The Toxicology of Glycol Ethers and its Relevance to Man (Fourth Edition) Volume II - Substance Profiles

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The Toxicology of Glycol Ethers and its Relevance to Man

CONTENTS - VOLUMES I AND II

EXECUTIVE SUMMARY

This report provides an update of an earlier ECETOC review [a] of a number of important ethylene and propylene glycol mono-ethers and di-ethers (glymes). It includes substantial new information concerning the human health consequences of exposure to this class of chemicals. The report presents toxicity data profiles for each individual compound.

Glycol mono-ethers are liquids that combine the solubility characteristics of ethers and alcohols since both functional groups are present. As a result, they are widely used in solvent applications, including formulations such as paints, inks and cleaning fluids. Non-solvent applications include uses as anti-icing agents in jet fuel, hydraulic system fluids and as chemical intermediates.

The hazard assessment of several glycol ethers can be based on short-term exposure studies because long-term exposure have not lead to more severe or different systemic effects. Glycol ethers have the potential to penetrate the skin (as a liquid or vapour) and this, therefore, represents a potentially significant route of exposure.

The majority of glycol ethers are of low acute toxicity; the main effect seen in laboratory animals at high doses is narcosis, typical of many solvents. Some glycol ethers are eye irritants. Overall, numerous studies with glycol ethers show that they do not exhibit genotoxic activity. The results of carcinogenicity studies with glycol ethers are consistent with this lack of genotoxic activity.

The systemic toxicity of the ethylene-based glycol ethers is mediated by their metabolism to the corresponding alkoxyacetic acids. Methyl- and ethyl-substituted ethylene glycol ethers can cause bone marrow depression, testicular atrophy, developmental toxicity, and immunotoxicity in animals. It should be noted that methyl- and ethyl-ethers of ethylene glycol are not used in consumer products in Europe. In contrast, the longer chain ethylene glycol ethers (ethylene glycol butyl ether, -propyl ether, -isopropyl ether and -phenyl ether) do not cause any of these effects. Toxicity commonly associated with the longer chain homologues involves red blood cell haemolysis (anaemia), to which humans are resistant. The alkoxyacetic acid metabolites of glycol ethers are responsible for the haemolysis.

None of the ethylene-bond effects have been observed for the propylene glycol ethers (α-isomers in commercial products); they are secondary alcohols and cannot be metabolised to their corresponding alkoxypropionic acids. Propylene glycol ethers are dealkylated to propylene glycol and then oxidised. The only change observed with propylene glycol ethers is an adaptive liver response and male rat kidney toxicity, which is not considered relevant to humans.

[a] ECETOC. 1995. The toxicology of glycol ethers and its relevance to man. Technical Report 64. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium [ISSN-0773-8072-64]

Reports of a number of effects in humans have been associated with glycol ether exposure, such as anaemia, granulocytopenia and leukopenia, increased risk of abortion or reduced sperm count in painters. Many such reports relate to methyl- and ethyl-substituted glycol ethers and are confounded by simultaneous exposures to other chemicals as well as limited information on exposure levels, which do not allow firm conclusions to be made concerning the contribution of glycol ethers to the observed effects. The toxicological findings reported to date indicate that, except for haemolytic anaemia and the liver and kidney effects in long-term studies, the effects seen in animals are also relevant to humans.

SUMMARY AND CONCLUSIONS

Glycol mono-ethers are liquids that combine the solubility characteristics of ethers and alcohols since both functional groups are present in the molecule. They are therefore widely used in solvent applications, including formulations such as paints, inks and cleaning fluids. Non-solvent applications include uses as anti-icing agents in jet fuel, hydraulic system fluids and as chemical intermediates.

The majority of glycol ethers are of low acute toxicity. Clinical signs of acute intoxication in animals are consistent with non-specific depression of the central nervous system, which is typical of many solvents. Lethargy and haemoglobinuria have been observed in glycol ethers that produced haemolysis in rodents. Although some glycol ethers are irritant to the eye, most are not, and none are appreciably irritant to the skin on acute exposure. As with other solvents, prolonged or repeated skin exposure may lead to a severe skin irritation. It is recognised that the glycol ether class lacks specific determinants for either genotoxicity or carcinogenicity. Negative results obtained in conventional genotoxicity assays, both *in vivo* and *in vitro*, confirm the lack of genotoxic activity for this class of solvents. Some glycol ethers have been tested in life-time studies in rats and mice, including ethylene glycol ethyl ether, ethylene glycol *n*-butyl ether, diethylene glycol ethyl ether, 2-propylene glycol 1-methyl ether and propylene glycol *tert*-butyl ether. However, the tumour responses seen in these cases were probably caused by mechanisms that are species-specific or reflect a mode of action to which humans are resistant. Overall, glycol ethers do not pose a significant genotoxic or carcinogenic risk to humans.

For the ethylene-based glycol ethers, the major route of metabolism is via alcohol and aldehyde dehydrogenases to the corresponding alkoxyacetic acids. A secondary route involves O-dealkylation to ethylene glycol and its oxidation metabolites. The metabolism of propylene-based glycol ethers varies with the isomer type. The α-isomers, which are used commercially, cannot be oxidised to acids, and O-dealkylation by microsomal cytochrome P450 (CYP) is the predominant route of metabolism. The minor impurity β-isomers are, like the ethylene glycol ethers, substrates for alcohol and aldehyde dehydrogenases, producing the corresponding propoxyacetic acids. They may also undergo O-dealkylation. This explains the main difference in the toxicities of the ethylene-based and propylene-based glycol ethers.

Within the ethylene-based series, the short chain ethers, including methyl- and ethyl-ethers of ethylene glycol and their acetates, show different toxicity effects from the higher propyl and butyl homologues. Methyl- and ethyl-substituted ethylene glycol ethers and derivatives have been shown to cause bone marrow depression, testicular atrophy, developmental toxicity, and immunotoxicity in animals. The toxicological effects observed are due to the alkoxyacetic acid metabolites, methoxyacetic acid and ethoxyacetic acid, which show relatively slow excretion rates especially in larger animals. In contrast, the longer chain ethylene glycol ethers (ethylene

glycol butyl ether, -propyl ether, -isopropyl ether and -phenyl ether) do not cause these effects because methoxyacetic and ethoxyacetic acid are not formed. Methyl and ethyl ethers of ethylene glycol are not used in consumer products in Europe.

The toxicity commonly associated with the longer chain ethylene-series homologues involves red blood cell haemolysis with secondary effects relating to this haemosiderin accumulation in the spleen, liver and kidney, and a compensatory haematopoiesis displayed in bone marrow. Ethylene glycol butyl ether, the most studied in this series, produces haemolytic anaemia in rats, rabbits and mice, showing greater sensitivity than other species, including guinea pigs. Those glycol ethers that cause haemolytic effects are more toxic than the other glycol ethers in respective susceptible species. Humans exhibit a resistance to glycol ether-induced haemolytic anaemia.

The toxicity of the propylene glycol ethers with the alkoxy group at the primary position (α-isomers, main isomers found in commercial products) is quite different from that of the ethylene glycol ethers. These ethers cannot be metabolised to their corresponding alkoxypropionic acids. None of the effects mentioned above have been reported and the only evidence of toxicity is towards liver and kidney. In the case of propylene glycol methyl ether, developmental effects have been reported when the primary position is occupied by a hydroxyl group (β-isomer). The β-isomer is not produced as a commercial product, and is found as a minor component (< 0.5%) of commercial propylene glycol methyl ether.

Target organ toxicity for the lower molecular weight ethylene-series glycol ethers in animals has been related to the extent of formation of methoxyacetic acid or ethoxyacetic acid, which may affect one or more of testes, bone marrow, thymus or developing offspring. For example, administration of ethylene glycol methyl ether in rats produces thymic and testicular atrophy, lymphocytopenia, and neutropenia with a near complete failure of blood cell precursor development in the bone marrow. Methoxypropionic acid has also been shown to produce developmental effects. With the exception of the developmental toxicity, these adverse effects are reversed upon removal of exposure. In sharp contrast, ethylene glycol butyl ether does not produce these effects, but produces haemolytic anaemia in rodents, accompanied by a compensatory bone marrow hyperplasia. Butoxyacetic acid has been shown to induce haemolysis in several animal species. An exception in the ethylene glycol ether series is ethylene glycol phenyl ether (phenoxyethanol), which is a more potent haemolytic agent (in the rabbit) than its metabolite, phenoxyacetic acid.

The liver has frequently shown an increased weight, in the absence of significant pathological change, following high doses of ethylene- and propylene-series glycol ethers. This has been interpreted as an adaptive change. Kidney weight changes and histopathological changes have been identified following dipropylene glycol ethyl ether and 2-propylene glycol methyl ether administration. These changes are associated with the accumulation of $\alpha_{2\mu}$-globulin in the case of

2-propylene glycol 1-methyl ether only in male rats. Based on information from several other hazard assessments of chemicals, they are considered not to be relevant for humans. This is also most likely the case for dipropylene glycol ethyl ether, but definitive analytical confirmation is not available.

The hazard assessment of several glycol ethers can be based on systemic changes found in short-term exposure studies, such as haematological effects and organ weight changes. These effects do not appear to increase in studies of long-term duration. This observation, together with the overall absence of genotoxic effects, indicates that long-term exposure is unlikely to lead to more severe or different effects. In the specific case of ethylene glycol butyl ether, hepatic oxidative stress due to haemolysis has led to tumours following lifetime exposure. Repeated oral dosing of ethylene glycol butyl ether in mice resulted in irritation of the forestomach. Irritation has also been observed in inhalation studies, probably due to oral ingestion from grooming and muco-ciliary transfer, which progressed to hyperplasia and forestomach tumours on prolonged exposure in a cancer bioassay.

Glycol ethers have the potential to penetrate the skin and this, therefore, represents a potentially significant route of exposure. In studies conducted in animals, dermal exposures result in toxicities similar to those following oral administration. Some comparative *in vitro* data show that the degree of penetration varies with chemical structure, with the rate decreasing with increasing molecular weight. Recent studies with ethylene glycol butyl ether indicate that dermal absorption from the vapour phase is a minor but not insignificant component of total systemic exposure.

Systemic health effects in humans have been reported to be associated with exposures to ethylene glycol methyl ether, ethyl ether and their acetates and also diethylene glycol dimethyl ether based on evaluation of worker populations and case reports. Ethylene glycol methyl and ethyl ethers exposure has been associated with anaemia, granulocytopenia and leukopenia. All such reports of human related effects are confounded by simultaneous exposures to other chemicals as well as limited information of exposure levels. The number of observations and the limited information on the level of exposure do not allow firm conclusions to be made concerning the contribution of glycol ethers to the observed effects. Although the available literature concerning human exposures to ethylene glycol methyl ether, -ethyl ether and the acetates do not provide conclusive evidence, the data reported to date indicate that, with the exception of haemolytic anaemia and the liver and kidney changes seen in some of the carcinogenicity bioassay studies, effects seen in animals are likely to be relevant to humans.

Several epidemiological studies have investigated the possible association between exposure to glycol ethers and aspects of the male and female reproductive system. Some of these studies have found increased risks in workers exposed to glycol ethers. However overall conclusions are difficult to draw because of the strong inter-correlation between exposure to other agents, the

possibility of recall bias and the variety of endpoints investigated. Further epidemiological studies are needed to confirm or refute these findings.

Overviews of the hazards and available data on glycol ethers are presented in Table 2 and 3. The toxicological information on individual glycol ethers is detailed in their substance profiles (Section 4.1 to 4.44). The following abbreviations are used for the names of glycol ether compounds (Table 1).

Table 1: List of glycol ethers and abbreviations

Abbreviation	Name
Ethylene-based	
DEGBE	Diethylene glycol butyl ether
DEGBEA	Diethylene glycol (mono) *n*-butyl ether acetate
DEGDEE	Diethylene glycol diethyl ether
DEGDME	Diethylene glycol dimethyl ether
DEGEE	Diethylene glycol (mono) ethyl ether
DEGEEA	Diethylene glycol ethyl ether acetate
DEGHE	Diethylene glycol (mono) hexyl ether
DEGME	Diethylene glycol (mono) methyl ether
EGBE	Ethylene glycol (mono) *n*-butyl ether
EGBEA	Ethylene glycol (mono) *n*-butyl ether acetate
EGDEE	Ethylene glycol diethyl ether
EGDME	Ethylene glycol dimethyl ether
EGEE	Ethylene glycol ethyl ether
EGEEA	Ethylene glycol (mono) ethyl ether acetate
EGHE	Ethylene glycol (mono) *n*-hexyl ether
EGiPE	Ethylene glycol (mono) isopropyl ether
EGiPEA	Ethylene glycol (mono) isopropyl ether acetate
EGME	Ethylene glycol (mono) methyl ether
EGMEA	Ethylene glycol (mono) methyl ether acetate
EGnPE	Ethylene glycol (mono) *n*-propyl ether
EGnPEA	Ethylene glycol (mono) *n*-propyl ether acetate
EGPhE	Ethylene glycol (mono) phenyl ether
MAA	Methoxyacetic acid [a]
TEGBE	Triethylene glycol (mono) *n*-butyl ether
TEGDME	Triethylene glycol dimethyl ether
TEGEE	Triethylene glycol (mono) ethyl ether
TEGME	Triethylene glycol (mono) methyl ether
Propylene-based	
1PG2ME	1-Propylene glycol 2-methyl ether
1PG2MEA	1-Propylene glycol 2-methyl ether acetate
2PG1BE	2-Propylene glycol 1-*n*-butyl ether
2PG1EE	2-Propylene glycol (mono) 1-ethyl ether
2PG1EEA	2-Propylene glycol 1-ethyl ether acetate
2PG1ME	2-Propylene glycol 1-methyl ether
2PG1MEA	2-Propylene glycol 1-methyl ether acetate
2PG1PhE	2-Propylene glycol 1-phenyl ether
DPGBE	Dipropylene glycol (mono) *n*-butyl ether
DPGEE	Dipropylene glycol (mono) ethyl ether
DPGME	Dipropylene glycol (mono) methyl ether
DPGPE	Dipropylene glycol (mono) propyl ether
DPGTBE	Dipropylene glycol *tert*-butyl ether
PGPE	Propylene glycol *n*-propyl ether
PGTBE	Propylene glycol *tert*-butyl ether
TPGBE	Tripropylene glycol (mono) *n*-butyl ether
TPGME	Tripropylene glycol (mono) methyl ether

[a] Not a glycol ether, but has similar toxicity

Table 2: Summary of hazards[a] posed by glycol ethers

Section	Compound	CAS[b] number	Haemolysis	Haematopoietic toxicity	Testicular toxicity	Reproductive toxicity	Developmental toxicity	Immuno-toxicity	Geno-toxicity	Carcinogenicity	Other effects
	Ethylene-series										
4.1	EGME	109-86-4	–ve	+ve	+ve	+ve	+ve	+	–ve	No data	CNS[c]/behavioural effects
4.2	EGMEA	110-49-6	–ve	+ve	+ve	+ve (limited data)	+ve	+	–ve	No data	
4.3	EGDME	110-71-4	No data	No data	+ve	+ve	+ve	No data	–ve	No data	CNS/behavioural effects
4.4	DEGME	111-77-3	–ve	–ve	+ve	No data	+ve (weak)	–ve	–ve	No data	
4.5	DEGDME	111-96-6	–ve	+ve	+ve	+ve	+ve	No data	–ve	No data	
4.6	TEGME	112-35-6	–ve	–ve	–ve	No data	–ve	No data	–ve	No data	
4.7	TEGDME	112-49-2	–ve	–ve	+ve	+ve	+ve	(+ve)	No data	No data	
4.8	MAA[a]	625-45-6	–ve	+ve	+ve	+ve	+ve	+	–ve	No data	
4.9	EGEE	110-80-5	–ve	+ve	+ve	No data	+ve	–ve	–ve	–ve (limited data)	
4.10	EGEEA	111-15-9	–ve	+ve (limited data)	+ve	+ve	+ve	–ve	–ve	No data	
4.11	EGDEE	629-14-1	–ve	No data	No data	No data	+ve	–ve	No data	No data	
4.12	DEGEE	111-90-0	–ve	–ve	+ve	–ve	–ve	–ve	–ve	No data	
4.13	DEGEEA	112-15-2	No data	No data	No data	No data	No data	No data	–ve	No data	
4.14	DEGDEE	112-36-7	No data	No data	No data	No data	–ve	No data	No data	No data	
4.15	TEGEE	112-50-5	–ve	–ve	–ve	No data	–ve	No data	No data	No data	
4.16	EGiPE	109-59-1	+ve	–ve	–ve	No data	–ve	No data	-ve	No data	
4.17	EGiPEA	91598-97-9	No data	No data	No data	No data	No data	No data	No data	No data	
4.18	EGnPE	2807-30-9	+ve	–ve	–ve	No data	–ve	No data	No data	No data	
4.19	EGnPEA	20706-25-6	+ve	–ve	–ve	No data	–ve	No data	No data	No data	
4.20	EGPhE	122-99-6	+ve (rabbits)	–ve	–ve	–ve	–ve	No data	–ve	No data	

Table 2: Summary of hazards[a] posed by glycol ethers (cont'd)

Section	Compound	CAS[b] number	Haemolysis	Haematopoietic toxicity	Testicular toxicity	Reproductive toxicity	Developmental toxicity	Immuno-toxicity	Geno-toxicity	Carcinogenicity	Other effects
	Ethylene-series (cont'd)										
4.21	EGBE	111-76-2	+ve	–ve	–ve	–ve	–ve	–ve	–ve	±	
4.22	EGBEA	112-07-2	+ve	–ve	–ve (limited data)	No data	No data	No data	No data	No data	
4.23	DEGBE	112-34-5	–ve	–ve	–ve	–ve	–ve	No data	–ve	No data	
4.24	DEGBEA	124-17-4	+ve	–ve	No data	No data	No data	No data	No data	No data	
4.25	TEGBE	143-22-6	–ve	–ve	–ve	No data	–ve	No data	No data	No data	
4.26	EGHE	112-25-4	-ve	–ve	–ve	No data	–ve	No data	–ve	No data	
4.27	DEGHE	112-59-4	–ve	–ve	No data	No data	No data	No data	–ve	No data	
	Propylene-series										
4.28	2PG1ME	107-98-2	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	
4.29	2PG1MEA	108-65-6	–ve	–ve	–ve	–ve	–ve	No data	–ve	No data	
4.30	1PG2ME	1589-47-5	–ve	–ve	–ve	No data	+ve	No data	–ve	No data	
4.31	1PG2MEA	70657-70-4	–ve	–ve	–ve	No data	+ve	No data	No data	No data	
4.32	DPGME	34590-94-8	–ve	–ve	–ve	No data	–ve	No data	–ve	No data	
4.33	TPGME	25498-49-1	–ve	–ve	–ve	No data	–ve	No data	–ve	No data	
4.34	2PG1EE	1569-02-4	–ve	–ve	–ve	No data	–ve	No data	–ve	No data	
4.35	2PG1EEA	54839-24-6	–ve	–ve	–ve	No data	No data	No data	–ve	No data	
4.36	DPGEE	30025-38-8	–ve	–ve	–ve	–ve	No data	No data	–ve	No data	
4.37	PGPE	1569-01-3	–ve	–ve	–ve	No data	–ve	No data	–ve	No data	
4.38	DPGPE	29911-27-1	–ve	–ve	–ve	No data	No data	No data	–ve	No data	
4.39	2PG1PhE	770-35-4	–ve	–ve	–ve	No data	No data	No data	–ve	No data	
4.40	2PG1BE	5131-66-8	–ve	–ve	–ve	No data	–ve	No data	–ve	No data	

[a] Not a glycol ether, but has similar toxicity

Table 2: Summary of hazards[a] posed by glycol ethers (cont'd)

Section	Compound	CAS[b] number	Haemolysis	Haemato-poietic toxicity	Testicular toxicity	Reproductive toxicity	Developmental toxicity	Immuno-toxicity	Geno-toxicity	Carcinogenicity	Other effects
	Propylene-series (cont'd)										
4.41	DPGBE	29911-28-2	–ve	–ve	–ve	No data	–ve	No data	–ve	No data	
4.42	TPGBE	55934-93-5	–ve	–ve	–ve	No data	No data	No data	–ve	No data	
4.43	PGTBE	57018-52-7	–ve	–ve	–ve	–ve	–ve	No data	–ve	+ve	
4.44	DPGTBE	132739-31-2	–ve	–ve	–ve	No data	No data	No data	–ve	No data	

[a] –ve, negative: no effects, +ve, positive: effects on organ or system; ±, equivocal; (–ve) or (+ve), insufficient data

[b] Chemicals Abstracts Service

[c] Central nervous system

Table 3: Summary of available [a] data on glycol ethers

Section	Compound	CAS number	Acute toxicity	Irritation	Sensitisation	Reproductive toxicity	Developmental toxicity	Repeated-dose toxicity	Genotoxicity	Carcinogenicity	Other [b]
	Ethylene-series										
4.1	EGME	109-86-4	+	+	-	+	+	+	+	-	K/M, N, Im, H
4.2	EGMEA	110-49-6	+	+	-	-	+ (limited)	+ (limited)	+	-	Im, H
4.3	EGDME	110-71-4	+ (oral)	-	-	-	+	+ (limited)	+ (limited)	-	K/M, N
4.4	DEGME	111-77-3	+	+	-	-	+	+	+	-	Im
4.5	DEGDME	111-96-6	+	+	+	+	+	+	+	-	K/M, H
4.6	TEGME	112-35-6	+	-	-	-	+	+	+	-	K/M, N
4.7	TEGDME	112-49-2	+ (oral)	-	-	+	+	+	-	-	
4.8	MAA [c]	625-45-6	+	+ (skin)	-	+	+	+	+	-	K/M, Im, H
4.9	EGEE	110-80-5	+	+	-	+	+	+	+	+ (limited)	K/M, N, Im, H
4.10	EGEEA	111-15-9	+	+	+	+	+	+	+	-	K/M, Im
4.11	EGDEE	629-14-1	+	+	-	-	+	+ (limited)	-	-	K/M
4.12	DEGEE	111-90-0	+	+	-	+	+	+	+	+ (limited)	K/M, H
4.13	DEGEEA	112-15-2	+	+	+	-	-	-	+	-	
4.14	DEGDEE	112-36-7	+ (oral)	+ (eye)	-	-	+	+ (limited)	-	-	
4.15	TEGEE	112-50-5	+	+	-	-	+	+	-	-	K/M
4.16	EGiPE	109-59-1	+	+	+	-	+	+	+	-	K/M
4.17	EGiPEA	91598-97-9	-	-	-	-	-	-	-	-	
4.18	EGnPE	2807-30-9	+	+	+	-	+	+	-	-	K/M, N
4.19	EGnPEA	20706-25-6	+	+	+	-	+	+	-	-	K/M
4.20	EGPhE	122-99-6	+	+	+	+	+	+	+	-	K/M
4.21	EGBE	111-76-2	+	+	+	+	+	+	+	+	K/M, N, Im, H
4.22	EGBEA	112-07-2	+	+	-	-	-	+ (limited)	-	-	H

Table 3: Summary of available[a] data on glycol ethers (cont'd)

Section	Compound	CAS number	Acute toxicity	Irritation	Sensitisation	Reproductive toxicity	Developmental toxicity	Repeated-dose toxicity	Genotoxicity	Carcinogenicity	Other[b]
4.23	DEGBE	112-34-5	+	+	+	+	+	+	+	-	K/M, N, H
4.24	DEGBEA	124-17-4	+	+	-	-	-	+ (limited)	-	-	K/M
4.25	TEGBE	143-22-6	+	+	-	-	+	+ (limited)	-	-	K/M
4.26	EGHE	112-25-4	+	+	-	-	+	+	+	-	
4.27	DEGHE	112-59-4	+	+	-	-	-	+ (limited)	+	-	
	Propylene-series										
4.28	2PG1ME	107-98-2	+	+	+	+	+	+	+	+	K/M, N, H
4.29	2PG1MEA	108-65-6	+	+	+	-	+	+ (limited)	+	-	K/M
4.30	1PG2ME	1589-47-5	+	+	-	-	+	+	+	-	K/M
4.31	1PG2MEA	70657-70-4	+	+	-	-	+	+	-	-	
4.32	DPGME	34590-94-8	+	+	+	-	+	+	+	-	K/M
4.33	TPGME	25498-49-1	+	+	-	-	+	+	+	-	
4.34	2PG1EE	1569-02-4	+	+	-	-	+	+	+	-	
4.35	2PG1EEA	54839-24-6	+	+	+	-	-	+	+	-	
4.36	DPGEE	30025-38-8	+	+	+	+	-	+	+	-	K/M, N
4.37	PGPE	1569-01-3	+	+	-	-	+	+	+	-	
4.38	DPGPE	29911-27-1	+	+	-	-	-	+	+	-	
4.39	2PG1PhE	770-35-4	+	+	-	-	-	+	+	-	K/M
4.40	2PG1BE	5131-66-8	+	+	+	-	+	+	+	-	K/M
4.41	DPGBE	29911-28-2	+	+	+	-	+	+	+	-	K/M
4.42	TPGBE	55934-93-5	+	+	+	-	-	+	+	-	
4.43	PGTBE	57018-52-7	+	+	+	+	+	+	+	+	K/M
4.44	DPGTBE	132739-31-2	+	+	+	-	-	+	+	-	K/M, N

[a] +, data are available; -, no data are available
[b] Abbreviations: K/M, kinetics; N, neurotoxicity; Im, immunotoxicity; H, human data
[c] Not a glycol ether, but has similar toxicity

Recommendations for further work

Several glycol ethers are part of the International Council of Chemical Associations (ICCA) programme on High Production Volume chemicals, which requires a base set of data to be available on all chemical substances registered on the ICCA tracking system. Glycol ethers covered in several submissions that have been developed for the ICCA programme are: ethylene glycol phenyl ether, ethylene glycol propyl ether, ethylene glycol *n*-hexyl ether, diethylene glycol ethyl ether, diethylene glycol hexyl ether, ethylene glycol butyl ether acetate, diethylene glycol butyl ether acetate, triethylene glycol butyl ether, propylene glycol butyl ether and propylene glycol phenyl ether. The reports have been submitted to the US-EPA and OECD for review.

