040

4.1.3.2.1 Acute toxicity

According acute toxicity data in human (suicide attempts cases) a conservative LOAEL of 400 mg/kg by oral route can be taken into account. As limited data exist in humans by dermal or inhalation route an extrapolation route to route of this LOAEL is done. The MOSs obtained are compared with minimal MOS calculated as follows:

Table 4.90: Assessment factors applied for the calculation of minimal MOS for acute toxicity (for inhalation and dermal route)

Interspecies differences	1 (human data)
Intraspecies differences	3 (workers: homogen population)
Type of effect	1
Extrapolation LOAEL to NOAEL	5
Confidence of the database	1
Minimal MOS	15 for inhalation and dermal routes

Inhalation route:

Oral LOAEL of 400 mg/kg has to be extrapolate to a inhalation NOAEL to compare with inhalation exposure.

The dose of 400 mg/kg by oral route correspond to 400 mg/kg of internal dose (100 % absorption by oral route)

To obtain this internal dose by inhalation, it is necessary to add internal dose due to inhalation of vapours and internal dose due to percutaneous penetration of vapours. The ratio between these two doses has been calculated 61% / 39 % respectively. Leading to:

400 x 0.61 = 244 mg/kg of internal dose due only to inhalation and

400 x 0.39 = 156 mg/kg of internal dose due only to percutaneous absortion of vapours.

- To obtain 244 mg/kg of internal dose in one day, a worker of 70 kg with a respiratory volume of 10 m^3/worday, withan absorption factor of 60 % for inhalation uptake Should be exposed to

244 mg/kg * 70 kg / 10 m^3 / 0.60 = 2846 mg/m^3.

This value is the extrapolated LOAEC and should be compared with exposure levels.

The MOSs between the LOAEL and the inhalation exposure levels are mentioned in table 4.91. The MOSs are evaluated by comparison with the minimal MOS (table 4.90). The conclusions are given in the table 4.91. Based on the risk assessment for inhalation exposure, it is concluded that toxicity due to acute exposure are not expected.

Conclusion ii is reached for all occupational scenarios.

Table 4.91: Occupational risk assessment of EGBE for acute toxicity

Scenario		Risk assessment for inhalation exposure			Risk assessment for dermal exposure to liquid EGBE		
		8-hour TWA inhalation (mg/m^3)	MOS[1]	Conclusion	Estimated Skin exposure mg/day (mg/kg bw/d	MOS	Conclusion
1 - Manufacture		12	237	ii	42 (0.6)	2220	ii
2 - Formulation		15.7	181	ii	2,000 (28.57)	47	ii
3 - Use of end products	3.1 Coating/Painting						
	3.11 –Industrial						
	-spraying	58.1	49	ii	2000 (28.57)	47	ii
	-other works	30.4	94	ii	430 (6.14)	217	ii
	3.12 - decorative	30.4	94	ii	70 (1)	1333	ii
	3.2 Printing						
	3.21 Silk screening	20	142	ii	23 (0.33)	4039	ii
	3.22 General printing	5	569	ii	168 (2.4)	555	ii
	3.3 Cleaning						
	- spraying	49	58	ii	250 (3.57)	373	ii
	- wiping	49	58	ii	1040 (14.86)	90	ii

a: non dispersive use, b: wide dispersive use.

1- calculation based on a respiratory volume of 10 m3/workday, a worker bw of 70 kg, an absorption factor of 60 %, an internal dose due to inhalation uptake (61 %) and dermal uptake of vapour (39 %) and an oral LOAEL of 400 mg/kg bw.

2- calculation based on a worker bw of 70 kg, an absorption factor of 30 % and an oral LOAEL of 400 mg/kg bw. Worst case.

Dermal route:

Oral LOAEL of 400 mg/kg has to be extrapolate to a dermal LOAEL to compare with dermal exposure.

Oral dose of 400 mg/kg would give an internal dose of 400 mg/kg. To reach this dose with a dermal exposure to liquid EGBE, the external dose should be :

400 / 0.3 = 1333 mg/kg bw

because 30 is assumed to be the worst case percentage of absorption of liquid EGBE by dermal route.

This LOAEL of 1333 mg/kg bw should be compared with estimated skin exposures. The MOSs between the LOAEL and the dermal exposure levels are mentioned in table 4.91. The MOSs are evaluated by comparison with the minimal MOS (table 4.90). The conclusions are given in the table 4.91. Based on the risk assessment for dermal exposure, it is concluded that toxicity due to acute exposure are not expected.

Conclusion ii is reached for all occupational scenarios.

Combined exposure:

For the combined exposures the estimated internal doses are calculated from the biological exposure data. The oral LOAEL of 400 mg/mg would lead to an internal doses of 400 mg/kg because an absorption factor of 100 is taken into account for oral route. This LOAEL should be compared with internal doses calculated from exposures in each scenario (inhalation + dermal). The internal doses are calculated as follow :

Inhalation exposure will give internal dose of:

X (value of the 8-hour TWA inhalation (mg/m^3)) x 10 m^3 (inhaled air during a workday) x 0.6 (percentage of absorption by inhalation) / 70 (mean bw of a worker) = Y (inhalation internal dose).

This value does not take into account the possible dermal absorption of vapour during the 8hr TWA. It has been demonstrated that dermal absorption of vapour EGBE could count for 39 % of the internal dose of EGBE. To take into account this value (which is not negligible) the value of internal dose due to dermal exposure to vapours (Z) should be added to the former value (Y). Z represent 39 % of the total internal dose and can be calculated as follow :

Z = 0.39/0.61 x Y = 0.64 Y

The total internal dose due to inhalation exposure (inhalation output + dermal vapour penetration output) is Y + Z = 1.64 Y

For dermal exposure internal dose is calculated for a 70 kg bw worker with a percentage of absorption of 30 % (liquid EGBE, worst case).