The overall evaluation of these compounds indicates certain knowledge gaps and the need to:

- Develop biological action levels for ethylene glycol methyl ether and/or methoxyacetic acid that are based on biomonitoring data and which will help control dermal exposure situations. This would be based on the no-observed adverse effect level for methoxyacetic acid, which may require more data to derive a definitive value.
- Determine the role of haemolytic anaemia in inducing oxidative stress that could lead to toxicological effects, especially in the liver.
- Further validate biological monitoring methods, focusing on the relationship of biological effects to airborne exposure values.
- Obtain exposure and/or use data from downstream users and consumer groups covering both qualitative (for example frequency, duration and control measures used) and quantitative determinants that address personal air measurements and biological monitoring.

4. SUBSTANCE PROFILES

4.1 Substance profile: EGME

4.1.1 Identity

Name:	Ethylene glycol (mono) methyl ether
IUPAC name:	2-Methoxyethanol
CAS registry No.:	109-86-4
Molecular formula:	$C_3H_8O_2$
Structural formula:	$CH_3–O–CH_2–CH_2–OH$
Molecular weight:	76.1
Other components:	Water (≤ 0.1%), methanol (≤ 0.1%), methyl diglycol (≤ 0.1%), ethylene glycol (≤ 0.02%)

4.1.2 Physico-chemical properties

Melting point:	–85.1°C
Boiling point:	124°C
Vapour pressure:	6.2 hPa
Solubility in water:	Completely soluble
Relative density:	$D_4^{20} = 0.965$

4.1.3 Conversion factors

1 ppm = 3.164 mg/m^3
1 mg/m^3 = 0.316 ppm

4.1.4 Toxicological data

4.1.4.1 Acute toxicity

Oral

Rat:	LD_{50} 2,460 mg/kgbw (males); kidney damage was noted (Smyth *et al*, 1941). LD_{50} 3,250 mg/kgbw (males). LD_{50} 3,400 mg/kgbw (females). In either sex, narcosis, lung and kidney damage were seen (Carpenter *et al*, 1956).

Guinea pig: LD_{50} 950 mg/kgbw (Smyth *et al*, 1941; Carpenter *et al*, 1956).
Rabbit: LD_{50} 890 mg/kgbw (Carpenter *et al*, 1956).

Dermal

Rabbit: LD_{50} 1,300 mg/kgbw (Carpenter *et al*, 1956).

Inhalation

Rat: Survived exposure to 2,000 ppm (6,300 mg/m^3) for 7 hours. Signs of toxicity included an increase in osmotic fragility of erythrocytes (Carpenter *et al*, 1956). No mortality following 4-hour inhalation of up to 5,000 ppm (15,800 mg/m^3); signs of toxicity are discussed in Section 4.1.4.3 (Samuels *et al*, 1984). Over 60 % mortality after a single 4-hour inhalation of 5,656 ppm (17,900 mg/m^3) (Goldberg *et al*, 1962).

Intraperitoneal

Rat: LD_{50} about 2.5 ml/kgbw (2.4 mg/kgbw) (Goldberg *et al*, 1962).

Mouse: LD_{50} about 28.25 mmol/kgbw (2,150 mg/kgbw) (Karel *et al*, 1947).

EGME may cause testicular toxicity, even after a single administration (Foster *et al*, 1983). The effect, which is mediated by the oxidative product MAA, can also be demonstrated in testicular cell cultures if there is metabolic capability. Pachytene spermatocytes are most sensitive and predominantly affected.

Pachytene spermatocytes are the most sensitive and predominantly affected (Creasy and Foster, 1984; Creasy *et al*, 1985). Ultra-structural studies in male SD rats administered single oral doses of 250 or 500 mg EGME/kgbw revealed no effects at 6 hours after treatment but necrosis of spermatocytes and vacuolisation of Sertoli cells starting at 12 hours after treatment, suggesting a causal association of both effects (Creasy *et al*, 1986).

Single oral administration of 50 mg EGME/kgbw to male Wistar rats resulted in decreased capability for *in vitro* fertilisation for 5 weeks following administration. This effect was

reversible by week 8 after administration. At 100 mg/kgbw, reduced fertility was observed from week 4.5 to 6.5 after administration, and at 200 mg/kgbw from week 2 to 7 (Holloway *et al*, 1990). This corresponds to a dose-dependent impact on different stages of the spermatogenic cycle. The lag time gap of 5 weeks corresponds to pachytene cell injury. Higher dose levels (100 and 200 mg EGME/kgbw) also affect earlier and later stages of spermatogenesis such as leptotene and preleptotene spermatocytes and elongated spermatids, resulting in reduced fertility also after 2 and 3 and up to 10 weeks (Foster *et al*, 1984; Lee and Kinney, 1989; Holloway *et al*, 1990).

In CD rats 6 weeks after single oral doses of 750 to 1,500 mg/kgbw there was complete sterility; CD-1 mice under the same treatment regimen showed less effect on reproductive capacity, though decreased testes weights were observed after 2 to 5 weeks. A dose of 500 mg/kgbw decreased the total sperm count to about 50%, increased abnormal sperm from 2.84 to 27.29%, and separated sperm cell heads from 3.74 to 67.28%. Nevertheless, the fertility parameters were not markedly affected at that dose level (Anderson *et al*, 1987).

The calcium channel blockers verapamil and diltiazem, parenterally administered after single gavage administration of 200 mg EGME/kgbw to male F344 rats, protected against pachytene spermatocyte cell death in stage XIV seminiferous tubules. At 300 mg EGME/kgbw, verapamil was less effective (Ghanayem and Chapin, 1990).

Matsui and Takahashi (1999), using SD rats (7 or 9 weeks old) dosed once with 250 mg EGME/kgbw, showed in morphometric investigations that induced death of spermatogonia, pachytenic spermatocytes or round spermatids was exclusively apoptotic cell death.

The time-course development of spermatocyte toxicity and apoptosis was investigated in F344 rats and Hartley guinea pigs dosed once with 200 or 300 mg EGME/kgbw (or repeatedly with 3 daily doses of 200 mg/kgbw). Onset and severity of the effects and their morphological characteristics were different in both species: in rats, degenerating spermatocytes appeared necrotic after 24 hours following a single dose; in guinea pigs, they appeared apoptotic 96 hours after the start of 3 daily doses. Testes from rats given a single oral dose of 200 mg/kgbw showed extensive pachytenic spermatocyte degeneration 24 hours after dosing. The nuclear chromatin degradation was typical for necrosis, whereas the same dose in guinea pigs showed a nuclear chromatin condensation typical for apoptosis (Ku *et al*, 1994,1995).

The normal distribution of a protein-tyrosine kinase subtype (Src) in testicular tissue and its modification by EGME-induced apoptosis was investigated in SD rats. The normal immuno-reactivity of Scr was mostly detected in the cytoplasm of Sertoli cells with the maximum level around the lumen at spermiation. A single dose of 200 mg/kgbw EGME to rats administered via

the drinking water induced an increase of Src also in epithelium and interstitium of the testes. At 8 hours after treatment, the cytoplasm of dying spermatocytes showed intensive immuno-staining of Src (Wang *et al*, 2000).

Spermatocyte apoptosis was associated with cyclophylin A in germ cells of F344 rats treated with 200 mg EGME/kgbw. Nuclease activity was demonstrated in an extract from treated animals, but not in controls. The nuclease activity could be localised to an 18 kDa band; it was dependent on calcium and inhibited by zinc and taurine tricarboxylic acid. Amino acid sequence analysis showed that this protein was identical to cyclophylin A, which is specific for pachytenic spermatocytes, spermatids, interstitial cells and Sertoli cell nuclei. Cyclophylin A staining was present in control and in EGME-treated rats in a stage-dependent manner with a typical appearance in pachytenic spermatocytes (Wine *et al*, 1997).

Apoptosis of pachytene spermatocytes was also demonstrated *in vitro*, in testis cultures obtained from CD-1 mice killed 8 hours after gavage of 500 mg EGME/kgbw in distilled water. *In situ* hybridisation with antisense RNA probes revealed certain expression changes in clones and localised changes in multiple germ cell stages and other cell types. Thus, a change in gene expression level appeared to be an early sign of the subsequent testicular toxicity (Wang and Chapin, 2000).

4.1.4.2 Irritation and sensitisation

Skin irritation

Undiluted EGME (0.5 ml) was not irritant when applied to the intact dorsal skin of the rabbit for 4 hours (Jacobs *et al*, 1987).

Eye irritation

Undiluted EGME (0.1 ml) was not irritant to the rabbit eye, when applied into the conjunctival sac (Jacobs, 1992).

Sensitisation

No data are available.

4.1.4.3 Repeated-dose toxicity (Table 4.1.1)

Proliferating and/or differentiating tissues (with exception of the intestinal epithelium which is unaffected) are the targets of EGME related toxicity in all species investigated; the tissues are:

- Germinal epithelium of testes, where pachytenic spermatocytes show the greatest sensitivity (this section);
- bone marrow and thymus, as judged by depletion of RBC and WBC and alterations in immune competence, immunotoxic and immuno-modulating effects) (Section 4.1.4.9);
- embryonic tissues (Section 4.1.4.6).

In addition, detrimental effects on the central and peripheral nervous systems were frequently reported in humans (Section 4.1.5.2). These effects are also observed in animals: Savolainen (1980) reported glial cell toxicity in male Wistar rats following inhalation of 50, 100 or 400 ppm EGME for 2 weeks, while Goldberg *et al* (1962, 1969) demonstrated a specific loss of active avoidance-escape response in conditioned Wistar and Carworth Farms-Elias (CFE) rats exposed by inhalation to fairly low concentrations of 125, 250 or 500 ppm over up to 14 days in the absence of signs of motor imbalance. Thus, the effect was distinct from unspecific CNS depression commonly seen with many solvents at high dose levels. The percentage of animals showing inhibition of conditioned response increased with concentration and exposure duration.

The specific toxicity of EGME to certain cell stages and tissues has resulted in its use as a positive control and reference material for mechanistic research and methodology development. Consequently, several oral, inhalation and dermal studies have been conducted. Typical results are described as follows.

Subacute toxicity

Oral

EGME is a well-established testicular toxicant following repeated oral administration. Several studies demonstrated that EGME affects the germ cell population with a specific vulnerability of certain stages of the cell cycle, i.e. pachytenic spermatocytes, particularly at stages VII and VIII. Necrotic and apoptotic effects were both observed, depending on study design, species and dose.

Groups of Wistar rats receiving 300 mg EGME/kgbw/d for 1, 2, 5 or 20 days showed moderate decreases of liver, spleen and testes weights and a massive decrease of thymus weight after 2 days. After 5 days, thymus depletion was nearly complete and after 20 days, testes weights

were significantly reduced as were testes and thymus weight in a further group treated with 100 mg/kgbw for 20 days (Kawamoto *et al*, 1990a).

Oral administration of 0, 50, 100 or 200 mg EGME/kgbw/d to F344 rats for 5 days with post observation periods of up to 7 weeks showed no immediate effects at 50 mg/kgbw. After 3 weeks there was aggregation of detached spermatids with a maximum after 4 to 5 weeks. No effects were seen after 7 weeks. At 100 and 200 mg/kgbw, dead spermatocytes of different stages occurred in 90% of the tubules, but after 7 weeks 50% of the tubules were repopulated, while in the remaining tubules only spermatogonia and Sertoli cells were present; a third group of tubules was in gradual restoration. Prostate and seminal vesicle weights were unaffected (no indication of testosterone shortage) (Chapin *et al*, 1985a).

Adult Indian wild house rats treated with EGME (500 mg/kgbw/d) for 1, 6 or 11 days, followed by 4 and 8 weeks of recovery, developed testicular lesions in the seminiferous tubules, yet without histopathological changes of the Leydig and Sertoli cells (as judged by light microscopy) and without alterations in testicular steroidogenesis. The authors assumed that the depletion of germ cell populations was due to a maturation arrest at the zytogen stage. Testicular androgen level was equivalent to normal copulation behaviour. Recovery of spermatogenesis occurred after 8 weeks and the percentage of frequency of stage VII to VIII cells seemed to maintain or re-achieve its control level (Aich and Manna, 1996).

Testicular atrophy and effects on bone marrow and thymus were evidenced in earlier 2 to 5-week studies with EGME in ICL-ICR and $B6C3F_1$ mice (Nagano *et al*, 1979; House *et al*, 1985), SD rats (Foster *et al*, 1983), hamsters and guinea pigs (Nagano *et al*, 1984). Delayed dose-dependent decreases of cellularity and granulopoietic stem cells were seen in the bone marrow of $B6C3F_1$ mice following exposure to EGME for 4 days; the effects reversed by week 16 (Hong *et al*, 1988a).

Dermal

Treatment (5 d/wk) of male Porton-Wistar rats at 0, 100 or 1,000 mg/kgbw for 4 weeks had little effects under non-occlusive conditions, whereas testicular and bone marrow damage, as well as haematological effects were seen at 1,000 mg/kgbw under occlusive patch (Fairhurst *et al*, 1989).

Male SD rats received dermally applied doses of 0, 625, 1,250 or 2,500 EGME/kgbw (on occluded sites), or 0, 1,250, 2,500 or 5,000 mg/kgbw (on non-occluded sites) for 7 consecutive days. Investigations on sperms and fertility were performed up to week 14 after treatment. Deaths occurred at 2,500 mg/kgbw (occluded). There were dose-related decreases in sperm and spermatid counts. In addition, abnormal sperm morphology was increased and fertility reduced.

These effects were seen with or without occlusion, but were more severe and recovery proceeded at a slower rate under occlusion (Feuston *et al*, 1989).

Parenteral routes

Newborn B6C3F_1 mice receiving 100, 200 or 400 mg EGME/kgbw/d by s.c. injection from day 1 to 5 post partum showed, at both higher dose groups, a dose-dependent decrease of cellularity and granulopoietic stem cells in bone marrow after 8 weeks; this was reversible by week 16 (Hong *et al*, 1988b).

In female SD rats, administered EGME at 400 mg/kgbw, 5d/w over 2 weeks by i.p. injection, no indication of nephrotoxicity was determined by the measurement of urinary NAG activity, as well as β_2-microglobulin and albumin concentration (Bernard *et al*, 1989).

Inhalation

Inhalation exposure of male Crl:CD BR rats to 300 ppm EGME for 3 days produced injury initially limited to pachytene and stage XIV spermatocytes; at 1 to 2 days post exposure, other stages became affected. This time pattern did not become apparent after a 2-week exposure regimen, when 20 to 80% of the tubules showed atrophic germinal epithelium; some tubules only showed single layers of stem cells. Leydig cells were not significantly affected and showed only some hyperplasia and interstitial oedema 7 and 14 days post exposure. After 42 days, tubular populations were restored partially. After 84 days, 5% of the tubules were still atrophic (loss of stem cells). Sertoli cells in the post-exposure period showed loss of cell-cell contacts and cytoplasmatic vacuolisation (Lee and Kinney, 1989). These studies demonstrate, like the above-mentioned oral study of Aich and Manna (1996), that the testicular effects are principally reversible over a wide dose range.

Following 4-hour inhalation by Wistar rats of up to 5,000 ppm EGME, 1,250, 2,000 or 5,000 ppm caused testicular atrophy and 625 ppm damaged spermatids. In another study, there was a decrease in testes weight on day 2 and testicular atrophy from day 1 to 19 (Samuels *et al*, 1984).

F344 rats and B6C3F_1 mice were exposed to 0, 100, 300 or 1,000 ppm EGME for 9 days over a period of 2 weeks. Decreased RBC and WBC counts occurred at the two higher levels and testicular atrophy (microscopic changes) at 100 ppm (Miller *et al*, 1981). Similar results were obtained by Doe *et al* (1983), who observed testicular atrophy at 300 ppm in Wistar rats after exposure for 10 days.

Subchronic toxicity

Dermal

Porton-Wistar rats that received dermal doses of 100 or 1,000 mg/kgbw/d for 4 weeks under occlusive conditions showed decreased RBC and WBC, bone marrow cellularity and testicular atrophy. The effects were much weaker under non-occlusive conditions (Fairhurst *et al*, 1989).

Male guinea pigs were dermally treated with 1,000 mg EGME/kgbw/d for 13 weeks, under occlusive dressing. Marked growth retardation, mild anaemia, lymphopenia and testicular damage were observed (Hobson *et al*, 1986a).

Inhalation

SD rats were exposed to vapours of 0, 30, 100 or 300 ppm EGME for 13 weeks. There was no mortality, but the highest dose caused severe testicular atrophy with abnormal sperm cell morphology, thymus atrophy and decreased WBC, platelets, Hb, serum proteins and body weights. Exposure to 100 ppm was without apparent effects; at 30 ppm there was a reduced testicular weight (no consistent dose-response relationship) without a histological substrate, so that this might have been a casual finding without toxicological significance and 100 ppm the no-observed adverse effect level (NOAEL) for the rat (Miller *et al*, 1983a).

NZW rabbits were exposed to vapours of 0, 30, 100 or 300 ppm EGME. Deaths occurred at the two higher levels. Decreased testicular weights were observed at 100 and 300 ppm and in 2 animals at 30 ppm (Miller *et al*, 1983a). In a subsequent study, NZW rabbits were exposed to vapours of 0, 3, 10 or 30 ppm for 13 weeks without apparent effects (Miller *et al*, 1982a), so that - in terms of subchronic toxicity - the NOAEL was either 30 ppm or close to 30 ppm.

In dogs exposed by inhalation to 750 ppm EGME for 12 weeks, there was a reduction of RBC, Hb and Hct. The authors further reported microcytosis, hypochromasia and polychromatophilia, and noted immature granulocytes and decreased osmotic fragility of erythrocytes (Werner *et al*, 1943b).

4.1.4.4 Genotoxicity and cell transformation (Table 4.1.2)

In vitro

EGME was not mutagenic when tested in *Salmonella typhimurium* (Ames test) (McGregor *et al*, 1983; McGregor, 1984), *Schizosaccharomyces pombe* (Abondandolo *et al*, 1980), with and

without addition of a metabolic activation system, and the mouse lymphoma cell thymidine kinase (TK) assay without metabolic activation (McGregor, 1984). EGME did not induce unscheduled DNA synthesis in human embryonic fibroblasts with or without metabolic activation (McGregor *et al*, 1983).

EGME did not induce mutations at the HGPRT locus of CHO and V79 cells, as well as the GPT locus in CHO AS52 cells (Ma *et al*, 1993; Elias *et al*, 1996). In addition, it did not increase the frequency of sister chromatid exchange (SCE) rates in V79 cells. EGME had no effect on the rate of chromosomal aberrations in V79 cells and human lymphocytes (Elias *et al*, 1996). EGME concentrations of 65 to 260 mmol/l caused a significant but weak increase in the percentage of micronuclei (together with nuclear disorganisation) in V79 cells (Elias *et al*, 1996). At very high concentrations (131 - 394 mmol/l) EGME induced aneuploidy (Elias *et al*, 1996).

A cell transformation assay in Syrian hamster embryo (SHE) cells with EGME was negative, whereas a test on the inhibition of metabolic cooperation between V79 cells was positive at non-cytotoxic doses (Elias *et al*, 1996).

In vivo

Administration of EGME has been associated with reduced fertility in rats and abnormal sperm head morphology; there was a weak dominant lethal response (McGregor *et al*, 1983; Moss *et al*, 1985). Adverse toxic effects of the EGME metabolite MAA on sperm have been reported *in vitro* and *in vivo* (Gray *et al*, 1985). It is probable that the adverse effects of EGME *in vivo* are due to testicular toxicity rather than genotoxicity, particularly in view of the negative results by EGME in other genotoxicity assays.

In a micronucleus test with ddY mice no significant effects were seen at 100, 300, 1,200, 1,500, 1,800, and 2,000 mg/kgbw. At 1,000 mg/kgbw, there was a 2.5-fold increase ($p < 0.05$) above control micronucleus rate. It was concluded that EGME has a weak genotoxic potential (Arashidani *et al*, 1993).

In the mouse micronucleus test, EGME was ineffective in increasing the incidence of micronucleated polychromatic erythrocytes (PCE) when tested in both sexes up to a maximum tolerated dose of 2,500 mg/kgbw (Au *et al*, 1993, 1996).

There was no increase in the number of chromosome aberrations in the bone marrow of CD rats following inhalation of EGME (McGregor *et al*, 1983).

In the dominant lethal assay performed by Rao *et al* (1983) in CD rats, the strong testicular toxicity and impaired fertility interfered with the test methodology: the number of implants was probably insufficient and did not yield conclusive dominant lethal effects. In the other available dominant lethal tests conducted in CD rats and CD-1 mice, EGME was not genotoxic (McGregor *et al*, 1983; Anderson *et al*, 1987).

Fruit flies (*Drosophila melanogaster*) were briefly exposed by inhalation to 0, 25 or 500 ppm EGME (maximum tolerated concentration, prior established). Two independent tests were performed using different stocks of flies. The results are unclear. After longer exposures for up to 7 days, sex-linked recessive lethal mutations were seen, but not after exposure for 10 days (McGregor *et al*, 1983). The test data are difficult to interpret (compare Section 4.5.4.4).

In the fruit fly *Drosophila melanogaster* concurrent teratogenic and, at least phenotypically, "mutagenic activity" of EGME resulted in similar phenotypes with teratic adult flies. Also the fertility of both males and females was affected. Mutations were considered to be a sequel of 2-methoxyacetaldehyde (MAALD) (Section 4.1.4.7). Some (1.1 - 8.7%) of the affected females produced offspring with phenotypic similarity, a phenomenon that looked like a classical example of inheritance of an acquired type. The author also pointed out that transcribed genes are more susceptible to mutagens (Eisses, 1999).

The EGME metabolite methoxyacetic aldehyde (MAALD) (Section 4.1.4.7) exhibited some clastogenicity *in vitro* in CHO and AS52 cells, in human lymphocytes, but not *in vivo* in bone marrow cells of $B6C3F_1$ mice even at cytotoxic dose levels (Au *et al*, 1996). Furthermore, MAALD induced chromosome aberrations and SCEs in V79 cells and morphological transformation in SHE cells (Elias *et al*, 1996).

The frequency of SCE rates induced by EGME and MAA was evaluated *in vitro* in human peripheral blood and for EGME also in bone marrow cells of mice. In human peripheral blood, SCE rates were increased after the addition of MAA to the culture medium but not of EGME. An increased SCE rate in mouse bone marrow cells was observed after i.p. injection of EGME (treatment *in vivo*, labelling *in vitro*) (Arashidani *et al*, 1998).

A comet assay was employed for the evaluation of testicular cells and bone marrow cells after single gavage administrations of 0, 500, 1,000 or 1,500 mg EGME/kgbw to SD rats. Two studies were conducted, one for effects 2 weeks after treatment and a second study for effects at 5 and 6 weeks after treatment. After 2 weeks, but not after 5 and 6 weeks, a dose-related increase in damage (as measured by the mean and median tail moment) was noted for haploid testicular cells; in diploid bone marrow cells there were also increases but without a clear dose relation (Anderson *et al*, 1996). Although the authors debate the possibility that EGME may be genotoxic,

the results may also reflect cytotoxicity and apoptotic events which are known for EGME (and its metabolite MAA, Section 4.1.4.7) in these target cells.

The frequency of SCE rates induced by EGME and MAA was evaluated *in vitro* in human peripheral blood and for EGME also in bone marrow cells of mice. In human peripheral blood, SCE rates were increased after the addition of MAA to the culture medium but not of EGME. An increased SCE rate in mouse bone marrow cells was observed after i.p. injection of EGME (treatment *in vivo*, labelling *in vitro*) (Arashidani *et al*, 1998).

EGME was, on balance, not genotoxic in the above *in vitro* and *in vivo* assays.

4.1.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.1.4.6 Reproductive and developmental toxicity (Table 4.1.3)

Foetotoxicity, embryotoxicity and teratogenicity

In vitro

After culture for 46 hours, EGME (5 mmol/l) did not affect the development of post-implantation rat embryos (Yonemoto *et al*, 1984).

In vivo

EGME, through its metabolite MAA (Section 4.1.4.7), exerts pronounced foetotoxic, embryotoxic and teratogenic effects in all species investigated (rat, mouse, rabbit, monkey and *Drosophila*) and via all routes of exposure (oral, dermal, inhalation) in the presence and absence of maternal toxicity. The critical dose levels are approximately 10 mg/kgbw/d; the NOAEL after inhalation being 3 to 10 ppm (9 - 32 mg/m^3). For MAA, critical dose levels have not yet been established but appear to be lower than those for EGME.

Oral

Wistar rats dosed once with 158 or 315 mg EGME/kgbw by gavage on day 12 of gestation produced dead and/or malformed foetuses (Ritter *et al*, 1985).

Toraason *et al* (1985) reported electrocardiographic anomalies (prolonged QRS waves) and cardiovascular malformations in SD rat foetuses on day 20 of gestation after gavage administration of 25 or 50 mg EGME/kgbw for 7 days. No foetotoxicity was seen at 25 mg/kgbw/d, whereas 50 or 75 mg/kgbw/d showed foetotoxic effects (Toraasen *et al*, 1986a).

Pregnant SD rats received 0, 50 or 100 mg EGME/kgbw/d by gavage from day 9 to 15 of gestation. The lower dose of 50 mg/kgbw/d reduced litter size and foetal body weights. Among the live foetuses, a significant number had visceral abnormalities. The dose of 100 mg/kgbw/d caused complete resorption of all foetuses. Secondary to loss of litters, total and ionic calcium were increased and the 1,25-dihydroxy-vitamin D level was reduced. In non-pregnant virgin rats, 100 mg/kgbw/d for 7 days did not affect calcium or vitamin D metabolism (Toraason *et al*, 1986b).

Pregnant SD rats were exposed to EGME via a liquid diet at approximate doses of 0, 16, 31, 73, 140, 198, 290 or 620 mg/kgbw/d on days 7 to 18 of gestation. Doses of 73 mg/kgbw/d and higher produced total embryo-lethality. Lower doses produced cardiovascular malformations. A dose of 16 mg/kgbw/d was not teratogenic, however, mean survivor weights were still reduced. No significant behavioural effect in offspring was observed (Nelson *et al*, 1989).

In a developmental screening assay according to a modified Chernoff-Kavlock protocol, EGME was administered by oral gavage at dose levels of 0, 50, and 250 mg/kgbw/d to pregnant Wistar rats from g.d. 6 to 15. Maternal piloerection at both levels and vaginal bleeding at 250 mg/kgbw were observed. At both levels no litters were delivered (Leber *et al*, 1990).

SD rats were dosed by oral gavage with 0, 50, 100 or 250 mg EGME/kgbw on day 13 of gestation (limb sensitivity) and limb buds were examined on day 15 of gestation and paws on day 20 of gestation. Development was highly affected at 250 mg/kgbw in all foetuses and litter. The limb buds were also abnormal at 50 and 100 mg/kgbw (Sleet and Ross, 1997).

Female SD rats were administered EGME in a series of studies at dose levels ranging from 10 to 600 mg/kgbw for up to 14 days by oral gavage. EGME suppressed cyclicity, caused corpora lutea hypertrophy, and inhibited ovulation at 100 mg/kgbw and above. Treatment at 300 mg/kgbw revealed elevated serum progesterone levels, while serum oestradiol, FSH, LH, and prolactin remained at base line levels. At 10 mg/kgbw no effects were observed (Davies *et al*, 1997).

Pregnant Wistar rats were treated by oral gavage once daily from day 8 to 20 of gestation with 0.5 ml EGME/animal diluted at 5%, 10% or 20% (estimated daily doses: 100, 200 or 400 mg/kgbw). Dose-dependent increase (30 to 100%) in abnormal foetuses, growth retardation and increased resorption were reported (Saavedra *et al*, 1997).

Following oral gavage administration of EGME up to 1,000 mg/kgbw on day 7 to 14 of gestation, ICL-ICR mice showed developmental effects including skeletal malformations at doses of 31.25 mg/kgbw. A NOAEL was not achieved (Nagano *et al*, 1981, 1984).

A dose level of 1,400 mg EGME/kgbw/d administered on day 7 to 14 of gestation produced total embryo-lethality in CD-1 mice (Schuler *et al*, 1984).

In three studies with CD-1 mice, repeated doses of 250 or 500 mg EGME/kgbw administered during critical periods of gestation produced foetuses with gross malformations (exencephaly and paw lesions or digit anomalies), with a NOAEL of 100 mg/kgbw. There was a reduction in foetal weight and a loss of embryos. Single doses of EGME had the same effects, but no embryo-lethality was seen (Horton *et al*, 1985).

Similar paw malformations were seen in CD-1 mice, following administration of single oral doses of EGME on day 11 of gestation, by Hardin and Eisenmann (1987) and Greene *et al* (1987); the latter authors noted a dose-dependency from 175 to 500 mg/kgbw for this effect. In another study, a dose of 350 mg EGME/kgbw induced forelimb-bud cytotoxicty as early as 2 hours after treatment, with maximum effect at 6 hours (Greene *et al*, 1987).

CD-1 mouse embryos, taken from animals treated with 250 or 325 mg EGME/kgbw by gavage on day 8 of gestation, had expanded areas of cell death in neural crests and medial region of the anterior neural tube. Morphologic, immuno-histochemical and fluorochrome-staining characteristics for apoptosis were recorded. The effects were more pronounced at the higher dose (Ambroso *et al*, 1998).

Pregnant monkeys (*Macaca fascicularis*) received daily gavage doses of 0, 12, 24 or 36 mg EGME/kgbw during the organogenetic gestation period (day 20 to 45 of gestation). At the highest dose maternal toxicity was pronounced and all 8 pregnancies ended in complete embryonic death; one dead embryo showed external malformation (missing a digit on each forelimb). At the middle dose 3/10 and in the lowest dose 3/13 pregnancies ended in embryonic death. At the highest dose the animals became severely anorectic and required supportive treatment for survival. After the end of the treatment period the animals recovered. Moderate and slight anorexia were seen at the lower levels. The major metabolite, MAA, showed a biological half-life of about 20 hours and appeared to accumulate in all dosed groups (Scott *et al*, 1989).

The developmental toxicity of EGME was assessed in *Drosophila melanogaster* after exposure to 12.5 to 26 mg/culture throughout development. The incidence of wing blade notches was increased at 12.5 and 22 mg/vial. The incidence of bent humeral bristles was increased at all concentrations tested (Lynch and Toraason, 1996).

In *Drosophila melanogaster* concurrent teratogenic and, at least phenotypically, "mutagenic activity" of EGME resulted in similar phenotypes with teratic adult flies (wing notches and duplication of macrochaetae similar to feeding with MAA). Also the fertility of both males and females was affected. Mutations were considered to be a consequence of MAALD. A fraction (1.1 - 8.7%) of the affected females produced offspring with phenotypic similarity, a phenomenon that looks like a classical example of inheritance of an acquired type. The author also points out that transcribed genes are more susceptible to mutagens (Eisses, 1999). In this study, exceedingly high doses of EGME (20 to 60 mmol/l for ≥ 72 hours) were used.

Dermal

In a Chernoff-Kavlock assay, doses of 0, 300, 1,000, 3,000 or 10,000 mg EGME/kgbw were applied for 6 hours under occlusion to Wistar-derived Alpk/AP rats from day 6 to 17 of gestation. The rats were allowed to litter and rear their litters until day 5 *post partum.* The NOAEL was 300 mg/kgbw, whereas 1,000 mg//kgbw decreased the litter size and 3,000 mg/kgbw caused foetal death; 10,000 mg/kgbw was lethal to the pregnant animals as well (Wickramaratne, 1986).

An undiluted dose of 250 mg EGME/kgbw was reported as the NOAEL in SD rats for single open epicutaneous exposures on day 12 of gestation. Significant increases in external, visceral, and skeletal malformations were observed in foetuses at 500 to 2,000 mg/kgbw (Feuston *et al*, 1990).

Repeated occluded exposure of Wistar rats to 50 to 970 mg EGME/kgbw/d on day 6 to 15 of gestation led to complete resorption of all litters at 480 mg/kgbw or higher. From the low dose of 50 mg/kgbw/d there were malformed foetuses and foeto- and embryotoxicity without maternal effects, and from 100 mg/kgbw/d increased post-implantation losses. No NOAEL has been established in this study (Hellwig, 1993).

Inhalation

Wistar rats exposed by inhalation to airborne concentrations of 0, 100 or 300 ppm EGME on day 6 to 17 of gestation, displayed prolonged gestation and decreased numbers of pups and live pups.

At 300 ppm, there was a decrease in maternal body weight gain and 100% loss of embryos (Doe *et al*, 1983).

Nelson *et al* (1984a) exposed SD rats to EGME by inhalation from day 7 to 15 of gestation. The highest concentration of 200 ppm produced complete resorptions in the absence of maternal toxicity. The lower levels of 100 and 50 ppm caused increased incidence of resorptions and skeletal and cardiovascular defects. In another study, 25 ppm EGME caused behavioural effects in offspring of SD rats (Nelson *et al*, 1984b).

Hanley *et al* (1984a,b) exposed F344 rats by inhalation to 0, 3, 10 or 50 ppm EGME from day 6 to 15 of gestation and observed skeletal variations but no malformations in the highest exposure group. Similarly, a concentration of 10 ppm was a NOAEL in CD-1 mice (Hanley *et al*, 1984a,b).

In New Zealand white (NZW) rabbits exposed to 0, 3, 10 or 50 ppm EGME vapours from day 6 to 18 of gestation, a clear teratogenic effect was noted at 50 ppm (mainly cardiovascular, urogenital and skeletal defects); retarded ossifications (in relation to the actual, but not the historical control) were recorded at 10 ppm. The authors therefore considered 10 ppm as a NOAEL in rabbits (Hanley *et al*, 1984a,b).

Other routes

I.p. injection of pregnant Wistar rats with 380 mg EGME/kgbw on day12 of gestation caused resorptions and limb malformations in practically all foetuses; 11% showed ventral duplication of hind limb digits (Scott *et al*, 1987).

EGME elicited exencephaly in CD-1 mouse foetuses when given in a single maternal treatment (s.c. injection of 250 or 325 mg/kgbw) 2 hours prior to the beginning of day 8 of gestation. The effect on gross and microscopic neural development was examined in foetuses on day 9, 10 and 18 of gestation. Typical cell death patterns in the embryonic neural folds were noted. At 325 mg/kgbw there was a high resorption rate. Compared to the control group (saline), EGME increased the incidence of open neural tubes at all time points investigated (Terry *et al*, 1996).

Pregnant Wistar rats were injected i.p. with 0.5 ml EGME/animal (estimated doses 100, 200, or 400 mg/kgbw/d) from day 8 to 20 of gestation. Dose-dependent increases (30 - 100%) in abnormal foetuses, growth retardation and increased resorption were observed (Saavedra *et al*, 1997). The proportions of malformed and retarded foetuses were not different from those after oral treatment (see above).

Protection against EGME-induced teratogenicity

The dysmorphogenesis of rat embryos and foetuses induced by EGME could be partially restored by additional administration of serine to the dams. In the above study of Sleet and Ross (1997), concomitant oral administration of 250 mg EGME/kgbw and of serine (1,734 mg/kgbw) completely abolished the occurrence of paw malformations. A 25% incidence (75% reduction) of paw malformations was noted when serine was administered 4 hours after EGME administration, the incidence was 41 to 45% (55 - 59% reduction) after 8 to 12 hours delay and 76% (24% reduction) after 24 hours delay.

A reduction of teratogenicity was also reported in CD-1 mice after simultaneous oral administration of 250 or 350 mg EGME/kgbw and ethanol or the ADH inhibitor 4-methylpyrazole (Sleet *et al*, 1988) and also in Wistar rats at 315 mg EGME/kgbw with 4-methylpyrazole (Ritter *et al*, 1985). Oral co-administration of 760 mg EGME/kgbw and ethanol, *n*-propanol or *n*-butanol did not modify the testicular toxicity (as judged by testicular weight and urinary creatine/creatinine ratio) of EGME (Morel *et al*, 1996).

In the study of Nelson *et al* (1989), the teratogenicity in SD rats given 73 or 140 mg EGME/kgbw in the liquid diet was not significantly reduced by simultaneous administration of ethanol. The authors state no significant ADH inhibition was achieved with the ethanol treatment.

Protection against EGME-induced teratogenicity in mice was also partially achieved with several physiological compounds such as acetate, formate, glycine, sarcosine, glucose; serine enantiomers were most effective (Mebus and Welsch, 1989; Clarke *et al*, 1991a).

The metabolite MAA has a long biological half-life and may interfere with the availability of one-carbon units linked to tetrahydrofolate pathways. In fact, 5 mmol/l MAA produced a 50% reduction of ^{3}H-thymidine incorporation into mouse embryos in serum-free medium. This effect could be counteracted by formate, acetate or sarcosine (Stedman and Welsch, 1989).

Effects on fertility

In vivo/ex-vivo

Treatment of SD rats with 50 to 100 mg/kgbw EGME (single dose i.p.) reduced the sperm fertilising potential *in vitro* of zona-free oocytes. The doses had no effect on sperm motility (Berger *et al*, 2000).

In vivo

Oral

Following daily oral dosing of male F344 rats with ≥ 100 mg EGME/kgbw for 5 days, the fertility index was decreased. When the animals were then mated with untreated females, the viable offspring was reduced (Chapin *et al*, 1985b).

When EGME was added to the drinking water of female SD rats for 14 days, the number of ovulating oocytes was reduced (0.15 to 0.25%) or stopped (0.3%); the doses were equivalent to approximately 150, 250 or 300 mg/kgbw/d, respectively) (Berger *et al*, 2000).

In continuous breeding studies in SD rats receiving up to 0.1% EGME in the drinking water, a NOAEL of 0.012% was established; this is equivalent to 9.6 and 8.1 mg/kgbw/d for F_0 and F_1 males and 15.3 and 14.2 mg/kgbw/d for F_0 and F_1 females. At 0.024 and 0.03% the number of viable and surviving pups was reduced. At 0.1% only one litter was obtained; testicular weights, epididymis, prostate and relative kidney weights were reduced at that level in F_0 (and at 0.03% also in F_1) as well as number and motility of sperm cells. Fertility indices were not significantly affected in F_1, whereas in F_0 the fertility index at 0.1% was reduced to 5% (Gulati *et al*, 1990a,b). Since EGME causes both prenatal and paternal testicular toxicity the impact of either effect upon the fertility parameters is difficult to distinguish.

In preliminary continuous breeding studies, all CD-1 mice receiving 0.5% to 2.0% EGME in the drinking water, were infertile. In the follow-up study with concentrations of 0, 0.1, 0.2 or 0.4% EGME in the drinking water (equivalent to 0, 159, 336 or 619 mg/kgbw/d), the fertility index was reduced at 0.4% in the F_0. The number of litters per pair was reduced by 30% at 0.2% while the number of live pups per litter was reduced by 18% (0.1%) and 77% (0.2%). No live pups were delivered at 0.4% (at this dose level, paternal testicular toxicity and prenatal toxicity are not discernable from the study parameters). The number of cumulative days to litter was increased for all litters at all dose levels. Thus, concentrations of 0.1 to 0.4% produced marked reductions in fertility and reproductive indices. The NOAEL was below 0.1% (Gulati *et al*, 1985a,b; 1988a; Chapin and Sloane, 1997).

Continuous breeding studies were conducted in C57BL/6 and C3H mice with 0.03, 0.1 or 0.3% EGME in the drinking water (equivalent to 60, 200 or 600 mg/kgbw/d). In C57 BL/6 mice, in the 0.3% dose group, the number and viability of offspring were reduced in the F_1 generation; the fertility index was 25% in the F_0 generation. In the F_1 generation, fertility indices were 50% in the 0.03% group and 0% in the 0.1% group. In C3H mice, the fertility index was 0% in F_0 at 0.3% and 0% in F_2 at 0.1% (Gulati *et al*, 1988b; 1989).

Inhalation

In an inhalation study in male and female SD rats, F_1-generation animals exposed to 0, 30, 100 or 300 ppm EGME for 13 weeks were cross-mated with untreated animals for 2 to 19 weeks. Severe reduction of male fertility was noted at 300 ppm. This effect was only partially reversible after 19 weeks; 100 ppm was the NOAEL for male rat fertility and reproduction in terms of the functional (and morphological) parameters (Rao *et al*, 1983).

Following occluded dermal application of 625, 1,250, 2,500 or 5,000 mg EGME/kgbw/d to male SD rats for 7 consecutive days, there was lethality at the highest dose after 5 days and this dose group was therefore discontinued. After mating with untreated females during week 1, 4, 7, 10 and 14, dose-related testicular and epididymal weight decreases, decline in epididymal and testicular sperm count, an increase in the number of sperm with abnormal morphology as well as tubular atrophy and reduced fertility indices were observed at all dose levels. Similar effects were observed under non-occlusive conditions at higher dose levels (1,250, 2,500 and 5,000 mg/kgbw/d). Morphologically, at 1,250 mg/kgbw there was a loss of spermatids, but an influence on the fertility index could not be established (Feuston *et al*, 1989).

PBPK models on EGME and MAA disposition in pregnant rats were also developed (Hays *et al*, 2000), as well as models covering embryonic organogenesis (Welsch *et al*, 1995).

Summary and conclusion

In summary, the following LOAEL and/or NOAEL values are available (Table 4.1.0):

Table 4.1.0: LOAELs and/or NOAELs for reproductive and developmental toxicity

Species, strain	Endpoint	NOAEL (mg/kgbw/d)	LOAEL (mg/kgbw/d)	Reference
Oral, gavage				
Rat, SD	Foetotoxicity	-	25	Toraason *et al*, 1985
Rat, SD	Foetotoxicity	25	-	Toraason *et al*, 1986a
Rat, Wistar	Foetotoxicity, teratogenicity	-	158	Ritter *et al*, 1985
Rat, SD	Teratogenicity	-	50	Sleet and Ross, 1997
Rat, SD	↓ fertility, embryo-/foetotoxicity	-	50	Chapin *et al*, 1985b
Mouse, JCL-ICR	Foetotoxicity, teratogenicity	-	31.25	Nagano *et al*, 1984
Mouse, CD-1	Embryo-/foetotoxicity, teratogenicity	100	250	Horton *et al*, 1985
Mouse, CD-1	Teratogenicity	100	-	Horton *et al*, 1985
Mouse, CD-1	Foetotoxicity	100	200	Sleet *et al*, 1988
Oral, drinking water				
Rat, SD	↓ fertility, foetotoxicity may interfere with assessment of fecundity	8.1	16.2	Gulati, 1990a,b
Dermal, occluded				
Rat, Wistar	↓ fertility, embryo-/foetotoxicity	300 [a]	1,000 [a]	Wickramaratne, 1986

[a] Approximately

The lowest NOAEL was approximately 8 mg/kgbw/d in the rat, with a LOAEL of 16 mg/kgbw/d. The values apply to male offspring rats in a continuous breeding study (see also Section 4.8.4.6).