Table 4.91 bis: Occupational combined risk assessment for acute toxicity

Scenario	Internal dose after exposure to 8-hour TWA (mg/kg bw) Y+Z	Internal dose after Dermal exposure to liquid EGBE (mg/kg bw) worst case (based on maximal dose)	Total internal dose (inhalation + dermal exposure)	MOS	Conclusion
1 - Manufacture	1.69	0.18	1.87	213	ii
2 - Formulation	2.2	8.58	10.78	37	ii
3 - Use of products 3.1 Coating/Painting - industrial					
- Spraying	8.17	8.58	16.75	24	ii
- Other works	4.28	1.84	6.12	65	ii
- decorative	4.28	0.30	4.58	87	ii
3.2 Printing					
- silk screening	2.81	0.10	2.91	137	ii
- general printing	0.70	0.72	1.42	281	ii
3.3 Cleaning					
- spraying	6.89	1.07	7.96	50	ii
- wiping	6.89	4.46	11.35	35	ii

The calculated MOS should be compared with minimal MOS. To be in the worst case, the highest minimal MOS should be chosen for the comparison: 15.

For acute toxicity a conclusion ii is reached for all scenarios for combined exposure.

4.1.3.2.2 Irritation and corrosivity

Skin

EGBE is classified as a skin irritant, and the use of appropriate PPE is recommended. This endpoint is of concern, but that this concern is managed by the information given by the classification and labelling of the substance so that no further concern remain. (**conclusion ii)**.

Eye

Eye irritation (liquid) :

The substance is classified as a eye irritant, and the use of appropriate PPE is recommended This endpoint is of concern, but that this concern is managed by the information given by the classification and labelling of the substance so that no further concern remain. (**conclusion ii**).

Eye and respiratory tract irritation (vapours):

Since the NOEL for respiratory tract irritation is derived from humans, the only assessment factor needed is that to allow for possible intraspecies variation. This is particularly true since the effects are not physiological but more discomfort in nature. A factor of 3, and therefore minimal MOS of 3 is considered sufficient for this end point. The MOSs between the NOAEL and the inhalation exposure levels and the conclusions of the risk assessment are mentioned in table 4.92.

Table 4.92: Occupational risk assessment of EGBE for eye and respiratory irritation by vapour exposure.

Scenario		Risk assessment for eye and respiratory irritation by vapour exposure		
		8-hour TWA inhalation (mg/m^3)	MOS[1]	Conclusion
1 - Manufacture		12	20	ii
2 - Formulation		15.7	16	ii
3 - Use of end products	3.1 Coating/Painting			
	3.11 –Industrial			
	-spraying	58.1	4.2	ii
	-other works	30.4	8	ii
	3.12 - decorative	30.4	8	ii
	3.2 Printing			
	3.21 Silk screening	20	12	ii
	3.22 General printing	5	50	ii
	3.3 Cleaning	49	5	ii

For eye and respiratory tract irritation due to the exposure of EGBE in vapour form, **conclusion ii** is reached for all occupational scenarios.

4.1.3.2.3 Sensitisation

Given the results from the dermal sensitisation studies it is concluded that EGBE is of no concern for workers with regard to skin sensitisation **(conclusion ii)**.

There are neither data from human experience nor other indications for respiratory sensitisation but this toxicological end-point is not expected for EGBE **(conclusion ii).**

4.1.3.2.4 Repeated dose toxicity

It is clear from the available data that haemolysis is the primary and critical response elicited in the main animal test models (rats and mice) following inhalation, oral or dermal exposure to EGBE. Blood from humans, pigs, dogs, cats, and guinea pigs is less sensitive to haemolysis by BAA. In sensitive species, EGBE produces a characteristic toxicity that is revealed clinically by the appearance of haemoglobinuria and pathologically by changes in a variety of blood parameters. A 100-fold greater concentration of BAA (10mM) is required for human erythrocytes to develop pre-hemolytic changes consistent with those seen in the rat (0.1mM). Such *in vivo* blood concentrations are unlikely to occur under normal conditions of human exposure to BE. Haemolysis did not occur with any blood from any individual, even sensitive sub-populations, when exposed *in vitro* to BAA(10 mM). Studies have also shown that potentially sensitive human sub-populations, including the children, the elderly and those with sickle cell anaemia are also equally resistant to the effect.

PBPK models exist for the rat, mouse and human.

These can be applied to derived the appropriate toxokinetic element of the interspecies assessment factor . The use of the model is described in the General Aspects section and is applied here as follows:

Step 1: Calculate C_{max} for BAA in blood corresponding to female rat LOAEL

Female rat (critical species and sex) LOAEL = 31 ppm.
Resultant C_{max} BAA from such an exposure= 285 µM.

Step 2: Verify steady state.

There were no changes in the C_{max} of BAA in blood during any 24-hour simulation period using a 6 hours/day, 5 days/week exposure regime at the female rat LOAEL, indicating that steady state was achieved.

Steps 3 and 4: Calculate the C_{max} for BAA in blood for humans continuously exposed to EGBE vapour

and calculate the $LOAEL_{HEC}$ for EGBE for human exposures producing the same C_{max} of BAA in blood as that that produces effects in rats.

For a BAA concentration of 285 µM, the HEC = 98 ppm (474mg/m^3).

Table 4.92a: Equivalent human doses for LOAEL(C) / NOAEL(C)

	Oral	Inhalation	Dermal
End point	LOAEL	LOAEC	NOAEL
Value	69mg/kg/day (male rat)	31ppm (152mg/m³)	150mg/kg/day
Equivalent BAA C_{max} concentration	129 µM	285µM	
Equivalent human dose	9.5 mg/kg/day	97ppm (474mg/m³)	

A Risk Characterisation not using PbPk model is also proposed in Appendix B.

The risk characterisation for repeat dose then becomes as follows:

4.92b: Assessment factors for repeated dose toxicity

Interspecies differences	0.1 (toxicodynamic factor) Inhalation: toxicocinetic element taken into account by PBPK model Dermal: Additional factor of 4 to account for allometric scaling of rats to humans
Intraspecies differences	5 (default for workers)
Duration of study	No factor required. The critical study is 6 months in duration and the effects for this end-point (haemolysis) would not be expected to be more severe compared to lifetime exposure. (haemolysis due to EGBE is considered to be an acute or sub-acute effect on rodents, moreover in some studies animals tended to "recover" (red blood cells being less sensitive) with long times of exposure.
Type of effect	1
Extrapolation LOAEL to NOAEL	3 for oral and inhalation. The effects were mild at this dose and there is evidence to show that the LOAEL is near the threshold level for effects of concern (eg NOAECs from other studies) Factor of 1 for dermal exposure
Confidence of the database	1
Minimal MOS	1.5 for oral and inhalation 2.0 for dermal exposure

Note that the critical LOAEC used for the inhalation risk characterisation is derived from a whole body inhalation exposure study (as are all similar studies). These animals will have been subjected to exposure by both the dermal and inhalation routes. The dermal absorption rates in rats and mice are also higher than in humans. Therefore it is not necessary to make a correction for additional uptake from dermal absorption of vapours.