4.1.4.7 Kinetics and metabolism (Table 4.1.4)

Uptake

EGME is absorbed through human skin *in vitro* at a rate of 2.82 mg/cm²/h (permeability constant 28.9 cm/h x 10^4); the damage ratio (of final ^3H_2O permeability constant after 8 hours to initial value) was 3.51 (Dugard *et al*, 1984).

Subacute/subchronic and reproduction toxicity studies in rats, mice and guinea pigs also demonstrated ready dermal absorption of EGME *in vivo* even under non-occlusive conditions (Hobson *et al*, 1986a; Fairhurst *et al*, 1989; Feuston *et al*, 1989 and 1990) (Table 4.1.1 and 4.1.3).

Distribution and metabolism

The differential distribution of EGME was investigated following various routes of administration, using whole-body autoradiographic techniques. Male $B6C3F_1$ mice were treated with tracer i.v. or oral doses of [2-^{14}C]-EGME (4.05 µg/kgbw; equivalent to 0.8 mCi/kgbw) and killed at 1 and 24 hours following treatment. In both groups the highest levels of radioactivity were detected in the liver, urinary bladder, bone marrow, kidney and epididymis, at 1 and 24 hours. There was markedly higher deposition of EGME and/or its metabolites in various tissues of the orally treated animals than in animals treated i.v. The results suggest that EGME is rapidly distributed either from blood or stomach to various tissues. Preferential deposition of radioactivity in the peripheral tissues of the bone, with a progressive inward accumulation in the bone marrow, was observed. Selective permeability of EGME and/or its metabolites was indicated by the higher uptake by the epididymis than that by the testis. The high levels of radioactivity in biosynthetically active tissues such as liver, bone marrow and gastric mucosa, indicate persistent interaction of EGME with cellular components of these tissues. These interactions may lead to EGME toxicity (Ahmed *et al*, 1994).

Repeated oral treatment with 100 or 300 mg/kgbw/d for 20 days did not induce CYP or NADPH cytochrome *c* reductase in Wistar rats. The activity of cytosolic ADH however was increased (Kawamoto *et al*, 1990b).

After administration of single oral doses of 76 or 662 mg EGME/kgbw (^{14}C-Iabelled at both glycol C atoms) to male F344 rats, 12% of the radioactivity was exhaled as $^{14}CO_2$ within 48 hours. MAA was identified as the primary urinary metabolite accounting for 80 to 90% of urinary ^{14}C (Miller *et al*, 1983b; Miller, 1987).

Following a single oral dose of radiolabelled EGME (500 mg/kgbw) in rats (strain not specified), 58.5% of the dose was recovered within 24 hours, with a further 11.5 % from 24 to 48 hours. As much as 73% of the urinary radioactivity was related to MAA, 15% to EGME and 8% remained unidentified (Foster *et al*, 1984).

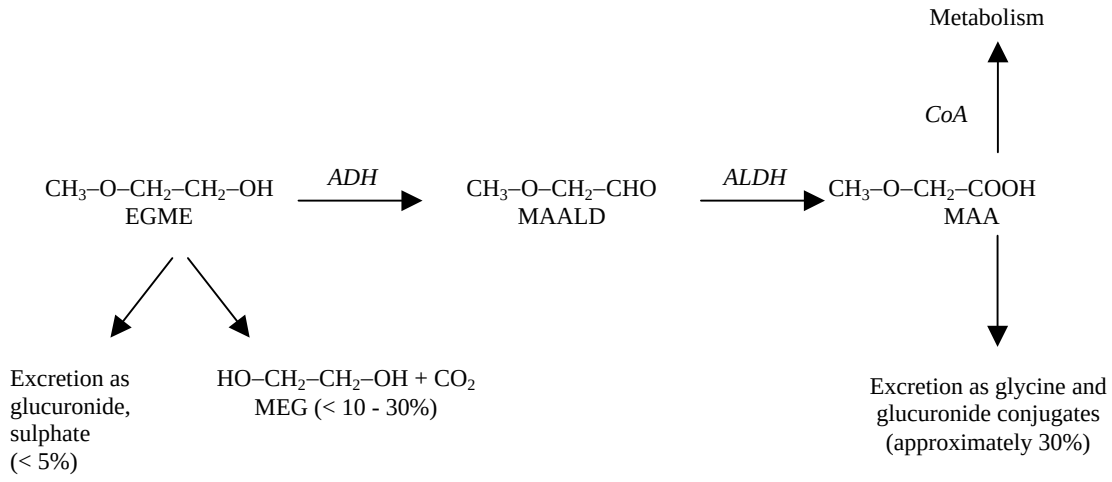
In the monkey *Macaca fascicularis*, blood sampling at 2, 4, 7.5 and 25 hours after each administration of oral doses of 12, 24 or 36 mg EGME/kgbw on day 1, 8, 15 and 22 of pregnancy, showed that maximal MAA concentrations were reached already after 2 hours. The levels decreased to about half the 2-hour values within 24 hours (Scott *et al*, 1989).

Following a teratogenic EGME oral dose (500 mg/kgbw), the plasma MAA levels in mice were approximately 5 mmol/l (450 mg/l) (Welsch *et al*, 1987).

Two major metabolic pathways were observed after 24 h-drinking water administration of ^{14}C-labelled EGME to male F344 rats dosed with 12.2, 26.2 or 110.3 mg/kgbw (180 - 1,620 ppm). Within 72 hours, following a dose-dependent increase, 40 to 50% of the radioactivity was excreted in the urine. Less than 34% of the total dose was oxidised to MAA, while 10 to 30% of the dose underwent cleavage of the ether bond with formation of MEG and consecutive exhalation of CO_2. Some of the parent compound (< 5%) and its glucuronide were also detected in the urine (Figure 4.1.4.7) (Medinsky *et al*, 1990).

The critical metabolite MAA appears to be less effectively cleared from larger species including monkeys and humans than rats and mice.

Figure 4.1.4.7: Metabolic pathways *(after Medinsky et al, 1990)*



Following semi-occluded dermal application of up to 321 mg ^{14}C-EGME/kgbw to F344 rats for 72 hours, 72% of the absorbed radioactivity was excreted in urine, of which 46% as MAA and up to 11% as MEG; the remainder urinary metabolites were not identified but were dose-dependent (Sabourin *et al*, 1992b).

Oxidation of EGME was investigated in liver ADH homogenates of Wistar rats after incubation at 0.50 to 20 mmol/l. Only the ADH-3 isoenzyme was able to oxidise EGME to MAA (Aasmoe *et al*, 1998).

EGME exerts its major toxic activity via its oxidative product MAA. Pretreatment of male SD rats with the ADH-inhibitor pyrazole protected against EGME (250 mg/kgbw i.p.) induced testicular toxicity and reduced the radioactivity in the 48-hour urine from 55.2 to 18% (Moss *et al*, 1985).

In rat primary testicular cell cultures, EGME was not active even at 50 mmol/l (3,800 mg/l) for 72 hours, due to lack of oxidation to MAA. In contrast, MAA at 2 to 10 mmol/l (180 to 900 mg/l) for 24 to 72 hours was toxic to testicular cell cultures (Gray *et al*, 1985).

The localisation of radioactivity from 2-methoxy[1,2-^{14}C]ethanol was examined in pregnant CD-1 mice and their conceptus after oral administration of a radioactive dose of 13 µCi/mouse (0.92 µmol/mouse) partly in combination with an unlabeled dose of 350 mg EGME/kgbw on day 11 of gestation. Radioactivity in the maternal compartment was most concentrated in the liver, blood, and gastro-intestinal tract, whereas conceptus ^{14}C was associated with the placenta, yolk sac, and embryonal structures such as limb buds, somites, and neuro-epithelium. Blood concentration plateau-ed within 30 minutes after dosing, remaining stable for 1.5 hours and then gradually declined, reaching 2 to 10% of the maximum concentration by 48 hours (Sleet *et al*, 1986).

Ethanol was the preferred substrate for ADH and pretreatment (20 mmol/kgbw i.p.) efficiently blocked oxidation of EGME (1,600 ppm for 2 hours by inhalation). This led to higher plasma levels of EGME in SD rats than expected from the original clearance rates. After single i.p. co-administration of 10 mmol/kgbw (761 mg/kgbw) with ethanol (20 mmol/kgbw) the blood levels of EGME remained nearly constant as long as ethanol blood levels were above 3 mmol/l, whereas repeated i.p. dosing plus ethanol resulted in an almost complete accumulation in the blood (Römer *et al*, 1985). Also Sleet *et al* (1988) observed higher plasma levels of EGME due to ethanol after oral treatment of CD-1 mice. In either case, the internal MAA dose is of toxicological relevance.

[1,2-Methoxy-^{13}C]-EGME was orally administered at developmentally toxic (250 mg/kgbw) or non-toxic (25 mg/kgbw) doses on day 11 of gestation and to male F344 rats. The mice were also dosed with L-serine, D-serine or acetate, which are known attenuators of EGME toxicity. Major metabolites identified were MEG, EGME-glucuronide and -sulphate and MAA. MAA was also partially further converted to glycine and glucuronide conjugates as well as to 2-methoxyacetyl-CoA and subsequent metabolites resulting from incorporation into the citrate cycle or the synthesis of fatty acids. The CoA cascade appeared to be reduced by acetate administration (Jenkins-Sumner *et al*, 1996).

A single dose of 760 mg EGME/kgbw in SD rats caused a 30-fold increase in the urinary creatine/creatinine ratio after 24 hours and a 5-fold increase after 48 hours. In the course of 48 hours, the urinary excretion of MAA was 64 mg/l/24 hour. The concomitant administration of either ethanol, *n*-propanol or *n*-butanol did not significantly modify this pattern. (Task Force comment: On the other hand, MAA has a long biological half-life, excretion rates are low and a moderately retarded conversion of EGME by other alcohols does not change the MAA excretion pattern.) Furthermore, the co-administration of the alcohols did not modify the testicular toxicity

(as judged by testicular weight and creatinine ratio) of EGME (Morel *et al*, 1996). Also Nelson *et al* (1989) did not observe an inhibitory activity of ethanol on the embryotoxicity in SD rats, whereas 4-methylpyrazol had been shown as effective (Ritter *et al*, 1985).

Testicular cell cultures from SD rats showed significant ADH capacity for EGME biotransformation (6-fold lower affinity than liver). EGME biotransformation could also be shown in testes from Wistar rats, one strain of mice, but not in testes from hamsters, guinea pigs, rabbits, dogs, cats or humans. However, the testes of all these species readily converted MAALD to MAA. The authors concluded that these differences do not provide a sufficient explanation for the reported species and strain differences in terms of susceptibility to EGME toxicity (Moslen *et al*, 1995).

Biomonitoring of EGME exposure can be carried out by determination of the MAA level and excretion rate in the urine, or by simultaneous measurements of EGME and MAA in human blood by GC-mass spectrometry (Shih *et al*, 1999a,b). Since MAA is only slowly excreted, especially in humans, there is a tendency for MAA to increase over time, e.g. in employees during the course of a working week.

Using PBPK models and a Monte Carlo simulation, an OEL value of 0.9 ppm (3 mg/m^3) for EGME was proposed by Sweeney *et al* (2001) and Gargas *et al* (2000a,b). This OEL practically excluded dermal exposure.

Human exposure was investigated employing 7 resting volunteers inhaling 16 mg EGME/m^3 (5 ppm) for 4 hours (total dose 0.25 mg EGME/kgbw). No toxicological signs or symptoms were seen. By this route and at this dose level 85.5% in average of EGME was transformed to MAA. MAA was detected in the urine up to 120 hours after the exposure. The elimination half-life was between 66 and 90 hours (mean 77.1 ± 9.5 sd) (Groeseneken *et al*, 1989a; Scott *et al*, 1989) (Section 3.1.1).

It should be noted that at inhalation exposure in humans, dermal exposure from the vapour phase may account for up to 55% of the internal dose (Kežic *et al*, 1997).

Five human volunteers were dermally exposed to vaporised and liquid 4,000 mg EGME/m^3 for 45 minutes and urinary excretion of MAA measured and compared with a reference inhalation exposure. Mean absorption rates were 36 cm/h (vapour) and 2.9 cm/h (liquid) (Kežic *et al*, 1997).

Dermal absorption of vaporous EGME was investigated in 7 volunteers after 4-hour single-arm exposure to 25 or 300 ppm. Uptake was 7.0 and 65.3 mg, respectively, with corresponding uptake rates of 1.36 and 13.2 μg/cm^2/h. The permeability constant was 14.0 cm/h, much higher than that of many widely used organic chemicals (Shih *et al*, 2000b).

4.1.4.8 Neurotoxicity

Inhibition of avoidance-escape response was observed in conditioned Wistar and CFE rats exposed (whole body, 4 h/d) for 7 days to concentrations of 500 to 4,000 ppm EGME (1,580 - 12,660 mg/m^3) or for 14 days (5 d/wk) to 125 to 500 ppm (395 - 1,580 mg/m^3), or for 10 exposure days to 1,000 to 8,000 ppm (3,160 - 25,310 mg/m^3). The effect was dose- and time-dependent. A concentration without such effect was not established (Goldberg *et al*, 1962, 1964).

Savolainen (1980) reported partial hind limb paresis in male Wistar rats and after whole-body vapour exposure to 400 ppm EGME (1,270 mg/m^3) for 2 weeks. Enzyme alterations in glial cells (increased acid proteinase, NADPH-diaphorase and 2',3'-cyclic nucleotide 3'-phosphohydrolase activities, decreased succinate dehydrogenase activity) were measured at all concentrations (50, 100, and 400 ppm). In addition, in the second week, body weight was reduced and spleens enlarged at 400 ppm.

4.1.4.9 Immunotoxicity

In vivo

EGME and its metabolite MAA have been shown to be immunotoxic in SD rats exposed via the drinking water (2,000 or 6,000 mg/l, equivalent to 150 or 450 mg/kgbw) and, to a lower extent, in B6C3F$_1$ mice (approximately 400 or 1,200 mg/kg/bw). They also noted dose-related increases of NK cell cytotoxic activity in SD rats exposed to EGME and MAA. The study pointed out the importance of the specific parameter that is applied for the immunological assessments (Exon *et al*, 1991).

Male C3H/HeN mice, orally administered with 500 or 1,000 mg/kgbw for 2, 5 or 10 days, showed cortical atrophy and markedly decreased cellularity of the thymus and increases in various *ex vivo* immunological assays, e.g. lympho-proliferative response to concanavalin A (Con A). In conjunction with *in vitro* studies (see below) these findings suggest that EGME selectively deplete immature thymocytes (Kayama *et al*, 1991).

In F344 rats, 10 daily oral administrations of 50, 100 or 200 mg/kgbw caused dose-dependent decreases of thymus weights, lymphocyte proliferation response upon B and T cell mitogen stimulation, antibody and interleukin-2 production. Similar effects were noted with MAA (Smialowicz *et al*, 1991a,b) (Section 4.8.4.9). In B6C3F$_1$ mice, only thymus atrophy was significant) after 10 oral dosages 500 or 1,000 mg EGME/kgbw over a 2-week period. Several examinations for alterations of immune function and host resistance did not reveal EGME-induced effects. At 250 mg/kgbw, thymus weight was not affected (House *et al*, 1985).

Treatment of female F344 rats and C57BL/6J mice by oral gavage with EGME at doses ranging from 50 to 400 mg/kgbw/d for 10 consecutive days revealed sensitivity differences between these species. Rats dosed at 100 to 400 mg/kgbw had decreased thymus weights and suppressed PFC response to TNP-LPS, and, at all dose levels, reduced lympho-proliferative (LP) response to Con A, PHA, PWM, and STM. No thymic involution, suppression of LP response or PFC response to TNP-LPS were observed in mice at these dose levels (Smialowicz *et al*, 1992a).

In a study comparing the immunosuppression of various glycol ethers, EGME was administered by oral gavage to male F344 rats at doses ranging from 50 to 400 mg/kgbw for 2 consecutive days. EGME (as well as MAA and EGMEA) dose-dependently suppressed the PFC response to TNP-LPS (Smialowicz *et al*, 1992b).

Inbred Lewis and Wistar-Furth rats were the more sensitive than inbred F344 and outbred SD rats in the suppression of the PFC response to TNP-LPS, showing effects at an oral dose level of 0.66 mmol/kgbw/d (50 mg/kgbw/d) EGME (or MAA, Section 4.8.4.9) administered for 10 days, whereas several strains of mice were comparably resistant. Female inbred C3H and C57BL/6J, hybrid $B6C3F_1$ and outbred CD-1 mice showed no immuno-suppression even at dose levels up to 5.28 mmol/kgbw (392 mg/kgbw) (Riddle *et al*, 1992; Smialowicz *et al*, 1994). Further studies revealed that the metabolites MAA and MAALD are equipotent immuno-suppressants in the rat (Smialowicz *et al*, 1993).

Oral dosing of adult male F344 rats with EGME (1 x 50, 100, 200 or 400 mg/kgbw) resulted in the suppression of the PFC response to TNP-LPS, MAA produced similar results (Smialowicz *et al*, 1992b) (Section 4.8.4.9).

After single EGME administration (125 or 500 mg/kgbw by gavage) apoptosis in the rat thymus may be observed within 3 hours; apoptotic cells on thymic slides were visible by means of colour-assisted computer image analysis. Phenobarbital pretreatment in drinking water (1 g/l) caused a decrease in the extent of thymic apoptosis. Further, liver cytosol from the PB-pretreated rats showed some reduction in the capacity to convert EGME to MAALD and a marked enhancement in conversion of MAALD to MAA. This might indicate that MAALD was a factor in thymus toxicity (Balasubramanian *et al*, 1996).

When undiluted EGME was applied to male F344 rats at dose levels of 0, 150, 300, 600, 900 or 1,200 mg/kgbw/d for 4 consecutive days on shaved skin (occluded test sites), thymus weights were decreased at all dose levels. Reduced spleen weights were observed at 900 and 1,200 mg/kgbw/d. Lympho-proliferative responses to phytohaemagglutinin (PHA) and pokewead mitogen (PWM) were enhanced at the top dose. Other groups of animals receiving dermal doses of 150, 300 or 600 mg/kgbw/d for 4 consecutive days were investigated for antibody PFC response to either the T-lymphocyte-independent antigen TNP-LPS or the T-lymphocyte-

dependent antigen sheep red blood cells (SRBC). A reduction in PFC response to TNP was observed at 600 and decreases of PFC responses to SRBC at 300 and 600 mg/kgbw (Williams *et al*, 1995).

Female C57Bl/6N mice were administered EGME by oral gavage at doses of 0, 100, 150 or 200 mg/kgbw/d from day 10 to 17 of gestation and the offspring was examined on day 18 of gestation. Significant thymic atrophy and cellular depletion, decreased percentages of $CD4^+8^+$ thymocytes and increased percentages of $CD4^-8^-$ thymocytes were seen in foetal mice. These data suggest that *in utero* treatment with EGME produces thymic hypocellularity and inhibit thymocyte maturation (Holladay *et al*, 1994).

Splenic reconstitution of irradiated hosts ($B6C3F_1$ mice) injected with foetal liver cells obtained from mice on day 18 of gestation (following treatment with EGME at 200 mg/kgbw/d from g.d. 10 - 17) was reduced as were the numbers of splenic T and B lymphocytes at day 15 (Holladay *et al*, 1994).

After gavage administration of 0, 25, 50, 100 or 200 mg EGME/kgbw/d to male F344 rats for 4 days, thymus weights were reduced at 50 to 200 mg/kgbw, while spleen weights were reduced only at 200 mg/kgbw. The lympho-proliferative responses to PHA, PWM and *Salmonella typhimurium* mitogens were increased at 200 mg/kgbw, whereas the PFC responses to TNP-LPS and SRBC were suppressed at 50 mg/kgbw and higher (Williams *et al*, 1995).

Thymic involution coincides with lympho-proliferative responses to B and T cells mitogens, decreased production of interleukin-2 by splenocytes, reductions in $CD8^-CD4^+$ helper/inducers and splenic T-lymphocytes and suppression of the primary antibody PFC response to SRBC and TNP-LPS. NK cell activities, one-way mixed lymphocyte reaction response or *in vitro* generated cytotoxic T-lymphocyte response remained unaffected; body and spleen weights were also normal. Enzyme inhibition experiments showed that either MAALD or MAA is required for the immunosuppressive activities. Mice were not significantly immuno-suppressed by EGME or MAA (Smialowicz, 1996).

In vitro

In cultured thymocytes and splenocytes from C3H/HaN mice orally treated with 1,000 mg/kgbw for 10 days, NP-specific cytotoxic T-cell activity was increased and several thymocyte surface markers (CD4+/CD8+, Thy-1+, PNA+) were relatively decreased (Kayama *et al*, 1991).

The immunosuppressive potential of EGME, MAALD and MAA was evaluated *in vitro* in human mononuclear WBC. The three test materials produced DNA fragmentation in the characteristic

pattern of apoptotic cells. Additionally, a dose-dependent increase in intracellular calcium concentrations was reported (Ju *et al*, 1998).

The immunosuppressive activity of EGME, MAA and MAALD was investigated in terms of polyclonal antibody responses of lymphocytes from F344 rats and $B6C3F_1$ mice. EGME had no effect on the antibody production of lymphocytes of either species. MAA and MAALD, however, suppressed IgM and IgG production by lymphocytes at non-toxic doses. Rat lymphocytes reacted at lower concentrations of MAA (0.5 and 1.0 mmol) than did mouse lymphocytes (2.0 mmol). MAALD at 0.3 mmol also showed a suppressive activity. In the case of EGME, immuno-suppression could be achieved by co-cultivation with hepatocytes from either species. Mouse hepatocytes had a greater capacity to metabolise EGME (Kim and Smialowicz, 1997). Due to the high concentrations employed, the results do not indicate a strong immunotoxicity as measured in the antibody production *in vitro*.

4.1.5 Human effects data

4.1.5.1 Accidental oral exposure

After accidental oral uptake of 100 ml EGME (approximately 1,500 - 1,800 mg/kgbw) by 2 persons, Nitter-Hauge (1970) observed high urinary excretion of oxalate levels in one case. Ingestion of 400 ml EGME (several thousand mg/kgbw) mixed with brandy, by a 44-year old man was fatal within 5 hours. Coma and severe liver and kidney injury were observed (Young and Woolner, 1946).

4.1.5.2 Occupational exposure

Several reports exist on the CNS toxicity of EGME in exposed workers.

Exposure to EGME may lead to a complex neurological disorder diagnosed and described as "toxic encephalopathy" (Donley, 1936; Greenburg *et al*, 1938; Parsons and Parsons, 1938; Groetschel and Schürmann, 1959; Zavon, 1963; Nitter-Hauge, 1970; Ohi and Wegman, 1978). Not all individuals showed the same signs and symptoms, but the following pattern is derived from different case studies of persons who were exposed at the workplace. The workers suffered from nausea, headache, drowsiness, irritated or burning eyes and impaired vision, deterioration of audition, loss of concentration and interest, states of agitation and in some cases hallucinations. Poor intellectual performance, stupor, disorientation, rigor and hypertonia of muscles, tremor, sometimes ataxia of arms and legs and spastic gait with clonic feet were also observed. In some cases the eye pupils were wide and reacted slowly. Romberg sign was occasionally positive, the

reflexes of the abdomen wall absent while patellae reflexes were either increased or decreased. Sabinsky reflex was always negative. The subjects also lost weight during their illness. Macrocytic anaemia, leukopenia, reactive lymphocytosis and premature leukocytes were diagnosed if investigated. All signs were gradually reversible after the cessation of exposure. Duration and intensity of exposure in these case reports were varied and ill-defined.

Air concentrations of EGME were measured under simulated conditions after anaemia and encephalopathy had occurred. The concentrations were 61 and 3,960 ppm (193 and 12,530 mg/m^3), depending of the kind of work. MAA was postulated as metabolite responsible for toxicity (Zavon, 1963).

Greenburg *et al* (1938) measured the concentration in workroom air after EGME intoxications had been diagnosed. They measured 25 ppm when the windows were open and 76 ppm when the windows were partially closed (80 and 240 mg/m^3, respectively). However, at the time of the intoxications (but not at the time of measurement), the ventilation had been defective.

According to Ohi and Wegman (1978) neurological symptoms were observed in 2 men exposed to an air concentration of 8 ppm (25 mg/m^3) over a period of a few months. Here, the principle source of exposure was thought to be skin contamination.

Macrocytic anaemia has been described in a worker who was exposed for 20 months to 35 ppm on average (range 18 to 58 ppm) (111 and 57 - 184 mg/m^3, respectively). Other solvents, methoxyethyl ketone and PGME, were also used but are unlikely to have caused this effect. After termination of exposure the patient recovered (Cohen, 1984).

In a cross-sectional epidemiology study in a chemical plant no signs of encephalopathy were detected among potentially exposed workers. However, smaller testicular size and possibly slight reduction in red and WBC counts could not be completely ruled out. The exposure levels monitored were below 20 ppm (63 mg/m^3) (Cook *et al*, 1982).

Alterations in the cellular immune response (decrease of T-helper cells, increase of NK cells, B lymphocytes and normal suppressor cells) in 9 workers exposed to EGME, EGEE and other solvents were observed (Denkhaus *et al*, 1986).

Three out of 7 workers with dermal and respiratory exposure to EGME, EGEE, DPGME and other solvents showed some evidence of bone marrow injury (Cullen *et al*, 1983).

Sparer *et al* (1988), Welch and Cullen (1988) and Welch *et al* (1988) investigated the effect of EGME and EGEE exposure on male reproductive factors and blood in 153 shipyard painters. Increased prevalence of oligo- and azoo-spermia (73%), odds ratio for lower sperm counts,

anaemia (10%) and granulocytopenia (5%) were noted. None of these effects occurred in 55 control workers.

Saavedra *et al* (1997) described congenital malformations (mostly of the craniofacial, musculosceletal and central nervous systems) with varying degrees of mental retardation in 44 persons whose mothers had been occupationally exposed during pregnancy to EGME and MEG through cutaneous, oral, and respiratory routes in a Mexican factory. A subsequent "case-control" study revealed causality with the EGME and MEG exposure. The workers themselves at times suffered to varying degrees from symptoms of intoxication, from strong headaches or cutaneous rash to repeated vomiting with dehydration, temporal loss of consciousness and coma; in the severe cases, intra-hospital treatment was needed. No analytical data on the level of exposure were presented but the described exposure and intoxication scenarios suggest high exposure.

In exposed workers anaemic effects of EGME exposure were observed and correlated with the biological monitoring of urinary MAA. Spermatotoxic effects were not noted (Shih *et al*, 2000a).

El-Zein *et al* (2002) investigated 41 offspring children of 28 women occupationally exposed to EGME (air concentrations and magnitude of dermal exposure not specified) for an average duration of 4.6 years. Six children of 5 women exposed during pregnancy showed dysmorphic features that were not observed among 35 children of 23 women who were not exposed during their pregnancy. Furthermore, all 6 affected children exclusively had persistent chromosome aberrations, including breaks, polyploid and endoreduplicated cells, but no translocations or inversions. The authors explained the pattern of their cytogenetic findings as a disposition for genetic instability characterised by delayed cell division.

4.1.6 Other information

EGME and MAA increased ovarian luteal cell progesterone production *in vivo* in female SD rats administered 300 mg EGME/kgbw/d at the onset of vaginal metoestrus, and *in vitro* in cultured SD rat luteal cells incubated with up to 10 mmol MAA/l for 3 to 48 hours and in cells from human oocyte donors (25,000 cells/well) treated with human chorionic gonadotropin and 0 to 5 mmol MAA/l for 6 to 48 hours. At 1 mmol MAA/l, the progesterone production into the culture medium was significantly increased after 24 hours. No effects on ATP levels were observed. Thus, EGME and MAA might have the potential to alter ovarian luteal function in women (Almekinder *et al*, 1997; Davis *et al*, 1997) (Section 4.8.6).

In vitro apoptotic changes could be replicated by MAA (5 mmol/l, 450 mg/l) in the adult rat seminiferous tubule culture model. The effects of MAA could be prevented by co-treatment with Geldanamycin, Herbimycin and other substances which are inhibitors of certain protein-tyrosine

kinases (Src). The data suggest, that pp60C-Scr mediates physiological Sertoli germ cell interaction and is forwardly involved in the MAA-induced germ cell apoptosis (Rawlings *et al*, 1985) (Section 4.8.4.6).

Cheever *et al* (2001) tried to investigate potential synergistic embryotoxicity between exposure to EGME and radiofrequency radiation-induced hyperthermia in rats. A possible interaction between radiofrequency radiation and EGME in developmental toxicity interaction in rats was also postulated by Nelson *et al* (1999).

In experimental leukaemia models, EGME revealed anti-tumorigenic properties. The effect was demonstrated in $B6C3F_1$ mice receiving 10 administrations of 300, 600 or 1,200 mg EGME/kgbw/d by gavage within 2 weeks prior to injection of leukaemic cells (Houchens *et al*, 1984) and with F344 rats receiving 15 or 100 mg EGME/kgbw/d in drinking water after injection of leukaemic cells. In the latter set of experiments, EGEE was 10-fold less active than EGME in rejecting the leukemia cells. The non-teratogenic homologues EGPE, EGBE, EGPhE and DEGME were totally ineffective (Dieter *et al*, 1990).

The toxicity of EGME and its acetate has been reviewed by Johanson (2000).

Table 4.1.1: Systemic toxicity of EGME

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**			
Rat, Wistar, 4 M	0	20 d	No effects	Kawamoto *et al*, 1990a
	100	20 d	↓ bw, ↓ relative thymus and testes weights	
	300	1 d	↓ bw	
	300	2 d	↓ bw, ↓ relative thymus and testes weights, ↓ relative liver, kidney, spleen and heart weights	
	300	5 d	Additionally strong depletion of lymphocytes in thymus cortex	
	300	20 d	↓ testicular weight	
Rat, SD, 6 M	0, 50	11 d	No effect	Foster *et al*, 1983
	100, 250, 500		Time- and dose-dependent testicular atrophy, beginning in pachytene	
Rat, F344, 20 M	0	5 d	No effects	Chapin *et al*, 1985a
	50		After 3 wk, aggregation of detached spermatids with a maximum after 4 - 5 wk. No effects after 7 wk	
	100, 200		Dead spermatocytes of different stages in 90% of tubules, but after 7 weeks 50% of the tubules repopulated. In 10% of tubules only spermatogonia and Sertoli cells. Other tubules in gradual restoration	

Table 4.1.1: Systemic toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage (cont'd)	**(mg/kgbw)**			
Rat, Indian house (wild), 5 M	0 500	1, 6, 11 d	No effects Testicular tubular atrophy, no histopathological changes of Leydig and Sertoli cells (light microscopy), no alterations in testicular steroidogenesis	Aich and Manna, 1996
Mouse, ICL-ICR, 5 M (20 M control)	0, 62.5, 125 250 500 1,000 2,000	1 x/d, 5 d/wk, 5 wk	No effects Testicular atrophy ↓ WBC, RBC and Hct; testicular effects ↓ WBC, RBC and Hct; testicular effects, no germ cells Died before completion	Nagano *et al*, 1979
Mouse, B6C3F$_1$, 10 F	0, 250 500 1,000	10 x, 2 wk	No effects. Cellularity ↓ in thymus and spleen ↓ WBC, platelets and Hb; thymic atrophy after 4 and 12 wk	House *et al*, 1985
Mouse, B6C3F$_1$, 5 M	0 50, 100, 250 250	4 d	No effects Dose-dependent ↓ of cellularity and granulopoietic stem cells in bone marrow after 8 wk, reversible by wk 16 Testicular atrophy, segmental degeneration of seminiferous tubules	Hong *et al*, 1988a
Hamster, Syrian golden, 4 M	0, 62.5, 125 500	5 d/wk, 5 wk	No effects ↓ WBC	Nagano *et al*, 1984
Guinea pig, NS, 3 M	0 250, 500	5 d/wk, 5 wk	No effects ↓ WBC at both concentrations, testicular atrophy	Nagano *et al*, 1984

Table 4.1.1: Systemic toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Dermal, occluded	**(mg/kgbw)**			
Rat, SD, 20 M	0 625; 1,250; 2,500 0 1,250; 2,500; 5,000	1 x	No effects Dose-related ↓ in sperm and spermatid count No effects ↑ abnormal sperm morphology; ↓ fertility	Feuston *et al*, 1989
Guinea pig, Hartley, 6 M	0, 1,000	6 h/d, 5 d/wk, 13 wk	Marked growth retardation, severe testicular atrophy, degeneration of seminiferous tubules with complete loss of spermatogenic cells; ↓ spleen weights, lymphopenia; ↓ RBC with ↑ mean corpuscular volume (MCV), ↑ serum creatine kinase and lactate dehydrogenase	Hobson *et al*, 1986a
Rat, Porton-Wistar, 8 M	0 100 1,000	5 d/wk, 4 wk	No effects ↓ bw gain, ↓ food intake ↓ bw gain, ↓ food intake, ↓ WBC, Hb, Hct, MCV, ↑ reticulocytes, ↓ pachytene spermatocytes and spermatids, bone marrow hypocellularity	Fairhurst *et al*, 1989
Dermal, non-occluded	**(mg/kgbw)**			
Rat, Porton-Wistar, 8 M	0, 100 1,000	5 d/wk, 4 wk	No effects ↓ bw gain, ↓ food intake, ↓ testes weight	Fairhurst *et al*, 1989

Table 4.1.1: Systemic toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, Wistar, 45 M	0 50, 100 400	(0 160, 320 1,270)	6 h/d, 5 - 10 d	No effects Dose-related ↑ in glial cell toxicity as indicated by altered enzyme activities Partial hind limb paralysis, ↓ growth	Savolainen, 1980
Rat, Wistar and CFE, NS	0 125, 250, 500, 1,000, 2,000, 4,000	(0 400, 790, 1,600, 3,200, 6,300, 12,700)	4 h/d, 2, 5, 11, 14 d	No effects Dose and exposure time-related inhibition on conditioned avoidance-escape behaviour. LOAEL = 125 ppm	Goldberg *et al*, 1962
Rat, Crl:CD BR, 25 M	0 300	(0 950)	6 h/d, 3 d	No effects Damage of pachytene and stage XIV spermatocytes; other stages affected at d 1 and 2 post exposure	Lee and Kinney, 1989
Rat, Crl:CD BR, 20 M	0 300	(0 950)	6 h/d, 5 d/wk, 2 wk	No effects 20 - 80% of tubules: atrophic germinal epithelium; Leydig cells not significantly affected. After 42 d, tubular populations restored partially. After 84 d, 5% of tubules still atrophic. Damaged Sertoli cells	Lee and Kinney, 1989

Table 4.1.1: Systemic toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Rat, Wistar, 20 M	0, 150, 300 625 1,250, 2,500, 5,000	(0, 470, 950 1,980 4,000, 7,900, 15,800	4 h; killed d 14	No effects Damaged spermatids Microscopic testicular changes and atrophy; damaged spermatids	Samuels *et al*, 1984
Rat, Wistar, 90 M	0 1,000, 2,500	(0 3,200, 7,900)	4 h, killed d 1, 2, 3, 4, 5, 8, 10, 15, 19	No effects ↓ testes weight at 48 h; testicular atrophy on d 1 - 19	Samuels *et al*, 1984
Rat, F344, 10 M, 10 F	0, 100 300 1,000	(0, 320 950 3,200)	6 h/d, 9 d	No effects Some thymic atrophy Lymphoid depletion in thymus cortex, spleen and lymph nodes; testicular degeneration, ↓ bone marrow cellularity	Miller *et al*, 1981
Rat, Wistar, 10 M	0, 100 300	(0, 320 950)	6 h/d, 10 d	No effects. Testicular atrophy	Doe *et al*, 1983
Rat, SD, 10 M, 10 F	0, 30, 100 300	(0, 95, 320 950)	6 h/d, 5 d/wk, 13 wk	No effects ↓ WBC, platelets, Hb, total protein, albumin and globulin; thymic and testicular atrophy	Miller *et al*, 1983a

Table 4.1.1: Systemic toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Mouse, B6C3F$_1$, 5 M, 5 F	0, 100 300 1,000	(0, 320 950 3,200)	6 h/d, 9 d	No effects Some thymic atrophy Testicular degeneration; ↓ bone marrow cellularity, WBC and RBC	Miller *et al*, 1981
Rabbit, NZW, 5 M, 5 F	0 30, 100, 300	(0 95, 320, 950)	6 h/d, 5 d/wk, 13 wk	No effects Dose-related increase in incidence and severity of testicular lesions. ↓ bw, thymus and testicular weights, microscopic lesions	Miller *et al*, 1983a
Rabbit, NZW, 10 M	0, 3, 10 30	(0, 9, 32 95)	6 h/d, 5d/wk, 13 wk	No effects ↓ bw, ↓ WBC, platelets and Hb, thymic atrophy after 4 and 12 wk	Miller *et al*, 1982a
Dog, 2 NS	0, 750	(0, 2,370)	7 h/d, 5 d/wk, 12 wk	↓ RBC, Hb, and Hct; microcytosis, hypochromasia, and polychromatophilia; immature granulocytes and ↓ osmotic fragility of erythrocytes	Werner *et al*, 1943b
Injection, s.c.	**mg/kgbw/d**				
Mouse, B6C3F$_1$, M, F, numbers NS	0		4 d, 14 d post-observation	No effects	Hong *et al*, 1988b
	100, 200, 400		1 d post-observation	↓ relative testes weight, loss of germinal epithelium, no other histopathological lesions	
			5 d post-observation	M ↓ leukocytes, ↓ granulopoietic stem cells already 1 d post-observation. F ↓ erythropoietic stem cells (1 d post-observation)	

Table 4.1.2: Genotoxicity of EGME

Endpoint / Organism	Strain or type / Target	Concentration	Exposure regime	Result	Remark	Reference
In vitro						
Gene mutation						
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98,	Up to 33 mg/plate	Liquid preincubation	–ve	+/– S9	McGregor *et al*, 1983
Salmonella typhimurium	TA102	NS	Liquid preincubation	–ve	+/– S9	McGregor, 1984
Schizosaccharomyces pombe	P1	Up to 48 mg/l		–ve	+/– S10 from induced and non-induced Swiss mice	Abondandolo *et al*, 1980
Schizosaccharomyces pombe	P1	NS		–ve	+ S9	Barale *et al*, 1979
Mouse lymphoma cell	L5178Y TK+/–	0.01 - 100 µg/ml	4 h	–ve	– S9	McGregor, 1984
CHO cells	K1-BH4; HGPRT locus	50 - 1,000 mmol/l	5 h	–ve	– S9	Ma *et al*, 1993
CHO cells	AS52; GPT locus	100 - 1,000 mmol/l	5 h	–ve	+/– S9	Ma *et al*, 1993
V79 cells	HGPRT locus	NS	3 h	–ve	– S9	Elias *et al*, 1996

Table 4.1.2: Genotoxicity of EGME (cont'd)

Endpoint / Organism	Strain or type / Target	Concentration	Exposure regime	Result	Remark	Reference
In vitro						
Chromosome aberration		**(mmol/l)**				
Human lymphocytes		10, 50, 125	1 h	–ve	– S9	Chiewchanwit and Au, 1994
Human lymphocytes		1, 5, 10, 50, 150 300, 600	24 h 24 h	–ve +ve	– S9 – S9	Chiewchanwit and Au, 1994
Human lymphocytes		NS	20 h	–ve	– S9	Elias *et al*, 1996
V79 cells		NS	19 h	–ve	– S9	Elias *et al*, 1996
Sister chromatid exchange						
Human lymphocytes		1, 10, 100	72 h	**±ve**	– S9	Arashidani *et al*, 1998
V79 cells		65 - 260	26 h	**–ve**	– S9	Elias *et al*, 1996
Micronucleus induction						
V79 cells		65 - 260	24 h	**±ve**	– S9	Elias *et al*, 1996
Aneuploidy						
V79 cells		137 - 396	26 h	±ve	– S9	Elias *et al*, 1996
Unscheduled DNA Synthesis		**(µg/ml)**				
Human fibroblasts		Up to 10	3 h	**–ve**	+/– S9. No cytotoxicity at 10 µg/ml	McGregor *et al*, 1983

Table 4.1.2: Genotoxicity of EGME (cont'd)

Endpoint / Organism	Strain or type / Target	Concentration	Exposure regime	Result	Remark	Reference
In vivo		**(mg/kgbw)**				
Mouse, 50 M/group	ddY Bone marrow	500, 1,000 i.p.				Arashidani *et al*, 1998
In vitro						
DNA damage, Comet assay		**(mg/kgbw)**				
Rat testicular cells	SD	500, 1,000, 1,500, oral gavage	1 x, 2 wk 1 x, 5 - 6 wk	**+ve** **–ve**		Anderson *et al*, 1996
Rat bone marrow cells	SD	500, 1,000, 1,500, oral gavage	1 x, 2 wk 1 x, 5 - 6 wk	**+ve** **–ve**		Anderson *et al*, 1996
In vivo						
Chromosome aberrations						
Rat, 10 M, 10 F	CD	25, 500 ppm, inhalation (80, 1,600 mg/m^3)	7 h or 5 d	**–ve**		McGregor *et al*, 1983
Mouse, 3 - 6 M	B6C3F$_1$ Bone marrow	35 - 2,500 mg/kgbw	1 x oral	**–ve**	6 h	Au *et al*, 1993
Mouse, 3 M	B6C3F$_1$ Bone marrow	1,200 - 1,900 mg/kgbw	1 x oral	**–ve**	17 h	Au *et al*, 1993
Mouse, 2 M	B6C3F$_1$ Bone marrow	800 - 1,400 mg/kgbw	1 x oral	**–ve**	17 h	Au *et al*, 1993

Table 4.1.2: Genotoxicity of EGME (cont'd)

Endpoint / Organism	Strain or type / Target	Concentration	Exposure regime	Result	Remark	Reference
Mouse, 2 M	$B6C3F_1$ Bone marrow	800 - 1,400 mg/kgbw	1 x i.v.	**–ve**	17 h	Au *et al*, 1993
Mouse, 4 M	$B6C3F_1$ Bone marrow	35 - 500 mg/kgbw	7 x oral	**–ve**	5 h after last treatment	Au *et al*, 1993
Dominant lethal mutations						
Rat, 10 M	CD	0 500, 750, 1,000, 1,500 mg/kgbw	1 x oral	**–ve**	No effects ↑ pre-implantation losses after 5 wk, sterility after 6 wk	Anderson *et al*, 1987
Rat, 20 - 30 M and F	CD	30, 100 and 300 ppm, inhalation (95, 320, 950 mg/m^3)	6 h/d, 5 d/wk, 13 wk	**±ve**	M ↓ fertility, no litters	Rao *et al*, 1983
Rat, 10 M	CD	25, 500 ppm, inhalation (80, 1,600 mg/m^3)	5 d	**–ve**	Pre-implantation losses at 500 ppm	McGregor *et al*, 1983
Mouse, 10 M	CD-1	0 500, 750, 1,000, 1,500 mg/kgbw	1 x oral	**–ve**	No effects No effects on fertility; degeneration and depletion of spermatocytes and spermatids	Anderson *et al*, 1987

Table 4.1.2: Genotoxicity of EGME (cont'd)

Endpoint / Organism	Strain or type / Target	Concentration		Exposure regime	Result	Remark	Reference
Sex-linked recessive lethal mutations		**(ppm)**	**(mg/m^3)**				
Drosophila melanogaster	*ORK m; M-5 F*	0	(0	1 h	No effects	Two independent tests using different stocks of flies. Test data difficult to interpret	McGregor *et al*, 1983
		25	80	1 h	Unclear		
		500	1,600	15 min	Unclear		
		48	150	7 h/d, 7 d	**+ve**		
		240	760	7 h/d, 5 d	**+ve**		
		120	380	7 h/d, 6 d	**+ve**		
		36	114)	7 h/d, 10 d	**–ve**		

Table 4.1.3: Reproductive and developmental toxicity of EGME

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**			
Rat, SD, 8 F	0	1 x/d, g.d. 7 - 13	No effects	Toraason *et al,* 1985
(11 controls)	25		↑ some foetuses with aberrant QRS complexes	
	50		Additional cardiovascular defects	
	100		100% resorption	
Rat, SD, 8 F	0, 25	1 x/d, g.d. 6 - 12	No effects	Toraason *et al,* 1986a
	50, 75		↑ foetotoxicity	
Rat, SD, 12 - 14 F	0	1 x/d, g.d. 9 - 15	No effects	Toraason *et al,* 1986b
	50		↑ foetotoxicity	
	100		100% resorption	
Rat, Wistar, 6 F	0	1 x/d, g.d.12	No effects	Ritter *et al,* 1985
(13 controls)	158		19.3% foetal death; 45.2% malformations	
	315		15.1% foetal death; 100% malformations	
	315 + 4-methylpyrazole (100 mg/kgbw) i.p.	1 x/d, g.d. 12	7.8% foetal death, 16.8% malformations	
Rat, Wistar, 15 F	0	1x/d, g.d. 8 - 20 (0.5 ml diluted/animal)	No effects	Saavedra *et al,* 1997
(20 F controls)	100, 200		Dose-dependent ↑ in malformations	
	400		↑ resorptions	