Risk characterisation

Oral exposure

No oral exposure has been identified for workers, therefore there are no concerns for this route of exposure

Inhalation

Table 4.92c: Risk assessment for inhalation and dermal exposure for RDT

Scenario		Risk assessment for inhalation exposure			Risk assessment for dermal exposure to liquid EGBE		
		8-hour TWA inhalation (mg/m^3)	MOS[1]	Conclusion	Estimated Skin exposure mg/day (mg/kg bw/d	MOS[2]	Conclusion
1 - Manufacture		12	39	ii	42 (0.6)	250	ii
2 - Formulation		15.7	30	ii	2000 (28.57)	5	ii
3 - Use of end products	3.1 Coating/Painting						
	3.11 –Industrial						
	-spraying	58.1	8	ii	2000 (28.57)	5	ii
	-other works	30.4	16	ii	430 (6.14)	24	ii
	3.12 - decorative	30.4	16	ii	70 (1)	150	ii
	3.2 Printing						
	3.21 Silk screening	20	24	ii	23 (0.33)	454	ii

	3.22 General printing	5	95	ii	168 (2.4)	62	ii
	3.3 Cleaning						
	spraying	49	10	ii	250 (3.57)	42	
	wiping	49	10	ii	1040 14.86)	10	ii

For exposure via the inhalation route, conclusion (ii) is drawn for all scenarios.

Dermal exposure:

Exposure values are compared with the NOAEL of 150 mg/kg.

It is not possible to use the PBPK approach for the dermal assessment and a more conventional approach is therefore used.

See previous table and table showing derivation of MOSs

For exposure via the dermal route, conclusion (ii) is drawn for all scenarios.

Combined exposure:

For the combined exposures the estimated internal doses are calculated from the biological exposure data. The inhalation LOAEC is chosen for comparison as this is lower than the equivalent for the dermal route. The LOAEC of 474mg/m³ would lead to an internal doses of 474 mg/m^3 x 10m³/day x 0.6/70kg = 40.6 mg/kg/day. This internal dose should be compared with internal dose calculated. This LOAEC should be compared with internal doses calculated from exposures in each scenario (inhalation + dermal). The internal doses are calculated as follow:

Inhalation exposure will give internal dose of:

X (value of the 8-hour TWA inhalation (mg/m^3)) x 10 m^3 (inhaled air during a workday) x 0.6 (percentage of absorption by inhalation) / 70 (mean bw of a worker) = Y (inhalation internal dose).

This value does not take into account the possible dermal absorption of vapour during the 8hr TWA. It has been demonstrated that dermal absorption of vapour EGBE could count for 39 % of the internal dose of EGBE. To take into account this value (which is not negligible) the value of internal dose due to dermal exposure to vapours (Z) should by added to the former value (Y). Z represent 39 % of the total internal dose and can be calculated as follow:

Z = 0.39/0.61 x Y = 0.64 Y

The total internal dose due to inhalation exposure (inhalation output + dermal vapour penetration output) is Y + Z = 1.64 Y

For dermal exposure internal dose is calculated for a 70 kg bw worker with a percentage of absorption of 30 % (liquid EGBE, worst case)

The values are summarized in the following table:

Table 4.92d: Risk characterisation for combined exposure and for RDT

Scenario	Internal dose after exposure to 8-hour TWA (mg/kg bw) Y+Z	Internal dose after Dermal exposure to liquid EGBE (mg/kg bw) worst case (based on maximal dose)	Total internal dose (inhalation + dermal exposure)	MOS	Ccl
1 - Manufacture	1.69	0.18	1.87	22	ii
2 - Formulation	2.2	8.58	10.78	3.8	ii
3 - Use of products					
3.1 Coating/Painting					
- industrial					
- Spraying	8.17	8.58	16.75	2.4	ii
- Other works	4.28	1.84	6.12	6.6	ii
- decorative	4.28	0.30	4.58	8.8	ii
3.2 Printing					
- silk screening	2.81	0.10	2.91	14	ii
- general printing	0.70	0.72	1.42	29	ii
3.3 Cleaning					
- spraying	6.89	1.07	7.96	5.1	ii
- wiping	6.89	4.46	11.35	3.6	ii

1) internal dose for exposure to EGBE vapour is due to inhalation uptake (10 m^3 a workday, a worker of 70 kg bw and 60 % of absorption.

The calculated MOS should be compared with minimal MOS. To be in the worst case, the highest minimal MOS should be chosen for the comparison: 2.

Conclusion ii is reached for all scenario for combined exposure. There are no concerns for repeat dose exposure for all scenarios. **(conclusion ii**).

4.1.3.2.5 Mutagenicity

Given the results from the mutagenicity studies it is concluded that EGBE is of no concern for workers with regard to mutagenicity **(conclusion ii)**.

4.1.3.2.6 Carcinogenicity

Inhalation exposure:

As the mechanism of haemangiosarcomas in male mice is related to haemotoxicity, the risk characterisation made for repeated dose toxicity (RDT) is also relevant for carcinogenicity. The other tumours seen in the animal studies being not relevant to humans no risk characterisation is needed for them.

See RDT risk characterisation section

Results:

Exposure cannot be excluded for:

There are no concerns for carcinogenicity for all scenarios. **(conclusion ii)**.

4.1.3.2.7 Toxicity for reproduction

It is possible to derive a no effect level of 720mg/kg/day for fertility effects based on a continuous breeding study in mice. The MOSs obtained are compared with minimal MOS calculated as follows:

Table 4.93: Assessment factors applied for the calculation of minimal MOS fertility effects.

Interspecies differences	10
Intraspecies differences	5 (workers: homogen population)
Type of effect	1
Confidence of the database	1
Minimal MOS	50

Internal dose due to 720 mg/kg bw (NOAEL) would be 720 mg/kg bw (100 % oral absorption). This value should be compared with estimated internal doses due to exposure (see RDT section for calculation).

The following table summarise risk characterisation for inhalation exposure, dermal exposure and combined exposure.

It is necessary in this case to correct the inhalation value for the dermal absorption of vapour during the 8hr TWA as it is being compared to a NOAEL derived from an oral study. It has been demonstrated that dermal absorption of vapour EGBE could count for 39 % of the internal dose of EGBE. To take into account this value (which is not negligible) the value of

internal dose due to dermal exposure to vapours (Z) should by added to the former value (Y). Z represent 39 % of the total internal dose and can be calculated as follow :

Z = 0.39/0.61 x Y = 0.64 Y

The total internal dose due to inhalation exposure (inhalation output + dermal vapour penetration output) is Y + Z = 1.64 Y. The values for Y are shown in the table used for the repeat dose combined route assessment.

Table 4.93bis: Risk characterisation for reprotoxicity

Scenario	Internal dose after exposure to 8-hour TWA (mg/kg bw) Y+Z [1]	MOS Inhalation only	Internal dose after Dermal exposure to liquid EGBE (mg/kg bw) worst case (based on maximal dose)	MOS dermal only	Total internal dose (inhalation + dermal exposure)	MOS Combined	Ccl inhalation, dermal and combined
1 - Manufacture	1.69	426	0.18	4000	1.87	385	ii
2 - Formulation	2.2	327	8.58	84	10.78	67	ii
3 - Use of products 3.1 Coating/Painting - industrial							
- Spraying	8.17	88	8.58	84	16.75	43	ii
- Other works	4.28	168	1.84	381	6.12	118	ii
- decorative	4.28	168	0.30	2286	4.58	157	ii
3.2 Printing - silk screening	2.81	256	0.1	6957	2.91	247	ii
- general printing	0.70	1029	0.72	1000	1.42	507	ii
3.3 Cleaning - spraying	6.89	104	1.07	640	7.96	90	ii
- wiping	6.89	104	4.46	154	11.35	63	ii

1) internal dose for exposure to EGBE vapour is due to inhalation uptake (10 m3 a workday, a worker of 70 kg bw and 60 % of absorption) and to percutaneous absorption of vapour EGBE (39 % of the total internal dose).

The calculated MOS should be compared with minimal MOS. To be in the worst case, the highest minimal MOS should be chosen for the comparison: 50.

Conclusion ii for all scenarios 3.1 industrial spraying.

For scenario 3.1. The calculated MOS is closed to the minimal MOS. Given the uncertainties of the dermal exposure assessment and the non specificity of the effects observed in the continuous breeding study, it can be considered that there is no concern for this end-point.

Conclusion ii is reached for all scenarios.

The developmental toxicity studies for EGBE clearly indicate that the developmental effects observed are a consequence of and secondary to maternal toxicity. Any formal risk characterisation for humans using this data would not be meaningful. The available data does not suggest that EGBE shares the developmental toxicity properties of certain other glycol ethers. There is no other data which indicates potential developmental toxicity concerns. Therefore the conclusion is that there is no concern for this end point, conclusion (ii). **(conclusion ii)**.

4.1.3.2.8 Summary of risk characterisation for workers

Conclusion (ii) applies for all scenarios concerning each endpoint.

4.1.3.3 Consumers

Assuming that oral exposure could only be accidental by ingestion of a product, the risk characterisation for consumers is limited to the dermal and the inhalation routes of exposure.

For risk characterisation, a value of 30 % for dermal absorption and a value of 60 % for inhalation exposure can be taken into account.

Table 4.94: Internal dose exposure depending on scenarios

Scenario	Inhalation (mg/kg/d)	Skin (mg/kg/d)	Sum of exposures (mg/kg/d)
1 - Household surface cleaners	0.054	2.1	2.154
2 – Indoor air	0.06		0.06
3 – Painting	6	1.3	7.3
4 - Household surface cleaners + indoor air	0.114	2.1	2.214
5 - Painting + indoor air	6.06	1.3	7.4

4.1.3.3.1 Acute toxicity

According to acute toxicity data in human (suicide attempts cases), a LOAEL of 400 mg/kg can be taken into account. As limited data exist in humans by dermal or inhalation route an extrapolation route to route of this LOAEL is done. The MOSs obtained are compared with minimal MOS calculated as follows:

Table 4.95: Assessment factors applied for the calculation of minimal MOS for acute toxicity and for inhalation and dermal route.

Interspecies differences	1 (human data)
Intraspecies differences	10
Type of effect	1
Extrapolation LOAEL to NOAEL	5
Confidence of the database	1
Minimal MOS	50

The MOSs between the LOAEL and the inhalation and skin exposures and for the sum of exposures are mentioned in table 4.96:

Table 4.96: MOSs and conclusion for acute toxicity

Scenario	Inhalation		Dermal		Sum of exposures	
	MOS	Conclusion	MOS	Conclusion	MOS	Conclusion
1 – Household surface cleaners	7407	ii	190	ii	185	ii
2 – Indoor air	6667	ii			6667	ii
3 – Painting	67	ii	308	ii	55	ii
4 – Household surface cleaners + indoor air	3508	ii	190	ii	180	ii
5 – Painting + indoor air	66	ii	308	ii	54	ii

Based on the risk assessment for the various scenarios, it is concluded that the toxicity due to acute exposure is not expected. So **conclusion ii** is reached for all scenarios.

4.1.3.3.2 Irritation and corrosivity

Skin

EGBE is classified as a skin irritant, and the use of gloves is recommended. This concern is managed by the information given by the classification and labelling of the substance so that no further concern remains (**conclusion ii**).

Eye

Eye irritation (liquid):

The substance is classified as an eye irritant but in normal use, liquid should not be in contact with eyes. This concern is managed by the information given by the classification and labelling of the substance so that no further concern remains (**conclusion ii**).

Eye and respiratory tract irritation (vapours):

Scenarios of concern are those for which an inhalation exposure by vapours of EGBE has been evaluated.

The NOAEL is 50 ppm that can be converted in mg/m^3. So the NOAEL for this effect is :

$$50 \times 4.9 = 245 \text{ mg/m}^3.$$

Table 4.97: Consumer risk assessment of EGBE for eye and respiratory irritation by vapour exposure.