Table 4.1.3: Reproductive and developmental toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage (cont'd)	**(mg/kgbw)**			
Rat, SD, 9 - 10 F	0 50, 100 250	1 x/d, g.d. 13	No effects Limb buds abnormal Limb and paw malformations (100%)	Sleet and Ross, 1997
Rat, SD, 4 - 8 F	250 + serine (1,734 mg/kgbw)	Serine given 0, 4, 8 - 12, 24 h after EGME administration	0%, 25%, 41 - 45%, 76% malformations	
Rat, SD, 5 M	0, 5, 15 50 100	1 x i.p.	Normal fertilisation *in vitro*, 95.4% zona-free and 64.4% cumulus-intact oocytes in controls ↓ fertilisation of zona-free oocytes (64.4%) ↓ fertilisation of oocytes, zona-free 64.4% and cumulus-intact 45.7%; no effect on sperm motility at all doses	Berger *et al*, 2000
Rat, F344, 20 M mated to 40 untreated F	0, 50 100, 200	1 x/d, 5 d before mating	No effects ↓ fertility index and viable offspring	Chapin *et al*, 1985b
Mouse, JCL-ICR, 21 - 24 F	0 31.25 62.5, 125 250 500 1,000	1 x/d, g.d. 7 - 14	No effects Skeletal variations Skeletal malformations Gross anomalies and skeletal malformations, embryonic death Embryonic death Up to 100% embryonic death	Nagano *et al*, 1984

Table 4.1.3: Reproductive and developmental toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage (cont'd)	**(mg/kgbw)**			
Mouse, CD-1, 30 F	0 1,400	g.d. 7 - 14	No effects 100% embryonic death	Schuler *et al*, 1984
Mouse, CD-1, 9 - 12 F	0	g.d. 7 - 14, 7 - 9, 8 - 10, 9 - 11	No effects	Horton *et al*, 1985
	250		Exencephaly and paw lesions; ↓ foetal weight; embryo-lethality	
	250, 500	g.d. 7 - 8, 9 - 10, 10 - 11	As above	
	250, 500	g.d. 12 or 13	As above, but no embryo-lethality	
Mouse, CD-1, 9 - 11 F (16 controls)	0 100 175, 250, 300, 350, 400, 450	g.d. 11	No effects NOAEL Digit anomalies	Horton *et al*, 1985
Mouse, CD-1, 9 - 12 F	0 250	Several intervals between g.d. 7 - 14	No effects Gross malformations (exencephaly and paw lesions).	Horton *et al*, 1985

Table 4.1.3: Reproductive and developmental toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, gavage (cont'd)	**mmol/kgbw**	**mg/kgbw**			
Mouse, CD-1, 10 - 11 F	0 3.3, 4.6 3.3, 4.6 + ethanol (43.3 mmol/kgbw), 5 - 10 h later 3.3, 4.6 + 4-methylpyrazole (0.12, 1.2 mol/kgbw), concomitantly	(0 250, 350 250, 350 250, 350)	g.d.11	No effects Near 100% teratogenicity 75% teratogenicity 60% (0.12 methyl pyrazole), 0% (1.2 methyl pyrazole) teratogenicity	Sleet *et al*, 1988
	(mmol/kgbw)	**(mg/kgbw)**			
Mouse, CD-1, 16 F	0 4	(0 304)	1 x, g.d. 11	No effects No maternal toxicity; paw malformations (68.5% of foetuses in 87.5% of litters)	Hardin and Eisenmann, 1987
Mouse, CD-1, 2 - 3 F per time point	0, 100, 250 350		1 x, g.d. 11, killed 2, 6, 24 or 48 h later and embryos removed	No effects No maternal toxicity; forelimb-bud cytotoxicty as early as 2 h post EGME treatment, with maximum effect at 6 h	Greene *et al*, 1987

Table 4.1.3: Reproductive and developmental toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, gavage (cont'd)	**mg/kgbw**				
Mouse, CF-1, 16 - 18 F	0, 100		1 x, g.d. 11, embryos removed 6 or 24 h later	No effects	Greene *et al,* 1987
	175, 250, 300, 350, 400, 450, 500			Paw malformations induced in dose-dependent manner	
Mouse, CD-1, 4 - 5 F	0		1 x, g.d. 8	No effects	Ambroso *et al*, 1998
	250, 325			Embryos with expanded areas of cell death (apoptosis) in neural crests and medial region of the anterior neural tube. Effects more pronounced at 325 mg/kgbw	
	(mmol/kgbw)	**(mg/kgbw)**			
Monkey, *Macaca fascicularis*, 8 - 14 F (6 controls)	0 0.16	0 12	g.d. 20 - 45	No effects Embryonic death: 3/12 (25%), 3/10 (30%), 8/8 (100%), respectively; 1 embryo missing 1 digit on each forelimb	Scott *et al*, 1989
	0.32, 0.47	24, 36			
	(mg/culture vial)				
Drosophila melanogaster	0		Throughout development	No effects	Lynch and Toraason, 1996
	12.5, 22			↑ incidence of wing notches	
	12.5, 15, 18, 22, 26			↑ bent humeral bristles	

Table 4.1.3: Reproductive and developmental toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
	(mmol/l)	**(mg/l**			
Drosophila melanogaster	20 - 60	(1,500 - 4,600)	Throughout development	↓ M and F fertility. 1.1 - 8.7% of affected F produced offspring with phenotypic similarity. Exceedingly high doses.	Eisses, 1999
Oral, liquid diet	**(%)**	**(mg/kgbw)**			
Rat, SD, 9 - 12 F	0	(0	g.d. 7 - 18	No effects	Nelson *et al*, 1989
	0.006	16		NOAEL	
	0.012	31		Skeletal and cardiovascular malformation rate 4% among survivors	
	0.025	73		Malformation rate 40%; no toxicity to dams	
	0.05, 0.1, 0.25, 0.5	140, 198, 290, 620		100% resorption; dose-dependent toxicity to dams at 0.25 and 0.5 %	
Oral, drinking water	**(%)**	**(mg/kgbw)**			
Rat, SD, 4 F	0	(0	*Ad libitum*, 14 d	No effects	Berger *et al*, 2000
	0.15	150		↓ ovulation rate	
	0.25	250		↓ ovulation rate ; ↓ number of oocytes	
	0.3	300)		No ovulation	

Table 4.1.3: Reproductive and developmental toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, drinking water (cont'd)	**(%)**	**(mg/kgbw)**			
Rat, SD, 20 M, 20 F	0	(0	*Ad libitum*, 2-generation, 22 wk	No effects	Gulati, 1990a
	0.01	10		↓ number of viable offspring in F_1	
	0.03	30		↓ number of viable offspring in F_0	
	0.1	100)		Marginal fertility in F_0	
Rat, SD, 20 M, 20 F	0, 0.006, 0.012	F_0 M 9.6, F 15.3 F_1 M 8.1, F 14.2	*Ad libitum*, 2-generation, 20wk	No effects	Gulati, 1990b
	0.024			↓ viable litter in F_0 and F_1	
Mouse, CD-1, 30 M / 30 F (40/sex controls)	0	(0	*Ad libitum*, 2-generation, 18 wk	No effects	Gulati *et al*, 1988a
	0.03	60		↓ number of viable litter in F_1	
	0.1	200		No fertility in F_1	
	0.3	600)		No fertility in F_0	
Mouse, CD-1, 20 M, 20 F (30/sex controls)	0	(0	*Ad libitum*, 2-generation, 18 wk	No effects	Gulati *et al*, 1985a
	0.1	200		↓ number of viable offspring.	
	0.2	400		Marginal fertility in F_1	
	0.4	800)		Marginal fertility in F_0; no viable litters	

Table 4.1.3: Reproductive and developmental toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, drinking water (cont'd)	**(%)**	**(mg/kgbw)**			
Mouse, CD-1, 20 M, 20 F (30/sex controls)	0	(0	*Ad libitum*, 98 wk, continuous breeding	No effects	Gulati *et al*, 1985b
	0.5, 1.0, 2.0	100, 200, 400)		Fertility index 0%	
Mouse, C57BL/6, 30 M, 30 F	0	(0	18 wk, continuous breeding	No effects	Gulati *et al*, 1988b
	0.03	60		F_1 fertility index 50%	
	0.1	200		F_1 fertility index 0%	
	0.3	600)		F_0 fertility index 25%, F_1 ↓ viable offspring	
Mouse, C3H, 30 M, 30 F	0, 0.03	(0, 60	18 wk, continuous breeding	No effects	Gulati *et al*, 1988b; 1989
	0.1	200		F_2 fertility index 0%	
	0.3	600)		F_0 fertility index 0%	
Dermal, non-occluded	**(%)**	**(mg/kgbw)**			
Rat, Wistar, 10 F	0, 3	(0, 300 [a]	6 h/d, g.d. 6 - 17 (solutions at 10 ml/kgbw)	No effects	Wickramaratne, 1986
	10	1,000		↓ litter sizes	
	30	3,000		Foetal deaths	
	100	10,000)		Maternal death rate 100%	

Table 4.1.3: Reproductive and developmental toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Dermal, non-occluded (cont'd)	**(%)**	**(mg/kgbw)**			
Rat, SD, 8 - 10 F	NS	0, 250	g.d. 12	No effects	Feuston *et al,* 1990
		500		↓ maternal bw gain; ↑ external, visceral, or skeletal malformations	
		1,000		↓ maternal bw gain; ↑ external, visceral, or skeletal malformations	
		2,000	g.d. 10, 11, 12, 13 or 14	↑ resorptions (g.d. 10); ↓ foetal weight (g.d. 10 and 12)	
Dermal, occluded	**(%)**	**(mg/kgbw)**			
Rat, SD, 20 M mated to untreated F	NS	0	7 d (daily doses administered as 4 aliquots at spaced intervals during the day)	No effects	Feuston *et al,* 1989
		625		↓ testicular weight and sperm numbers	
		1,250, 2,500		↓ fertility for all doses	
Rat, Wistar, 10 F	0, 3	0, 29	6 h/d, g.d. 6 - 17 (solutions at 10 ml/kgbw)	No effects	Wickramaratne, 1986
	10	97		↓ litter sizes	
	30	290		Foetal deaths	
	100	965		Maternal death rate 100%	

Table 4.1.3: Reproductive and developmental toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Dermal, occluded (cont'd)					
	(ml/kgbw)	**(mg/kgbw)**			
Rat, Wistar, 45 - 50 F	0	(0	6 h/d, d 6 - 15	No effects	Hellwig, 1993
	0.05	50		↑ malformation; foeto- and embryotoxicity; no maternal effects	
	0.1	100		26.5% post-implantation losses, ↑ malformations; ↓ maternal weight gain	
	0.3	290		99.4% post-implantation losses, 5 malformed foetuses	
	0.5, 0.8, 1.0	480, 770, 970)		↓ maternal bw, no litters, 100% resorption	
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, Wistar, 20 F	0	(0	6 h/d, g.d. 6 - 17, then litters delivered	No effects	Doe *et al*, 1983
	100	320		↓ prolonged gestation and number of pups and live pups	
	300	950)		↓ maternal bw gain; 100% embryonic death	
Rat, SD, 11 - 38 F	0	(0	7 h/d, g.d. 7 - 15	No effects	Nelson *et al*, 1984a
	50, 100	160, 320		↑ malformation and foetotoxicity	
	200	630)		No litters	

Table 4.1.3: Reproductive and developmental toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Rat, SD, 15 - 18 F	0 25	(0 80)	7 h/d, g.d. 7 - 13 or 14 - 20	No effects Significant differences in avoidance conditioning of offspring from mothers exposed on g.d. 7 - 13; neurobehavioral deviations in offspring	Nelson *et al*, 1984b
Rat, F344, 30 - 31 F	0, 3, 10 50	(0, 9, 32 160)	g.d. 6 - 15	No effects Minor skeletal variations	Hanley *et al*, 1984a,b
Rat, SD, 20 M, 20 F (30 controls)	0, 30, 100	(0, 95, 316	6 h/d, 5 d/wk, 13 wk, then paired with unexposed F for breeding	No effects	Rao *et al*, 1983
	300	950)		↓ male fertility, partially reversed when bred 13 and 19 wk after exposure	
Mouse, CF-1, 20 - 30 F	0, 10	(0, 32	6 h/d, g.d. 6 - 15, killed g.d. 18	No effects	Hanley *et al*, 1984a,b
	50	160)		Slight foetotoxicity; minor skeletal variations	
Rabbit, NZW, 29 - 30 F	0, 3 10	(0, 9 32	6 h/d, g.d. 6 - 18	No effects Reduced ossification in relation to actual (not historical) control	Hanley *et al*, 1984a,b
	50	160)		↓ maternal bw gain; ↑ absolute liver weight; ↑ resorptions; ↓ foetal bw; ↑ skeletal and visceral malformations	

Table 4.1.3: Reproductive and developmental toxicity of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Intraperitoneal	**(mmol/kgbw)**	**(mg/kgbw)**			
Rat, Wistar, 11 F	0 5	(0 380)	1 x, g.d. 12	No effects 28% resorptions; 86/87 foetuses showed limb malformations; 11% ventral duplication of hand limb digits	Scott *et al*, 1987
Rat, Wistar, 15 F (20F controls)	0 (0.5 ml EGME /animal diluted at 5, 10, or 20%) 100, 200 400		1x/d, g.d. 8 - 20	No effects Dose-dependent ↑ in malformations ↑ resorptions	Saavedra *et al*, 1997
Subcutaneous	**(mg/kgbw)**				
Mouse, CD-1, 7 - 11 F	0 250 325		1 x, 2 h before g.d. 8	No effects ↑ incidence of open neural tubes. Typical cell death patterns in the embryonic neural folds ↑ incidence of open neural tubes. ↑ resorption rate	Terry *et al*, 1996

[a] Internal dose difficult to estimate; peak and AUC levels of accumulating MAA will determine effects

Table 4.1.4: Absorption (uptake), distribution, metabolism and elimination of EGME

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral	**(mmol/kgbw)**	**(mg/kgbw)**			
Rat, Wistar, 4 or 8 M	NS	100, 300	1, 2, 5, 20 d	↓ liver weight at high dose (20 d). ↑ cytosolic ADH, but no effect on other enzyme parameters	Kawamoto *et al*, 1990b
Rat, F344, 2 x 3 M	1, 8.7	76, 662	1 x	At 48 h, 80 - 90% of radioactivity eliminated, of which 80 - 90% as MAA in urine, 10 - 12% exhaled as CO_2	Miller *et al*, 1983b
Rat, NS, 6 M	NS	500	1 x	Within 24 h, 58.5% of radioactivity eliminated in urine, 70% within 48 h. Relative amounts: 73% MAA, 15% EGME, 8% unidentified	Foster *et al*, 1984
Rat, F344, 3 M	NS	25, 250	1 x,	Major urinary metabolites MEG, EGME-glucuronide and sulphate and MAA and N-(methoxyacetyl) glycine. MAA partially further converted to glycine and glucuronide conjugates as well as to 2-methoxyacetyl-CoA and subsequent metabolites	Jenkins-Sumner *et al*, 1996
Rat, SD, 10 M	10	760		At 24 h, 30-fold ↑ in the urinary creatine/creatinine ratio, 5-fold after 48 hours. Urinary excretion was 0.71 ± 0.042 mmol MAA/l for 24 h. Concomitant ethanol, *n*-propanol or *n*-butanol (10 or 30 mmol/kgbw oral) did not significantly modify this pattern	Morel *et al*, 1996
Mouse, $B6C3F_1$, NS M	NS	4	1 x	Highest levels of radioactivity in liver, urinary bladder, bone marrow, kidney and epididymis. Rapid distribution from stomach to tissues	Ahmed *et al*, 1994

Table 4.1.4: Absorption (uptake), distribution, metabolism and elimination of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral (cont'd)	**(mmol/kgbw)**	**(mg/kgbw)**			
Mouse, CD-1, 3 - 5 F	NS	25, 250	1 x, g.d. 11	Major metabolites MEG, EGME-glucuronide and sulphate and MAA. MAA partially further converted to glycine and glucuronide conjugates as well as to 2-methoxyacetyl-CoA and subsequent metabolites	Jenkins-Sumner *et al*, 1996
		250 + L-/D-serine (16.6/16.5 mmol/ kgbw) or acetate (43.3 mmol/ kgbw)		↓ CoA cascade by acetate	
Monkey, *Macaca fascicularis*, 3 - 6 F pregnant	NS	12, 24, 36	25 d	Blood sampling 2, 4, 7.5 and 25 h after each administration on d 1, 8, 15 and 22. Maximal MAA concentration already after 2 h, 50% ↓ after 24 h. ↑ MAA levels measured (µg/ml):	Scott *et al*, 1989

Dose (mg/kgbw)	Time (h)	Day 1	8	15	22
12	2	16	29	43	38
	24	12	16	16	20
24	2	41	73	86	85
	24	20.5	47	34.5	46
36	2	75	192	200	191.5
	24	25	71	98.5	107

Table 4.1.4: Absorption (uptake), distribution, metabolism and elimination of EGME (cont'd)

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Oral, drinking water	**(mg/l)**	**(mg/kgbw)**			
Rat, F344, 4 M	180, 450, 1,620	12.2, 26.2, 110.3	*Ad libitum*, 24 h	At 72 h; 40 - 50% of radioactivity recovered in urine. Relative amounts 34 - 35% MAA, 42 - 60% MEG, 6 - 8% EGME. ↑ relative amount of MAA with dose, ↓ MEG, 20 - 30% exhaled as CO_2 and 5% EGME	Medinsky *et al*, 1990
Dermal, *in vitro*	**(mg/kgbw)**				
Human abdominal skin	1 or 5 ml/1.8 cm^2		8 h, diffusion cell	Permeability constant 28.9x 10^4 cm/h. Penetration rate 2.82 $mg/cm^2/h$	Dugard *et al*, 1984
Dermal, non-occluded	**(mg/kgbw)**				
Rat, F344, 4 M	9, 26, 78 (disposition part), 35, 109, 321(metabolism part)		72 h	Concomitant sampling: 19.4 - 26.9% of radioactivity resorbed, 51 - 61% evaporated. Of resorbed activity 67 - 72% excreted in urine; 8.8 - 10% in faeces; 14 - 16% remained in carcass. In urine 23 - 46% MAA; 8.6 - 11% as MEG; 32 - 58% unidentified ↑ with dose	Sabourin *et al*, 1992b
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, SD, 3 F	1,600 1,600 + ethanol 20 mmol/kgbw i.p. prior to inhalation	5,060 5,060	2 h 2 h	86 µg EGME/ml blood 243 µg EGME/ml blood	Römer *et al*, 1985

Table 4.1.4: Absorption (uptake), distribution, metabolism and elimination of EGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Intraperitoneal	**(mg/kgbw)**			
Rat, SD, 3 M	250	1 x	At 24 h, 40.4% of radioactivity recovered in urine. At 48 h, 55.2% recovery; 50 - 60% identified as MAA, 18 - 25% as MAA glycine	Moss *et al*, 1985
	250 (pyrazole prior to injection)	1 x	Only 18% recovery after 48 h ↑ EGME plasma elimination half-time from 0.6 to 42.6 h ↓ radioactivity plasma elimination half-time from 19.7 to 51.0 h	
Intravenous	**(mg/kgbw)**			
Mouse, B6C3F_1, NS M	4	1 x	Highest levels of radioactivity in liver, urinary bladder, bone marrow, kidney and epididymis. Rapid distribution from blood to tissues	Ahmed *et al*, 1994

4.2 Substance profile: EGMEA

4.2.1 Identity

Name:	Ethylene glycol (mono) methyl ether acetate
IUPAC name:	2-Methoxyethyl acetate
CAS registry No.:	110-49-6
Molecular formula:	$C_5H_{10}O_3$
Structural formula:	$CH_3–O–CH_2–CH_2–O–CO–CH_3$
Molecular weight:	118.1
Other components:	No data

4.2.2 Physico-chemical data

Melting point:	–65°C
Boiling point:	145°C
Vapour pressure:	9.3 hPa
Solubility in water:	Complete
Relative density:	$D_4^{20} = 1.0074$

4.2.3 Conversion factors

1 ppm = 4.909 mg/m^3
1 mg/m^3 = 0.204 ppm

4.2.4 Toxicological data

4.2.4.1 Acute toxicity

Oral

Rat:	LD_{50} 3,930 to 4,300 mg/kgbw (Smyth *et al*, 1941; BASF, 1966).
Guinea pig:	LD_{50} 1,250 mg/kgbw (Kirk-Othmer, 1980 cited by IPCS, 1990).

Dermal

Rabbit:	LD_{50} 5,250 to 5,560 mg/kgbw (Kirk-Othmer, 1980 cited by IPCS, 1990).

Inhalation

Rat:	Survived 4-hour exposure to 1,500 ppm EGMEA (7,400 mg/m^3); death occurred after 8 hours (Carpenter *et al*, 1956).
Mouse:	Survived exposure to 4,500 ppm (22,100 mg/m^3) for 8 hours (Gross, 1938).
Guinea pig:	Survived exposure to 4,500 ppm for 1 hour, lethality after 3-hour exposure (Gross, 1938).
Rabbit:	Survived exposure to 4,500 ppm for 1 or 3 hours (Gross, 1938).
Cat:	Nearly saturated vapour concentrations (about 4,500 ppm) caused lethality due to bronchopneumonia (Gross, 1938).

4.2.4.2 Irritation and sensitisation

Skin irritation

EGMEA was not irritant or slightly irritant to rabbit skin (Carpenter and Smyth, 1946; BASF, 1966).

Eye irritation

Whereas no irritation of the rabbit eye due to EGMEA was observed by Carpenter and Smyth (1946), irritant effects on the eye were observed in another experiment (BASF, 1966).

Sensitisation

No data are available.

4.2.4.3 Repeated-dose toxicity (Table 4.2.1)

Subacute toxicity

Male ICL-ICR mice received oral dose levels of 62.5 to 4,000 mg EGMEA/kgbw/d for 5 weeks. Dose-dependent testicular atrophy and leukopenia were noted (Nagano *et al*, 1979, 1984).

Repeated whole-body inhalation (8 h/d) of 500 or 1,000 ppm EGMEA (2,500 or 4,900 mg/m^3) for up to 6 days was lethal for cats and rabbits, but guinea pigs and mice survived, all showing kidney damage. Injuries of typical target organs (testes, bone marrow, and thymus) were not reported in these early studies (Gross, 1938).

Subchronic toxicity

No data are available.

4.2.4.4 Genotoxicity (Table 4.2.2)

Only spurious data are available (see below). However, due to the rapid cleavage of EGMEA to EGME the database and conclusions for the latter should serve as a surrogate (Section 4.1.4.4).

In cytogenetic assays with EGME in the fruit fly *Drosophila melanogaster*, aneuploidy and a loss of X-chromosome were present after feeding young ZESTE stock adults. EGME did not show inhibition in the *D. melanogaster* or mouse brain microtubular assembly tests (Sehgal and Osgood, 1990; Osgood *et al*, 1991).

Increased SCE rates and increased chromosome aberrations were reported in CHO cells after metabolic activation (Loveday *et al*, 1990) and in *Saccharomyces cerevisiae* (without S9 mix) (Whittacker *et al*, 1989). Aneuploidy occurred in diploid *Saccharomyces cerevisiae*, but no gene recombination or gene mutation (Zimmermann *et al*, 1985).

Single i.p. injection of 1,333 mg EGMEA/kgbw to Chinese hamsters did not induce an increase in the frequency of micronucleated PCE of the bone marrow at 12, 24, 48 or 72 hours after administration (Basler, 1986).