Scenario	Inhalation (mg/m3)	MOS	Conclusion
1 – Household surface cleaners	0.27	906	ii
2 – Indoor air	0.3	816	ii
3 – Painting	30	8	ii
4 – Household surface cleaners + indoor air	0.57	430	ii
5 - -Painting + indoor air	30.3	8	ii

For eye and respiratory tract irritation due to the exposure of EGBE in vapour form, **conclusion ii** is reached for all consumer scenarios.

4.1.3.3.3 Sensitisation

Skin

Given the results from the dermal sensitisation studies it is concluded that EGBE is of no concern for consumers with regard to skin sensitisation **(conclusion ii)**.

Respiratory tract

There are neither data from human experience nor other indications for respiratory sensitisation.

4.1.3.3.4 Repeated dose toxicity

It is clear from the available data that haemolysis is the primary and critical response elicited in the main animal test models (rats and mice) following inhalation, oral or dermal exposure

to EGBE. Blood from humans, pigs, dogs, cats, and guinea pigs is insensitive to haemolysis by BAA. In sensitive species, EGBE produces a characteristic toxicity that is revealed clinically by the appearance of haemoglobinuria and pathologically by changes in a variety of blood parameters. A 100-fold greater concentration of BAA (10mM) is required for human erythrocytes to develop pre-hemolytic changes consistent with those seen in the rat (0.1mM). Such *in vivo* blood concentrations are unlikely to occur under normal conditions of human exposure to BE. Haemolysis did not occur with any blood from any individual, even sensitive sub-populations, when exposed *in vitro* to BAA(10 mM). Studies have also shown that potentially sensitive human sub-populations, including the children, the elderly and those with sickle cell anaemia are also equally resistant to the effect.

The same models will be used for consumers than for workers to calculate the equivalent human EGBE concentration that would produce this dose, and finally extrapolate this on the assumption. The NOAEL(C)/LOAEL(C) were calculated using the PBPK model in humans.

Table 4.97 bis: Equivalent human doses for NOAEL(C) / LOAEL(C)

	Oral	Inhalation	Dermal
End point	LOAEL	LOAEC	NOAEL
Value	69 mg/kg/day (female rat)*	31 ppm (152mg/m³)	150mg/kg/day
Equivalent BAA C_{max} concentration	129 µM	285 µM	
Equivalent human dose	9.5 mg/kg/day**	97 ppm (474mg/m³)	

* as quoted by US EPA. **assuming 70kg person drinking 2 litres water/day

To select an appropriate interspecies chemical safety assessment factor (CSAF), we use the same method than in the workers' risk characterisation leading to an interspecies factor of 0.1 (see Table 4.97 ter).

The risk characterisation for repeat dose then becomes as follows:

Table 4.97 ter: Assessment factors for oral exposure

Interspecies differences	0.1 (toxicodynamic factor based on the difference in sensitivity between rodents and humans as described in the risk characterisation for workers part) Inhalation: toxicokinetic element taken into account by PBPK model Dermal: Additional factor of 4 to account for allometric scaling of rats to humans
Intraspecies differences	10 (default for consumers)

Duration of study	No factor required. The critical study is 6 months in duration and the effects for this end-point (haemolysis) would not be expected to be more severe compared to lifetime exposure. (haemolysis due to EGBE is considered to be an acute or sub-acute effect on rodents, moreover in some studies animals tended to "recover" (red blood cells being more resistant) with long times of
Type of effect	1
Extrapolation LOAEL to NOAEL	3 for oral and inhalation The effects were mild at this dose and there is evidence to show that the LOAEL is near the threshold level for effects of concern (eg NOAECs from other studies)
Confidence of the database	1
Minimal MOS	3 for oral and inhalation 4 for dermal exposure

Risk characterisation

For repeated dose toxicity, daily exposure level has to be averaged over a year. So the internal exposure dose used for risk characterisation will be :

$$\frac{\text{internal dose x number of events over a year}}{365}$$

Table 4.98: Internal dose exposure depending on scenarios average over a year

Scenario	Number of events	Inhalation (mg/kg/d)	Skin (mg/kg/d)	Sum of exposures (mg/kg/d)
1 – Household surface cleaners	1.8-4.8 events/week (1) = 94-250 events/year	0.036	1.44	1.476
2 – Indoor air	Each day	0.06		0.06
3 – Painting	10 events/year	0.16	0.036	0.196
4 – Household surface cleaners + indoor air	1.8-4.8 events/week (1) = 94-250 events/year	0.096	1.44	1.536
5 – Painting + indoor air	10 events/year	0.22	0.036	0.256

(1) Data from the Technical Guidance Document

The NOAEL is 474 mg/m^3 by inhalation and 150 mg/kg/day for dermal exposure. There is no oral exposure in normal use of products containing EGBE.

Internal NOAELs have to be calculated and to be compared with the internal daily exposures by skin and by inhalation and to the sum of internal exposures.

For combined exposure, the NOAEL concerning the dermal exposure will be chosen as it is more protective for consumers.

The respiratory volume for an adult is 20 m^3 and the mean bodyweight is 60 kg. The absorption of EGBE by inhalation is 60 %. So the internal NOAEL by inhalation will be :

$$\frac{474 \times 20 \times 0.6}{60} = 94.8 \text{ mg/kg/d}$$

The dermal absorption factor is 30%. So the internal NOAEL by dermal route will be :

$$150 \times 0.3 = 45 \text{ mg/kg/d}$$

Table 4.99: MOSs and conclusion for repeated dose toxicity

Scenario	Inhalation		Dermal		Sum of exposures	
	MOS	Conclusion	MOS	Conclusion	MOS	Conclusion
1 – Household surface cleaners	2633	ii	31	ii	30	ii
2 – Indoor air	1580	ii			750	ii
3 – Painting	592	ii	1250	ii	230	ii
4 – Household surface cleaners + indoor air	987	ii	31	ii	29	ii
5 – Painting + indoor air	431	ii	1250	ii	176	ii

Conclusion **ii** is reached for all scenarios**.**

4.1.3.3.5 Mutagenicity

Given the results from the mutagenicity studies it is concluded that EGBE is of no concern for consumers with regard to mutagenicity **(conclusion ii)**.