The limited relevance of some of the positive tests and the comprehensive database on EGME genotoxicity suggest that EGMEA has no genotoxic potential *in vivo* in mammals.

4.2.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.2.4.6 Reproductive and developmental toxicity

Pregnant CD-1 mice (number and sex/group not stated) received 1,225 mg EGMEA/kgbw/d by oral gavage from day 6 to 13 of gestation. Following evaluation by means of the Chernoff-Kavlock protocol, dams appeared to be unaffected. In 31 litters no viable foetus was born (Hardin *et al*, 1987; NTIS, 1984).

By analogy to EGME, EGMEA should be considered as a developmental toxicant.

4.2.4.7 Kinetics and metabolism

The half-life time for hydrolysis of EGMEA in rat plasma (37°C) *in vitro* was determined to be 11.75 minutes, with subsequent formation of MAA (Hoffmann and Jäckh, 1985).

The primary urinary metabolite of EGMEA in workers printing silk screens was MAA. Post-shift (14 - 16 hours after exposure) excretion of 3 mmol MAA/mol creatinine corresponded to an inhalation exposure to 0.5 cm^3 EGMEA/m^3 (0.5 ppm, 2.5 mg/m^3). This should be considered as the biological action level (Laitinen, 1998).

4.2.4.8 Neurotoxicity

No data are available.

Since EGME shows distinct CNS-toxic properties and EGMEA is rapidly cleaved to EGME, this effect may also be assumed for EGMEA (Section 4.1.4.8).

4.2.4.9 Immunotoxicity

In an experiment designed to compare the potential immunosuppressive activity of various glycol ethers EGMEA was administered orally to male F344 rats at dosages ranging from 50 to 400 mg/kgbw on 2 consecutive days. EGMEA (together with EGME and MAA) suppressed PFC response to TNP-LPS (Smialowicz *et al*, 1992b).

4.2.5 Human effects data

Dermal and inhalation exposure to EGMEA of a female worker involved in cleaning laboratory glassware and other equipment (using 1 - 2 l EGMEA/d, concentration levels not stated) was reported as the suspected cause of hypospadia in 2 male offspring (Bolt and Golka, 1990).

Table 4.2.1: Systemic toxicity of EGMEA in ICL-ICR mice dosed by oral gavage

Number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
5 M	0	1 x/d, 5 d/wk, 5 wk	No effects	Nagano *et al*, 1979, 1984
	62.5, 125, 250, 1,000, 2,000		Dose-dependent testicular atrophy, leukopenia	

Table 4.2.2: Genotoxicity of EGMEA

Endpoint / Species	Strain or type / Target	Dose or concentration	Result	Remark	Reference
In vitro					
Gene mutation					
Salmonella typhimurium	TA1535, TA100, TA1537, TA98	50 - 5,000 µg/plate	–ve	+/– S9	Bootman and May, 1985
Saccharomyces cerevisiae		2.91 - 5.66%	–ve	+/– S9	Zimmermann *et al*, 1990
Sister chromatid exchange					
CHO cells		151 - 1,530 µg/plate	–ve	– S9	Loveday *et al*, 1990
		1,000 - 3,000 µg/plate	+ve	+ S9	
Chromosome aberration					
CHO cells		501 - 5,010 µg/plate	–ve	– S9	Loveday *et al*, 1990
		998 - 5,010 µg/plate	+ve	+ S9	
Saccharomyces cerevisiae	Mitotic chromosome loss	12.4 - 83.3 mg/ml	+ve (weak)	– S9	Whittacker *et al*, 1990

Table 4.2.2: Genotoxicity of EGMEA (cont'd)

Endpoint / Species	Strain or type / Target	Dose or concentration	Result	Remark	Reference
Aneuploidy					
Saccharomyces cerevisiae	D61.M	2.91 - 5.66%	+ve	+/– S9	Zimmermann *et al*, 1990
Recombination					
Saccharomyces cerevisiae	D61.M	2.91 - 5.66%	–ve	+/– S9	Zimmermann *et al*, 1990
In vivo					
Micronucleus frequency		**(mg/kgbw)**			
Hamster, 10 M and F	Chinese Bone marrow	1 x 1,333, s.c. injection	–ve	Sampling 12, 24, 48 and 72 h	Basler, 1986
Aneuploidy		**(ppm)**			
Drosophila melanogaster	Flies Flies Larvae	4,200, 42,000, feeding 3,200, 32,000 500, 5,000	+ve –ve –ve	ZESTE gene FIX test (microtubular assembly) ZESTE gene	Sehgal and Osgood, 1990

4.3 Substance profile: EGDME

4.3.1 Identity

Name:	Ethylene glycol dimethyl ether
IUPAC name:	1,2-Dimethoxyethane
CAS registry No.:	110-71-4
Molecular formula:	$C_4H_{10}O_2$
Structural formula:	$CH_3–O–CH_2–CH_2–O–CH_3$
Molecular weight:	90.1
Other components:	No data

4.3.2 Physico-chemical properties

Melting point:	–58°C to –71°C
Boiling point:	84°C
Vapour pressure:	64 hPa
Solubility in water:	Soluble
Relative density:	$D_4^{20} = 0.8501$

4.3.3 Conversion factors

1 ppm = 3.745 mg/m^3
1 mg/m^3 = 0.267 ppm

4.3.4 Toxicological data

4.3.4.1 Acute toxicity

Oral

Mouse: LD_{50} 2,525 mg/kgbw (Plasterer *et al*, 1985).

Dermal

No data are available.

Inhalation

No data are available.

4.3.4.2 Irritation and sensitisation

Skin irritation

No data are available.

Eye irritation

No data are available.

Skin sensitisation

No data are available.

4.3.4.3 Repeated-dose toxicity (Table 4.3.1)

Subacute toxicity

A gavage study in ICL-ICR mice dosed with 250, 500 or 1,000 mg EGDME/kgbw/d for 5 weeks reported dose-dependent decreases in testicular weight and WBC (Nagano *et al*, 1984).

In CFE rats whole body-exposed via inhalation to concentrations of up to 8,000 ppm EGDME, dose-dependent behavioural changes, mortality and haemorrhages of the lungs and gastro-intestinal tract were seen (Goldberg *et al*, 1964).

Subchronic toxicity

No data are available.

4.3.4.4 Genotoxicity (Table 4.3.2)

EGDME was not mutagenic in *Salmonella typhimurium* TA100, the only strain tested, when tested in a standard plate incorporation (Ames) assay and a pre-incubation assay, both in the absence or presence of a metabolic activation system, up to 500 μg/plate (Arimoto *et al*, 1982).

4.3.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.3.4.6 Reproductive and developmental toxicity (Table 4.3.3)

SD rats were dosed by gavage with 0, 30, 60, 120, 250, 500 or 1,000 mg EGDME/kgbw/d on day 8 to 18 of gestation. At the highest dose, 4 out of 6 dams died. Doses of 120 mg/kgbw and above were associated with a near 100% resorption rate. A dose of 60 mg/kgbw led to an increased resorption rate of 16% versus 3% in untreated controls, which mainly corresponded with late foeto-lethality (g.d. 16 - 18). Furthermore, there were abnormalities (oedema) in the litters produced. A dose of 30 mg/kgbw still showed some treatment-related oedema and small reductions in live births and survival rate (Leonhardt *et al*, 1991).

EGDME was teratogenic in ICL-JCR mice following oral doses from 250 to 490 mg EGDME/kgbw on day 7 to 10 of gestation, which caused no apparent maternal toxicity (Uemura, 1980; Nagano *et al*, 1984).

Crl:CD-1 mice dosed with a variety of structurally related glycol ethers, including EGDME, showed characteristic paw malformations in the offspring similar to those induced by EGME and DEGDME, after a single oral dose of 361 mg EGDME/kgbw/d on day 11 of gestation (Hardin and Eisenmann, 1987).

4.3.4.7 Kinetics and metabolism

Skin permeation was calculated using the Franz cell method with human skin. EGDME was tested in pure form and with 70% acetone. In pure form, the lag time was 39 minutes, flux at steady state 3.434 mg/cm^2/h, and permeation rate 3.396 cm/h x 10^{-3}. In mixture with acetone the respective values were 35 minutes, 0.837 mg/cm^2/h and 1.089 cm/h x 10^{-3} (Larese *et al*, 1999).

No other data are available.

4.3.4.8 Neurotoxicity

Dose-dependent and progressive behavioural changes were seen in CFE rats. The effects were reversible (Goldberg *et al*, 1964) (Table 4.3.1).

4.3.4.9 Immunological data

No data are available.

4.3.5 Human effects data

No data are available.

Table 4.3.1: Systemic toxicity of EGDME

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Oral, gavage	**(mg/kgbw/d)**				
Mouse, ICL-JCR, 5 M	0 250, 500, 1,000		1 x/d, 5 d/wk, 5 wk	No effects Dose-dependent reductions in testicular weight and WBC	Nagano *et al*, 1984
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, CFE, 8 - 10 F	0 1,000, 2,000 4,000, 8,000	(0) (3,750, 7,500) (15,000, 30,000)	4 h/d, 5 d/wk, 10 d	No effects Dose-dependent and progressive inhibition of conditioned avoidance response. ↓ growth in all treatment groups Deaths	Goldberg *et al*, 1964

Table 4.3.2: Genotoxicity of EGDME in vitro

Endpoint / Species	**Strain**	**Concentration (µg/plate)**	**Result**	**Remark**	**Reference**
Gene mutation					
Salmonella typhimurium	TA100	0, 50, 200, 500	**–ve**	+/– S9. inconsistent reporting of dose levels and results	Arimoto *et al*, 1982
Salmonella typhimurium	TA100	0, 50, 100, 500	**–ve**	+/– S9, pre-incubation.	Arimoto *et al*, 1982

Table 4.3.3: Reproductive and developmental toxicity of EGDME by oral gavage

Species, strain, number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
Rat, SD, 6 - 28 F	0	1 x/d, g.d. 8-18	No effects	Leonhardt *et al*, 1991
	30, 60		Foetotoxicity: retarded ossification, ↓ pup bw	
	120, 250, 500		Maternal toxicity, 100% foeto-lethality.	
	1,000		Maternal mortality	
Mouse, ICL-JCR, 23 - 28 F	0	1 x/d, g.d. 7-10	No effects	Uemura, 1980; Nagano *et al*, 1984
	250, 350, 490		No maternal toxicity. Dose-related foetal mortality. Dose-related external deformations in offspring. Developmental NOAEL not established	
Mouse, CD-1, 20 F	0, 361	1 x/d, g.d. 11-18	No maternal toxicity. ↓ foetal bw, paw malformations	Hardin and Eisenmann, 1987

4.4 Substance profile: DEGME

4.4.1 Identity

Name:	Diethylene glycol (mono) methyl ether
IUPAC name:	2,(2-Methoxyethoxy)ethanol
CAS registry No.:	111-77-3
Molecular formula:	$C_5H_{12}O_3$
Structural formula:	$CH_3–(O–CH_2–CH_2)_2–OH$
Molecular weight:	120.1
Other components:	Monoethylene glycol, EGME, TEGME

4.4.2 Physico-chemical properties

Melting point:	–85°C
Boiling point:	190 - 196°C
Vapour pressure:	0.24 hPa at 25°C
Solubility in water:	Miscible
Relative density:	$D_4^{20} = 1.021$

4.4.3 Conversion factors

1 ppm = 4.993 mg/m^3
1 mg/m^3 = 0.200 ppm

4.4.4 Toxicological data

4.4.4.1 Acute toxicity

Oral

Rat:	LD_{50} 9,210 mg/kgbw when administered as 50% aqueous solution (Smyth *et al*, 1941).
Rabbit:	LD_{50} 7,200 mg/kgbw (Gingell *et al*, 1994).
Guinea pig:	LD_{50} 4,200 mg/kgbw when administered as 50% aqueous solution (Smyth *et al*, 1941).

Dermal

Rat: LD_{50} 20 ml/kgbw (20,400 mg/kgbw) (Browning, 1965).

Inhalation

Rat: LC_{50} greater than saturated atmosphere (Gingell *et al*, 1994).

4.4.4.2 Irritation and sensitisation

Skin irritation

DEGME was not appreciably irritant to rabbit skin (Wolfe, 1954).

Eye irritation

DEGME caused transitory irritation of conjunctival membranes (Carpenter and Smyth, 1946; Wolfe, 1954).

Sensitisation

No data are available.

4.4.4.3 Repeated-dose toxicity (Table 4.4.1)

Subacute toxicity

No mortalities were seen in rats receiving DEGME by gavage at doses from 190 up to 1,830 mg/kgbw/d for 30 days. Unspecified microscopic changes were reported in the liver, kidneys and gastro-intestinal tract at all dose levels (Smyth and Carpenter, 1948).

DEGME administered by gavage to Wistar rats at doses of 0, 500, 1,000 or 2,000 mg/kgbw/d showed at the highest dose some reduction in thymus, liver and testicular weight. Furthermore, at this dose level, an increase in microsomal liver protein was seen. According to the authors and the data presented, the NOAEL was presumably 1,000, but at least 500 mg/kgbw (Kawamoto *et al*, 1990a).

In a limited drinking water study in ICL-ICR mice, 0.2% DEGME (400 mg/kgbw/d) administered for 25 days was without testicular or other effects (Nagano *et al*, 1984).

Subchronic toxicity

No adverse treatment-related effects were seen in F344 rats exposed for 13 weeks to up to 216 ppm DEGME (NOAEL), the maximum achievable vapour concentration (Miller *et al*, 1985a).

Hartley guinea pigs treated dermally with DEGME at doses of 40, 200 or 1,000 mg/kgbw/d for 13 weeks showed some liver affection (fatty changes) at all dose levels. At 200 and 1,000 mg/kgbw/d lower spleen weights were noted. The fatty changes were considered to be of minimal significance (Hobson *et al*, 1986a).

On balance the data show that DEGME is very different from EGME and also DEGDME.

4.4.4.4 Genotoxicity (Table 4.4.2)

In vitro

DEGME was not mutagenic in the standard Ames test and the pre-incubation test using *Salmonella typhimurium* (BASF, 1989). There was no indication of clastogenic effects in Chinese hamster V79 cells at concentrations of up to 1,200 mg/l (Hoechst, 1996 cited by ECB, 2000).

4.4.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.4.4.6 Reproductive and developmental toxicity (Table 4.4.3)

DEGME was foetotoxic in 3 different experiments with pregnant SD rats dosed orally at 720 mg/kgbw/d and above on day 7 to 16 of gestation. At 2,165 mg/kgbw/d, a dose without clear maternal toxicity, there was an increased incidence of malformed ribs and malformations of the cardiovascular system, together with reduced foetal body weights and a reduced implantation frequency (Hardin *et al*, 1986).

DEGME was foetotoxic in Wistar rats following oral doses of 600 mg/kgbw/d, and teratogenic with slight maternal toxicity at 1,800 mg/kgbw/d. The most sensitive organ to DEGME exposure was the thymus in both adults and foetuses (Yamano *et al*, 1993).

By contrast, s.c. injection of DEGME in pregnant Wistar rats caused no statistically significant treatment-related malformations at doses up to 1,000 µl/kgbw/d (Doe, 1984b), which is close to 1,000 mg/kgbw/d.

Pregnant NZW rabbits treated dermally with DEGME showed a selective foetal effect (delayed ossification) under treatment conditions causing no maternal toxicity (Scortichini *et al*, 1986).

4.4.4.7 Kinetics and metabolism (Table 4.4.4)

No studies detailing the metabolic fate of DEGME in animals were located.

Studies of the hepatic alcohol/ADH and MFO systems in Wistar rats pre-treated with DEGME or EGME indicated different substrate responses. DEGME induced the MFO system, whereas EGME increased the activity of the ADH system (Kawamoto *et al*, 1990b). Further work by Kawamoto *et al* (1991) failed to show induction of rat hepatic microsomal γ-glutamyl transpeptidase following single or repeated oral doses of DEGME.

Comparative skin penetration studies showed that DEGME crosses human skin *in vitro* at a rate of 0.206 mg/cm^2/h, i.e. 13-fold less than that of EGME (Dugard *et al*, 1984).

4.4.4.8 Neurotoxicity

No data are available.

4.4.4.9 Immunotoxicity

In an experiment designed to compare the potential immunosuppressive activity of various glycol ethers, DEGME was administered orally to male F344 rats at dosages ranging from 50 to 400 mg/kgbw on 2 consecutive days. DEGME (in contrast with EGME, EGMEA and MAA) did not suppress PFC response to TNP-LPS (Smialowicz *et al*, 1992b).

4.4.5 Human effects data

(Penetration of DEGME through excised human skin *in vitro* is described in Section 3.6).

DEGME, when applied at a concentration of 20% in petrolatum in a closed patch test, caused no irritation nor sensitisation in 25 human subjects (Opdyke, 1974).

Table 4.4.1: Systemic toxicity of DEGME

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**				
Rat, 5 NS	0 190 740 1440, 1,830		1 x/d, 30 d	No effects At all dose levels: unspecified micro-pathological changes to liver, kidneys and gastro-intestinal tract ↓ palatability ↓ growth. No mortality	Smyth and Carpenter, 1948
Rat, Wistar, 4 M, control 8 M	0, 500, 1,000, 2,000		1, 2, 5, 20 d	Time and dose-dependent effects on relative organ weights (thymus, testes, spleen, liver and kidney). NOAEL 1,000 or 500 mg/kgbw	Kawamoto *et al*, 1990a
Oral, drinking water	**(%)**	**(mg/kgbw)**			
Mouse, ICL-ICR, 5 M	0, 0.2	(0, 400)	*Ad libitum*, 25 d	No adverse treatment-related effects. Study limited to examination of bw, testis weight, seminal residue and coagulating gland weight, and mean WBC count.	Nagano *et al*, 1984
Dermal, occluded	**(mg/kgbw)**				
Guinea pig, Hartley, 6 M, control 7 M	0 40 200, 1,000		6 h/d, 5d/wk, 13 wk	No effects Undiluted application. No treatment-related effects on bw at any dose. Fatty change in liver in all treatment groups. ↓ spleen weight. NOAEL not established	Hobson *et al*, 1986a
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, F344, 10 M, 10 F	0, 30, 100, 216	(0, 150, 500, 1,080)	6 h/d, 5 d/wk, 13 wk	No effects. NOAEL 216 ppm	Miller *et al*, 1985a

Table 4.4.2: Genotoxicity of DEGME in vitro

Endpoint / Organism	Strain or type / Target	Concentration		Result	Remark	Reference
Gene mutation		**(µg/plate)**				
Salmonella typhimurium	TA1535, TA100, TA1537, TA98	5,000		**–ve**	+/– S9	BASF, 1989
Salmonella typhimurium	TA1535, TA100, TA1537, TA98	5,000		**–ve**	+/– S9 pre-incubation	BASF, 1989
Chromosome aberration		**(mmol/l)**	**(mg/l)**			
Chinese hamster V79 cells		10	(1,200)	**–ve**	+/– S9	Hoechst, 1997

Table 4.4.3: Reproductive and developmental toxicity of DEGME

Route / Species, strain, number and sex/group	Dose (mg/kgbw/d)	Exposure regime	Result	Reference
Oral, gavage				
Rat, SD, 21 - 23 F	0	1x/d, g.d. 7 - 16	No effects	Hardin *et al*, 1986
	720		Slight foetotoxicity	
	2,165		Malformations of ribs and cardiovascular system	
Rat, SD, CR:CD(SD)BR	0, 1,000	1 x/d, g.d. 7 - 16	No effects. Range-finding study	Hardin *et al*, 1986
9 F	1,495, 2,235		Skeletal ossification impaired, also at higher doses	
	3,345		Minimal maternal toxicity	
	5,175		Maternal mortality	

Table 4.4.3: Reproductive and developmental toxicity of DEGME (cont'd)

Route / Species, strain, number and sex/group	Dose (mg/kgbw/d)	Exposure regime	Result	Reference
Oral, gavage (cont'd)				
Rat, SD, CR:CD(SD)BR 12 - 13 F	0 720 2,165	1 x/d, g.d. 7 - 16	No effects. Teratology study, dose levels based on prior range-finding Developmental LOAEL for rib and carcio-vascular malformations; maternal NOAEL ↓ maternal bw, ↓ foetal bw and litter size; skeletal malformations: rudimentary cervical ribs and bilateral wavy ribs	Hardin *et al*, 1986
Rat, Wistar, 22 F	0 200 600, 1,800	1 x/d, g.d. 7 - 17	No effects NOAEL Foetotoxicity and teratogenicity	Yamano *et al*, 1993
Subcutaneous				
Rat, Wistar (Alpk/Ap), 14 - 15 F	0, 250, 500 1,000	1 x/d, g.d. 6 - 20	No clear treatment-related findings ↓ pup survival	Doe, 1984b
Dermal, occluded				
Rabbit, NZW, 25 F	0 50 250 750	g.d. 6 - 18	No effects NOAEL Delayed ossification ↑ resorptions	Scortichini *et al*, 1986

Table 4.4.4: Absorption (uptake), distribution, metabolism and elimination of DEGME

Route / Species, strain, number and sex/group	**Dose**	**Exposure regime**	**Result**	**Reference**
Oral	**(mg/kgbw)**			
Rat, Wistar, 4 or 8 M	0, 500 1,000, 2,000	1, 2, 5 or 20 d	No effects ↓ liver weight at high dose (20 d). ↑ hepatic microsomal protein content and induction of CYP. No effect on cytosolic ADH and cytochrome C reductase	Kawamoto *et al*, 1990b
Dermal, *in vitro*				
Human abdominal skin	1 or 5 ml/1.8 cm^2	8 h, diffusion cell	Permeability constant 2.06 cm/h x 10^4. Penetration rate 0.206 $mg/cm^2/h$	Dugard *et al*, 1984

4.5 Substance profile: DEGDME

4.5.1 Identity

Name:	Diethylene glycol dimethyl ether
IUPAC name:	Bis(2-methoxyethyl)ether
CAS registry No.:	111-96-6
Molecular formula:	$C_6H_{14}O_3$
Structural formula:	$CH_3–(O–CH_2–CH_2)_2–O–CH_3$
Molecular weight:	134.2
Other components:	No data

4.5.2 Physico-chemical properties

Melting point:	–64°C
Boiling point:	162°C
Vapour pressure:	2.27 hPa
Solubility in water:	Miscible
Relative density:	$D_4^{20} = 0.9434$

4.5.3 Conversion factors

1 ppm = 5.579 mg/m^3
1 mg/m^3 = 0.179 ppm

4.5.4 Toxicological data

4.5.4.1 Acute toxicity

Oral

Rat:	LD_{50} 5,000 mg/kgbw (US-EPA, 1982).
Mouse:	LD_{50} 2,980 mg/kgbw (Plasterer *et al*, 1985).

Dermal

No data are available.

Inhalation

Rat: 4-h LC_{50} 4,300 ppm (24,000 mg/m^3) (males) (Du Pont, 1987).