4.1.3.3.6 Carcinogenicity

As the mechanism of haemangiosarcomas in male mice is related to haemotoxicity, the risk characterisation made for repeated dose toxicity can also be used for carcinogenicity. The other tumours are not relevant to humans so no risk characterisation is needed for them.

For carcinogenicity, daily exposure level has to be averaged over a year as for repeated dose toxicity. So the same values of internal exposures will be compared to the NOAEL defined for carcinogenicity.

See RDT risk characterisation.

The values are summarised in the following table:

Table 4.99 bis: Risk characterisation for carcinogenicity

Scenario	Inhalation		Dermal		Sum of exposures	
	MOS	Conclusion	MOS	Conclusion	MOS	Conclusion
1 – Household surface cleaners	2633	ii	31	ii	30	ii
2 – Indoor air	1580	ii			750	ii
3 – Painting	592	ii	1250	ii	230	ii
4 – Household surface cleaners + indoor air	987	ii	31	ii	29	ii
5 – Painting + indoor air	431	ii	1250	ii	176	ii

Conclusion **ii** is reached for all scenarios.

4.1.3.3.7 Toxicity for reproduction

It is possible to derive a no effect level of 720 mg/kg/day for fertility effects based on a continuous breeding study in mice. Daily exposure level has been averaged over a year as reported in Table 4.98.

The MOSs obtained are compared with minimal MOS calculated as follows:

Table 4.100: Assessment factors applied for the calculation of minimal MOS fertility effects.

Interspecies differences	10
Intraspecies differences	10
Type of effect	1
Confidence of the database	1
Minimal MOS	100

Risk characterisation

Table 4.101: MOSs and conclusion for reproduction

Scenario	Inhalation		Dermal		Sum of exposures	
	MOS	Conclusion	MOS	Conclusion	MOS	Conclusion
1 – Household surface cleaners	20000	ii	500	ii	488	ii
2 – Indoor air	12000	ii			12000	ii

3 – Painting	4500	ii	20000	ii	3673	ii
4 – Household surface cleaners + indoor air	7500	ii	500	ii	469	ii
5 – Painting + indoor	3273	ii	20000	ii	2813	ii

Conclusion **ii** is reached for all scenarios:

The developmental toxicity studies for EGBE clearly indicate that the developmental effects observed are a consequence of and secondary to maternal toxicity. Any formal risk characterisation for humans using this data would not be meaningful. The available data does not suggest that EGBE shares the developmental toxicity properties of certain other glycol ethers. There is no other data which indicates potential developmental toxicity concerns. Therefore the conclusion is that there is no concern for this end point, conclusion (ii). **(conclusion ii)**.

4.1.3.4 Humans exposed via the environment

The key health effect is repeated dose toxicity. Irritation (via dermal or ocular routes)) is of low concern where exposure is dissipated throughout the environment. Comparison of the total internal dose of 94.8 $mg.kg^{-1}$ (corresponding to the LOAEL of 31 ppm for RDT via inhalation route corrected with PbPk modelling to obain human internal dose see also calculation of internal NOAEL by inhalation in the consumer part in chapter 4.1.3.3.4.) with the highest estimated exposure at regional ($3.22.10^{-4}$ $mg.kg^{-1}.day^{-1}$) and local ($3.73.10^{-2}$ $mg.kg^{-1}.day^{-1}$) levels leads to margins of safety of, respectively, $2.9.10^{5}$ and $2.5.10^{3}$ which do not lead to concern (compared to the minimal MOS of 3 calculated for consumers).

4.1.3.4.1 Summary of risk characterisation for exposure via the environment

(ii) There is at present no need for further information and/or testing and or risk reduction measures beyond those applied already.

This conclusion applies for all endpoints in relation to local and regional exposure.

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

EGBE has a low vapour pressure and is moderately flammable (flash point is 60°C). It has no explosive or oxidising properties. However, it is noted that oxidation by air may involve peroxidation of the substance, which may increase explosive properties. A general warning to this effect is recommended. Use of antioxidants reduces the potential to peroxidation.

It can be concluded that there is no concern for human health with regard physico-chemical properties (conclusion ii).

5 RESULTS [6]

5.1 INTRODUCTION

5.2 ENVIRONMENT

5.3 HUMAN HEALTH

5.3.1 Human health (toxicity)

5.3.1.1 Workers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.3.1.2 Consumers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to all end points and all scenarios:

5.3.1.3 Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

[6] Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

5.3.2 Human health (risks from physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

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ABBREVIATIONS

ADH	Alcohol Dehydrogenase
ADI	Acceptable Daily Intake
AF	Assessment Factor
ALP	Alcaline Phosphatase
ARDS	Adult Respiratory Distress Syndrome
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
ATSDR	Agency for toxic substances and disease registry (USA)
AUC	Area Under The Curve
B	Bioaccumulation
β2-m	β2-microglobulin
BAA	Butoxy Acetic Acid
BAL	Buthoxy aldehyde
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BEG	Glucuronide conjugate of EGBE
BEI	Biological Exposure Indice
BES	Sulfo conjugate of EGBE
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
BSA	Bovine Serum Albumin
bw	body weight / *Bw*, *b.w.*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CAT	Carnitine Acetyl Transferase
CBC	Complete Blood Count
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CEPE	European council of the paint, printing ink and artists' colours industry
CI	Clearance

CMNI	Maximal Concentration with No Irritation
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 50 percent dissipation / degradation
DTH	Delayed Time Hypersensibility
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC50	Effect Concentration measured as 50 % reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10 % effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EG	Ethylene Glycol
EGBE	2-Butoxyethanol
EGBEA	2-Butoxyethanol acetate
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50 % reduction in growth rate in algae tests
ESD	Emission Scenario Document
EtOH	Ethanol
EU	European Union

EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
G6P	Glucose 6 Phosphate
G6PD	Glucose 6 Phosphate Dehydrogenase
GE	Glycol Ether
GGT	Gamma Glutamyl Transpeptidase
GI	Gastro Intestinal
GLP	Good Laboratory Practice
GLU	Glucose
GP	Guinea Pig
Hb	Haemoglobin
HCT	Haematocrit
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HGB	Haemoglobin
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
HSE	Health and Safety Executive (UK)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
IL2	Inter Leukine 2
ILO	International Labour Organisation
INF	Interferon
INRS	Institut National de Recherche et de Sécurité
IP	Intra Peritoneal
IPA	Intra Peritoneal
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
IV	Intra Venous
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues

Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LAS	Alkyl Sulfonate
LC50	median Lethal Concentration
LD50	median Lethal Dose
LDH	Lactate DeHydrogenase
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LN	Lymph Node
LOAEC	Low Observable Adverse Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MDA	Malonyl DiAldehyde
MeOH	Methanol
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC
NAEL	No Adverse Effect Level
nBuOH	n-Butanol
NICNAS	National Industrial Chemicals Notification and Assessment Scheme (Australia)
NIOSH	National Institute for Occupational Safety and Health (USA)
NK	Natural Killer
NOAEC	No Observable Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level

NOEC	No Observed Effect Concentration
nPrOH	n-Propanol
NTP	National Toxicology Program (USA)
NZ	Natural Killer
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PCV	Packed cell volume
PEC	Predicted Environmental Concentration
PEG	PolyEthylene Glycol
PGME	Propylene Glycol Methyl Ether
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
PO	Per os
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RBC	Red Blood Cell
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex III of Directive 67/548/EEC
SAR	Structure-Activity Relationships

SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SEM	Scanning electron microscopy
SETAC	Society of Environmental Toxicology And Chemistry
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STEL	Short-Term Exposure Limit
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TLV	Threshold Limit Value
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
TWA	Time-Weighted Average
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
Vd	Volume of Distribution
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WBC	White Blood Cell
WEC	Whole Embryo Culture
WHO	World Health Organization
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations

	according to Annex III of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)

Appendix A

Methods of calculation of consumer exposures

Scenario 1 : Household surface cleaners

inhalation

$$E_{ei} = \frac{V_r \times C_{air} \times t_e}{W_b \times h_d}$$

E_{ei} = external exposure by inhalation

V_r = respiratory volume in one day

C_{air} = concentration in air

t_e = duration of exposure

W_b = mean bodyweight for a consumer (60 kg)

h_d = hours in one day

$$E_{ii} = E_{ei} \times abs_i$$

E_{ii} = internal exposure by inhalation

abs_i = percentage of absorption by inhalation

Dermal

$$E_{ed} = \frac{C_p \times Q_p \times Q_h}{W_b}$$

E_{ed} = external dermal exposure

C_p = concentration of EGBE in the cleaning product

Q_p = Quantity of product used

Q_h = Quantity of EGBE transferred to the hands

W_b = mean bodyweight for a consumer (60 kg)

$$E_{id} = E_{ei} \times abs_i$$

E_{id} = internal dermal exposure

abs_i = percentage of absorption by skin

Scenario 2 : indoor air

inhalation

$$E_{ei} = \frac{V_r \times C_{air} \times t_e}{W_b \times h_d}$$

E_{ei} = external exposure by inhalation

V_r = respiratory volume in one day

C_{air} = concentration in air

t_e = duration of exposure

W_b = mean bodyweight for a consumer (60 kg)

h_d = hours in one day

$$E_{ii} = E_{ei} \times abs_i$$

E_{ii} = internal exposure by inhalation

abs_i = percentage of absorption by inhalation

Scenario 3 : painting

Inhalation

$$E_{ei} = \frac{V_r \times C_{air} \times t_e}{W_b \times h_d}$$

E_{ei} = external exposure by inhalation

V_r = respiratory volume in one day

C_{air} = concentration in air

t_e = duration of exposure

W_b = mean bodyweight for a consumer (60 kg)

h_d = hours in one day

$$E_{ii} = E_{ei} \times abs_i$$

E_{ii} = internal exposure by inhalation

abs_i = percentage of absorption by inhalation

CONSEXPO model parameters:

Contact scenario: evaporation due to Painting

Duration of contact per event: 133 min

Frequency of contact: 10/year

Person uses product (room volume, ventilation and release area personal).

Personal volume=5.000000 m3.

Mean event concentration (average case): 3.772e+02 mg/m3

Year average (average case): 9.539e-01 mg/m3

Mean event concentration (cumulative worst case): 3.772e+02 mg/m3

Year average (cumulative worst case): 9.539e-01 mg/m3

Exposure estimates based on the following parameters:

Release area: 28.000 m2

Room volume: 25.000 m3

Room Ventilation rate: 12.5 m3/hr

Product amount: 5.000 kg

product density: 1.300 mg/cm3

Weight fraction: 3%

Fraction to upper layer: 1.000 fraction

Layer exchange rate: 1.000 1/min

Temperature: 293.000 Kelvin

Molecular weight matrix: 400.000 g/mol

Uptake

Model: fraction model

Cumulative worst case estimate: 7616 mg/year : 0.2979 mg/(kg.day)

Uptake estimates based on the following parameters:

Absorbed fraction: 60 %

Inhalation rate: 25300 cm3/min

Respirable fraction: 100%

WALLPAINT EXPOSURE MODEL (WPEM)

Resident DIY model

Room volume 25m^3

Painted area 28m^2

Air changes 0.5 per hour

Paint quantity:5kg, density 1.3, type: flat

Model type: empirical

Body mass: 60kg

Events per year: 10

Active/total lifetime: 40/70 years

No sinks

EGBE content: 3%

Painting time: 133 minutes

Dermal

$$E_{ed} = \frac{C_{prod} \times TH_{der} \times AREA_{der} \times n}{D \times BW}$$

where :

E_{ed} = external exposure

C_{prod} = concentration of EGBE in paint (kg/m^3)
TH_{der} = thickness of product layer on skin
$AREA_{der}$ = area of contact between paint and skin
n = number of events (n = 1)
D = dilution. There is no dilution so D = 1
BW = mean bodyweight for a consumer (60 kg)

$$E_{id} = E_{ei} \times abs_i$$

E_{id} = internal dermal exposure

abs_i = percentage of absorption by skin

Appendix B

Risk characterisation without using PbPk extrapolation

In the kinetic studies, if the pbpk is not taken into account, some data can be used to derive MOS for interspecies differences. It is clearly demonstrated that rodent blood cells are much more sensitive than human ones (at least 100 times), for this effect, a conservative factor of 0.1 will be used. For dermal penetration is was also demonstrated than rat skin was 2-3 more permeable than human one, for this end-point a correcting factor of 0.5 will be taken into account.

The risk characterisation for repeat dose then becomes as follows:

Table: Assessment factors applied for the calculation of minimal MOS for occupational exposure

Interspecies differences	0.1 for greater sensitivity of rodents blood cells 0.5 for dermal absorption Dermal: Additional factor of 4 to account for allometric scaling of rats to humans
Intraspecies differences	5 (default for workers)
Duration of study	No factor required
Uncertainties concerning dermal exposure	0.2 for dermal and for scenarios using data derived from measurements with a less volatile solvent.*
Type of effect	1
Extrapolation LOAEL to NOAEL	3 for oral and inhalation The effects were mild at this dose and there is evidence to show that the LOAEL is near the threshold level for effects of concern (eg NOAECs from other studies) Factor of 1 for dermal exposure
Confidence of the database	1
Minimal MOS	1.5 for oral and inhalation 1 for dermal exposure

Note that the critical LOAEC used for the inhalation risk characterisation is derived from a whole body inhalation exposure study (as are all similar studies). These animals will have been subjected to exposure by both the dermal and inhalation routes.

Exposure will be compared with the LOAEC of 31 ppm (152 mg/m^3) derived from a 14-week study in rats.

Risk characterisation

Oral exposure

No oral exposure has been identified for workers, therefore there are no concerns for this route of exposure.

Inhalation

Scenario		Risk assessment for inhalation exposure			Risk assessment for dermal exposure to liquid EGBE		
		8-hour TWA inhalation (mg/m^3)	MOS[1]	Conclusion	Estimated Skin exposure mg/day (mg/kg bw/d	MOS[2]	Conclusion
1 - Manufacture		12	12.7	ii	42 (0.6)	250	ii
2 - Formulation		15.7	9.7	ii	2000 (28.57)	5.3	ii
3 - Use of end products	3.1 Coating/Painting						
	3.11 –Industrial						
	-spraying	58.1	2.6	ii	2000 (28.57)	5.3	ii
	-other works	30.4	5	ii	430 (6.14)	24	ii
	3.12 - decorative	30.4	5	ii	70 (1)	150	ii
	3.2 Printing						
	3.21 Silk screening	20	7.6	ii	23 (0.33)	454	ii
	3.22 General printing	5	30.4	ii	168 (2.4)	63	ii

	3.3 Cleaning - spraying	49	3.1	ii	250 (3.57)	42	
	3.3 Cleaning - wiping	49	3.1	ii	1040 (14.86)	10	ii

For exposure via the inhalation route, conclusion (ii) is drawn for all scenarios.

Dermal exposure:

Exposure values are compared with the NOAEL of 150 mg/kg.

See previous table

For exposure via the dermal route, conclusion (ii) is drawn for all scenarios.

Combined exposure:

For the combined exposures the estimated internal doses are calculated from the biological exposure data. The inhalation LOAEC is chosen for comparison as this is lower than the equivalent for the dermal route. The LOAEC of 152 mg/m³ would lead to an internal doses of 152 mg/m^3*10m³/day*0.6/70kg = 13 mg/kg/day This internal dose should be compared with internal dose calculated . This LOAEC should be compared with internal doses calculated from exposures in each scenario (inhalation + dermal). The internal doses are calculated as follow :

Inhalation exposure will give internal dose of:

X (value of the 8-hour TWA inhalation (mg/m^3)) x 10 m^3 (inhaled air during a workday) x 0.6 (percentage of absorption by inhalation) / 70 (mean bw of a worker) = Y (inhalation internal dose).

This value does not take into account the possible dermal absorption of vapour during the 8hr TWA. It has been demonstrated that dermal absorption of vapour EGBE could count for 39 % of the internal dose of EGBE. To take into account this value (which is not negligible) the value of internal dose due to dermal exposure to vapours (Z) should by added to the former value (Y). Z represent 39 % of the total internal dose and can be calculated as follow :

Z = 0.39/0.61 x Y = 0.64 Y

The total internal dose due to inhalation exposure (inhalation output + dermal vapour penetration output) is Y + Z = 1.64 Y

For dermal exposure internal dose is calculated for a 70 kg bw worker with a percentage of absorption of 30 % (liquid EGBE, worst case)

The values are summarized in the following table:

Scenario	Internal dose after exposure to 8-hour TWA (mg/kg bw) Y+Z	Internal dose after Dermal exposure to liquid EGBE (mg/kg bw) worst case (based on maximal dose)	Total internal dose (inhalation + dermal exposure)	MOS	Ccl
1 - Manufacture	1.69	0.18	1.87	7.0	ii
2 - Formulation	2.2	8.58	10.78	1.2	iii
3 - Use of products					
3.1 Coating/Painting					
- industrial			16.75		
- Spraying	8.17	8.58	6.12	0.77	iii
- Other works	4.28	1.84	4.58	2.1	ii
- decorative	4.28	0.30		2.8	ii
3.2 Printing			2.91		
- silk screening	2.81	0.10	1.42	4.5	ii
- general printing	0.70	0.72	7.96	9.2	ii
3.3 Cleaning spraying	6.89	1.07	11.35	1.63	ii
3.3 Cleaning wiping	6.89	4.46		1.14	iii

1) internal dose for exposure to EGBE vapour is due to inhalation uptake (10 m^3 a workday, a worker of 70 kg bw and 60 % of absorption.

The minimal MOS required for the inhalation route is 1.5 whilst for the dermal route it is 1.

According to the results obtained a conclusion iii is reached for scenario 2, scenario 3.1: industrial spraying and scenario 3.3 cleaning wiping.

There are no concerns for repeat dose exposure for the other scenarios. **(conclusion ii)**.

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The report provides the comprehensive risk assessment of the substance 2-butoxyethanol. It has been prepared by France in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to man and the environment, laid down in Commission Regulation (EC) No. 1488/94.

This part of the evaluation considers the emissions and the resulting exposure to human populations in all life cycle steps. The scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The human health risk assessment concludes that there is no concern for workers, consumers, for humans exposed via the environment and for human health (physico-chemical properties).