4.5.4.2 Irritation and sensitisation

Skin irritation

DEGDME was not irritant to rabbit skin (US-EPA, 1982).

Eye irritation

DEGDME was not irritant to the rabbit eye (US-EPA, 1982).

Sensitisation

DEGDME was not a sensitiser when tested by the Buehler method or alternative footpad method (Shepard, 1993a,b).

4.5.4.3 Repeated-dose toxicity (Table 4.5.1)

Subacute toxicity

Repeated inhalation of 600 ppm DEGDME by Alderley Park rats caused irregular body-weight gain, atrophy of the thymus and congested adrenals. The NOAEL was 200 ppm (Gage, 1970).

Repeated inhalation exposure of SD rats to concentrations ranging from 98 to 1,100 ppm DEGDME for 2 weeks caused testicular atrophy (DuPont 1988a,b; Lee *et al*, 1989; Valentine *et al*, 1998). Oral dosing of SD rats with 684 mg DEGDME/kgbw/d for 20 days led to primary and secondary spermatocyte degeneration, spermatidic giant cells, reduced testes weight, as well as reduced testicular LDH activity (Cheever *et al*, 1989a).

The similarity of toxic effects seen and knowledge of the metabolic fate of DEGDME indicate that the formation of MAA mediates the toxic effects observed.

Subchronic toxicity

Groups of 20 male and 20 female SD rats were exposed via inhalation (nose-only) for 2 weeks (5 days/week, 6 hours/day) to 0, 110, 370 or 1,100 ppm. 300 ppm EGME were included as positive control. A 14-day recovery period was included for both sexes and 42 and 84 days of recovery for males only. Atrophy of the germinal epithelium was detected in the testes, the seminal vesicles and epididymides; prostate weights were about 30% lower in the top dose after 2 weeks. By day 84, there was partial or complete recovery. Furthermore, there was toxicity to the bone marrow with typical effects seen in RBC and WBC, spleen and thymus. The testicular effects were somewhat less pronounced than with those obtained with EGME. The NOAEL was 370 ppm in females, NOAEL < 110 ppm in males (Lee *et al*, 1989; Valentine *et al*, 1998).

4.5.4.4 Genotoxicity (Table 4.5.2)

In vitro

DEGDME was not mutagenic in the standard *Salmonella typhimurium* assay (Ames test) and it did not induce unscheduled DNA synthesis in human embryonic fibroblasts; both tests were conducted with and without addition of a metabolic activation system (McGregor *et al*, 1983).

In vivo

There was a small increase in the number of chromosome aberrations in the bone marrow of CD rats following inhalation of DEGDME at 250 ppm for 1 or 5 days, but not at 1,000 ppm (McGregor *et al*, 1983).

When tested on germ cells of $B6C3F_1$ mice exposed to 1,000 ppm DEGDME for 4 days, a statistically significant increase in sperm abnormalities (all categories) was seen, particularly those with amorphous heads. Inhalation of 250 ppm was without effects (McGregor *et al*, 1983). Sperm abnormalities are not a defined genotoxity endpoint since they can be induced by non-specific testicular damage. These results, therefore, must be interpreted with caution.

In CD rats exposed by inhalation to 0, 250 or 1,000 ppm DEGDME for 5 days, pregnancies were reduced at week 4 to 9 after exposure to 1,000 ppm, while total implantations were significantly reduced in weeks 6 and 7. Since the high proportion of early deaths could be partly explained in terms of low implantation frequency, it was not possible to demonstrate conclusively that a dominant lethal effect occurred at 1,000 ppm. Control and 250 ppm groups were normal with the exception of a statistically significant decrease in early death frequency at week 7 in 250 ppm group (McGregor *et al*, 1983).

Fruit flies (*Drosophila melanogaster*) were exposed by inhalation to 0 or 250 ppm DEGDME for 2.75 hours (maximum tolerated concentration, prior established). Two independent tests were performed using different stocks of flies. Following the same mating and breeding protocol, 6 recessive lethal mutations were seen in the F_2 generation in the first test; in the other test, there was 1 recessive lethal. The negative controls showed 3 and 2 recessive lethals, respectively, in the F_2 generation. In the F_3 generation, there were 6 recessive lethals in the first experiment and 0 in the second, both at 0 and 250 ppm (McGregor *et al*, 1983). The test data are difficult to interpret.

Despite some ambiguous results (which can be essentially explained by the marked effects on fertility) the weight of evidence suggests that DEGDME has no significant genotoxic potential.

4.5.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.5.4.6 Reproductive and developmental toxicity (Table 4.5.3)

When tested for genotoxicity (Section 4.5.4.4: McGregor *et al*, 1983) DEGDME revealed a strong anti-fertility effect.

Developmental toxicity was noted in CD-1 mice after oral administration of 3,000 mg DEGDME/kgbw/d. From 37 dams no litters were produced (Schuler *et al*, 1984; Hardin *et al*, 1987). After oral treatment of CD-1 mice with dose levels 0, 62.5, 125, 250 or 500 mg/kgbw/d from day 6 to 15 of gestation, 62.5 mg/kgbw was the developmental NOAEL. At 250 and 500 mg/kgbw various malformations and an increase in post-implantation loss were observed. At 125 mg/kgbw foetal body weight was reduced (Price *et al*, 1987).

Crl:CD-1 mice dosed with a variety of structurally related glycol ethers, including DEGDME, showed similar characteristic paw malformations in the offspring after a single oral dose of 537 mg DEGDME/kgbw/d on day 11 of gestation (Hardin and Eisenmann, 1987).

In NZW rabbits, dose-related developmental toxicity was seen at 50, 100 and 175 mg/kgbw/d, with a NOAEL of 25 mg/kgbw/d (Schwetz *et al*, 1992).

Pregnant Crl:CDBR rats were exposed by inhalation to 0, 25, 100 and 400 ppm DEGDME on day 7 to 16 of gestation. A concentration of 25 ppm EGME served as positive control. At 400 ppm DEGDME, the dams exhibited reduced food consumption and there were no live foetuses. Maternal liver weights were increased at 100 and 400 ppm. At 100 ppm, foetal weight

was decreased. An increased incidence of structural malformations (essentially brain and skeletal) was observed at 100 ppm and in the rats exposed to EGME. Variations (primarily delayed skeletal ossifications and rudimentary ribs) were increased at 25 and 100 ppm. A foetal NOAEL could not be established (Driscoll *et al*, 1998).

The proximate reproductive and developmental toxicant in rodents is MAA (Cheever *et al*, 1988; Daniel *et al*, 1986, 1991).

4.5.4.7 Kinetics and metabolism (Table 4.5.4)

Skin permeation was calculated using the Franz cell method with human skin. DEGDME was tested in pure form and with 70 % acetone. In pure form the lag time was 36 minutes, flux at steady state was 0.952 mg/cm^2/h, and permeation 1.016 cm/h x 10^{-3}. In mixture with acetone, the respective values were 49 minutes, 0.647 mg/cm^2/h and 1.141 cm/h x 10^{-3} (Larese *et al*, 1999).

Considerable metabolism data exist for DEGDME in SD rats (Cheever *et al*, 1988) and CD-1 mice (Daniel *et al*, 1991). The principal pathway of biotransformation of DEGDME involves O-demethylation with subsequent oxidation to form 2-methoxy-ethoxyacetic acid (MEAA). In addition, cleavage (O-dealkylation) of the central ether bond resulted in formation of 2-methoxyethanol, which was subsequently oxidised to the toxic metabolite, MAA. The major route of elimination was through the urine.

Repeated doses of DEGDME or of phenobarbitone, an inducer of CYP, administered to SD rats increased the rate of cleavage of the central ether bond resulting in an increased formation of MAA (Cheever *et al*, 1989b). Thus in conditions where MFO induction may occur, the toxic effects of MAA may be more apparent.

4.5.4.8 Neurotoxicity

No data are available.

4.5.4.9 Immunotoxicity

No data are available.

4.5.5 Human effects data

DEGDME had a low order of haemolytic activity in human blood *in vitro* (Mottu *et al*, 2001).

A field study and a case-control study were carried out in order to identify congenital malformations and mental retardations in 44 children of ex-workers of the same factory in a Mexican city. The workers had been in direct unprotected contact with MEG and DEGDME. The children exhibited signs and symptoms of peculiar faces, mental retardations as well as musculo-skeletal and sensorial abnormalities (Saavedra-Ontiveros *et al*, 1996).

Table 4.5.1: Systemic toxicity of DEGDME in rats

Route / Strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**				
Crt:CD(SD)BR, 5 M	0		1 x/d, 20 d; animals killed 1 x/2 d during dosing, and 1 x/wk to 8 wk post-exposure	No effects	Cheever *et al,* 1989a
	684			Primary and secondary spermatocyte degeneration and spermatidic giant cells observed after 6 - 8 treatments. ↓ testes/bw ratio by d 10 of treatment until 8 wk post-exposure. Decreased activity of testicular lactate dehydrogenase (LDH)-X, a pachytene spermatocyte marker enzyme, by d 18 of treatment	
Inhalation	**(ppm)**	**(mg/m^3)**			
Alderley Park, 4 M, 4 F	0	(0	6 h/d, 15 d	No effects	Gage, 1970
	200	1,100		NOAEL	
	600	3,300)		Irregular ↑ bw gain; blood and urine tests normal; autopsy: atrophied thymus, congested adrenal	
Crl:CDBR, 10 M	0, 3.1, 9.9	(0, 17.3, 55	6 h/d, 5 d/wk, 2 wk, nose only	No effects. 10 rats necropsied immediately, 10 rats maintained for 2 wk recovery	DuPont, 1988a,b
	30	170		NOAEL	
	98	550)		↓ bw. Testicular atrophy both immediately post exposure and after 2 wk	

Table 4.5.1: Systemic toxicity of DEGDME in rats (cont'd)

Route / Strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Crl:CDBR, 20 M	0	(0	6 h/d, 5 d/wk, 2 wk; animals necropsied at 2 wk and 2, 6, 12 wk post-exposure	No effects	Lee *et al,* 1989; Valentine *et al,* 1998
	110, 370 1,100	610, 2,060 6,000)		Slight/moderate testicular effects Testicular atrophy affecting all stages of spermatogenesis (300 ppm EGME +ve control). Some reversibility of response seen. NOAEL not established	

Table 4.5.2: Genotoxicity of DEGDME

Endpoint / Organism	Strain or type / Target	Concentration	Exposure regime	Result	Remark	Reference
In vitro						
Gene mutation						
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	Up to 94,000 µg/plate	Plate incorporation	**–ve**	+/– S9	McGregor *et al*, 1983

Table 4.5.2: Genotoxicity of DEGDME (cont'd)

Endpoint / Organism	Strain or type / Target	Concentration		Exposure regime	Result	Remark	Reference
Unscheduled DNA Synthesis							
Human	Embryonic intestinal fibroblasts	Up to 19,000 µg/l		3 h at 37°C	**–ve**	+/– S9	McGregor *et al,* 1983
In vivo							
Sperm abnormality		**(ppm)**	**(mg/m^3)**				
Mouse, 10 M	B6C3F$_1$ Germ cells	0, 250	(0, 1,400	Inhalation, 7 h/d, 4 d	**–ve**	Mouse killed 35 d after treatment.	McGregor *et al,* 1983
		1,000	5,600)		**+ve**. ↑ sperm abnormalities from 5.14 to 32.30% (all categories), significant ($p < 0.001$) particularly those with amorphous heads		
Chromosome aberration		**(ppm)**	**(mg/m^3)**				
Rat, 10 M, 10 F	CD bone marrow	0	(0	Inhalation, 7h/d, 1 or 5 d	–ve	Rats killed 6, 24 or 48 h after 1 d of exposure, and 6, 24 h after 5 d of exposure.	McGregor *et al,* 1983
		250	1,400		Weakly +ve. Small ↑ total aberrations, not dose-related		
		1,000	5,600)		**–ve**		

Table 4.5.2: Genotoxicity of DEGDME (cont'd)

Endpoint / Organism	Strain or type / Target	Concentration		Exposure regime	Result	Remark	Reference
Dominant lethal mutations		**(ppm)**	**(mg/m^3)**				
Rat, 10 M	CD germ cells	0	(0	Inhalation, 7 h/d, 5 d	No effects	Serially mated 1 x/wk (1 M: 2 F) for 10 wk. F examined 17 d after first caging with M	McGregor *et al*, 1983
		250	1,400		Statistically significant ↑ early death frequency at wk 7		
		1,000	5,600)		↓ pregnancies 4 - 9 wk after exposure, total implantations significantly ↑ ($p < 0.01$) in wk 6 and 7; overall ±ve		
Sex-linked recessive lethal mutations		**(ppm)**	**(mg/m^3)**				
Drosophila melanogaster	ORK m; M-5f	0	(0	Inhalation, 2.75 h	F_2 showed 3 recessive lethals (2 in other test); F_3: 6 (0 in other test)	Two independent tests using different stocks of flies	McGregor *et al*, 1983
		250	1,400)		F_2: 6 recessive lethals (1 in other test); F_3: 6 (0 in other test) Results unclear		

Table 4.5.3: Reproductive and developmental toxicity of DEGDME

Route / Species, strain, number and sex/group	**Dose or concentration**	**Exposure regime**	**Result**	**Reference**
Oral, gavage	**(mg/kgbw)**			
Mouse, CD-1, 49 - 50 F	0 3,000	1 x/d, g.d. 7 - 14, observed until d 3 post partum	No effects Maternal mortality 41%; no viable litters	Schuler *et al*, 1984; Hardin *et al*, 1987
Mouse, Crl:CD-1 (1CR) BR Swiss albino, 13 - 15 F	0 62.5 125 250, 500	1 x/d, g.d. 6 - 15, killed g.d. 17	No effects. No maternal toxicity at all dose levels. Developmental NOAEL ↓ foetal bw/litter Significant ↑ percentage of post implantation loss/litter and of malformed live foetuses/litter Developmental effects involved neural tube, limbs, digits, cranio-facial structures, abdominal wall, cardiovascular system, urogenital organs, and both the axial and appendicular skeleton. Maternal NOAEL	Price *et al*, 1987
Mouse, CD-1, 20 F	0 537	1 x/d, g.d. 11, killed g.d. 18	No effects. No effect on foetal bw Gross foetal malformations (paw defects). No maternal toxicity	Hardin and Eisenmann, 1987

Table 4.5.3: Reproductive and developmental toxicity of DEGDME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, gavage (cont'd)	**(mg/kgbw)**				
Rabbit, NZW, 15 - 22 F	0		1 x/d, g.d. 6 - 19, killed g.d. 30	No effects	Schwetz *et al*, 1992
	25			Maternal and developmental NOAEL	
	50			Maternal toxicity (↓ bw gain)	
	100			↑ incidence of resorptions and malformed live foetuses. Malformations of digits, cranio-facial structure, abdominal wall, cardiovascular system, urogenital organs and axial skeletal	
	175			As for 100 mg/kgbw. Maternal mortality (15%) at 175 mg/kgbw/d	
nhalation	**(ppm)**	**(mg/m^3)**			
⟨at, 25-26 F Crl:CDRBR)	0	(0	6 h/d (nose-only), g.d. 7 - 16	No effects	Driscoll *et al*, 1998
	25, 100	140, 560		↑ of structural malformations (essentially brain and skeletal) at 100 ppm; ↑ variations (primarily delayed skeletal ossification and rudimentary ribs) at 25 and 100 ppm	
	400	2,200)		No live foetuses	

Table 4.5.4: Absorption (uptake), distribution, metabolism and elimination of DEGDME

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Oral	**(mmol/kgbw)**	**(mg/kgbw)**			
Mouse, CD-1 Swiss, 60 F, time-mated	3.73	(500)	1 x, g.d. 11	Excreta sampling 0 - 48 h. Rapid elimination of radioactivity via urine (63% dose within 48 h). Principal metabolites 2-MEAA (63% dose) and MAA (28% dose)	Daniel *et al*, 1991
Rat, SD, 10 M	0.051, 5.1	(6.84, 684)	1 x	Sampling 0 - 96 h. Rapid elimination of radioactivity at both dose levels. 0 - 24 h urine samples contained > 70% of dose. Profile of metabolites in urine similar at each dose level. Major metabolite: 2-MEAA (70% of dose). Second metabolite: MAA (6% of dose)	Cheever *et al*, 1988
Rat, SD, 5 M	5.1	(684)	1 x/d, 22 d	1 x ^{14}C-DEGDME to naïve rats and rats pre-treated with phenobarbitone (0.1% in drinking water) or non-radioactive DEGDME. Sampling 0 - 96 h. Urine was major route of elimination in each case. Metabolic profile qualitatively similar in naïve and pre-treated animals. Quantitative ↑ in MAA content of 0 - 96 h urine between naïve rats (6.2% dose) and rats pretreated with DEGDME (10% dose) or phenobarbitone (13.4% dose)	Cheever *et al*, 1989b

4.6 Substance profile: TEGME

4.6.1 Identity

Name:	Triethylene glycol (mono) methyl ether
IUPAC name:	2-[2-(2-methoxyethoxy)ethoxy]-ethanol
CAS registry No.:	112-35-6
Molecular formula:	$C_7H_{16}O_4$
Structural formula:	$CH_3–(O–CH_2–CH_2)_3–OH$
Molecular weight:	164.2
Other components:	Tetraethylene glycol monomethyl ether, pentaethylene glycol monomethyl ether, etc.

4.6.2 Physico-chemical properties

Melting point:	–44°C
Boiling point:	249.2°C
Vapour pressure:	< 0.013 hPa
Solubility in water:	Soluble

4.6.3 Conversion factors

1 ppm = 6.826 mg/m^3
1 mg/m^3 = 0.147 ppm

4.6.4 Toxicological data

4.6.4.1 Acute toxicity

Oral

Rat:	LD_{50} 11,800 mg/kgbw (Smyth *et al*, 1962).

Dermal

Rabbit:	LD_{50} 7,400 mg/kgbw (Smyth *et al*, 1962).

Inhalation

Rat: No significant signs of toxicity following 8 hours of exposure to saturated vapour (actual level not reported) (Smyth *et al*, 1962).

4.6.4.2 Irritation and sensitisation

Skin irritation

No data are available.

Eye irritation

No data are available.

Sensitisation

No data are available.

4.6.4.3 Repeated-dose toxicity (Table 4.6.1)

Subacute toxicity

In a 2-week study conducted in SD rats to investigate the effect of TEGME on the palatability of the diet or drinking water, a dietary dose of 5,000 mg TEGME/kgbw/d was without effect. The same dose applied in drinking water caused diminished food consumption and body weight, and increased water consumption (Cosse and Atkin, 1989).

A 14-day exposure of male SD rats to doses up to 8 g/kgbw/d resulted in clinical signs including cachexia, pilo-erection and laboured breathing; altered gait, FOB changes (lowered grip strength); lowered food and water intake and body weight loss. Lowered food intake and body weight loss were also seen at a dose of 4 g/kgbw/d but not at 1.6 g/kgbw/d (Gill and Hurley, 1990).

A dose of 1,000 mg/kgbw TEGME was applied daily to the shaved skin of male and female NZW rabbits for 21 days. The only effect attributed to treatment was slight irritation at the site of application (Leber *et al*, 1990).

Subchronic toxicity

TEGME applied to the shaved skin of SD rats at dose levels of 0, 400, 1,200, or 4,000 mg/kgbw/d for 13 weeks produced local irritation only on abraded skin areas. There was no evidence of systemic toxicity (Corley *et al*, 1990; Gill *et al*, 1998).

A 13-week drinking water study is reported in Section 4.6.4.8 (Gill and Negley, 1990; Gill *et al*, 1998).

4.6.4.4 Genotoxicity (Table 4.6.2)

In vitro

EGME was not mutagenic in *Salmonella typhimurium* using a preincubation protocol, in the presence or absence of metabolic activation, at concentrations of 2,000 to 5,000 µg/plate (Samson and Gollapudi, 1990) and the HGPRT test in CHO cells, at 2,000 to 5,000 µg/ml (Linscombe and Gollapudi, 1990).

In vivo

In CD-1 mice orally dosed at 500, 1,667 or 5,000 mg TEGME/kgbw, there was no evidence of increased incidence of micronucleated PCE in the bone marrow at any of the dose levels compared with controls (McClintock and Gollapudi, 1990).

4.6.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.6.4.6 Reproductive and developmental toxicity (Table 4.6.3)

In a screening study using the Chernoff-Kavlock protocol, TEGME was administered by gavage to pregnant Wistar rats at doses of 0, 250 or 1,000 mg/kgbw/d from day 6 to 15 of gestation. The animals were allowed to litter. The growth and viability of the offspring was assessed at days 1 and 5 post partum. No adverse effects were reported in the dams or their offspring (Leber *et al*, 1990; Hoberman *et al*, 1990a; 1996).

Pregnant Crl:CD(SD)BR rats were dosed orally with 0, 625, 1,250, 2,500 or 5,000 mg TEGME/kgbw/d, administered on day 6 to 15 of gestation. Maternal death and embryo-foetal lethality were reported at 5,000 mg/kgbw. There was a slightly reduced food consumption in the dams and decreased foetal weight with delayed foetal ossification at doses of 1,250 mg/kgbw/d and above. At 2,500 mg/kgbw/d and above, an increase in foetal variations was observed and at 5,000 mg/kgbw/d a slight increase in resorptions (Hoberman *et al*, 1990a; 1996). The results do not demonstrate specific foetal toxicity of TEGME.

Pregnant NZW rabbits were dosed with 0, 250, 500, 1,000 or 1,500 mg/kgbw/d of TEGME, administered orally from day 6 to 18 of gestation. At 1,500 mg/kgbw/d various signs of maternal toxicity were reported, including death and elevated incidences of angulated hyoid alae and delayed ossification of the xyphoid in the progeny. At 1,000 mg/kgbw/d one death was reported, possibly related to treatment, but no foetal effects (Hoberman *et al*, 1990b; 1996). In all, there is no evidence of selective toxicity to the foetus (including teratogenesis) in rats or rabbits.

A review of the reproductive and developmental toxicity of TEGME was published by Kimmel (1996).

4.6.4.7 Kinetics and metabolism

The rate of skin permeability of undiluted TEGME in isolated human epidermis was determined to be 0.034 mg/cm²/h (Leber *et al*, 1990).

No information is available on the metabolism of TEGME.

4.6.4.8 Neurotoxicity (Table 4.6.1)

When SD rats were applied TEGME (undiluted) onto their skin, under occlusive patch, at nominal doses of 0, 400, 1,200 and 4,000 mg/kgbw/d for 90 days (Section 4.6.4.3), there was no evidence of neurotoxicity (Gill *et al*, 1998).

A 13-week drinking water study was conducted in CD rats at target doses of 0, 400, 1,300 or 4,200 mg/kgbw/d. The protocol was specifically designed to investigate possible neurotoxicity, including observation of behavioural changes and detailed pathological examination of the nervous system. Food consumption, body weight and body weight gain were reduced in both sexes at the highest dose and to a lesser extent at the mid dose. Decreased water consumption was seen only in females at 4,200 mg/kgbw. Dose-related decreases in liver weight were noted in males at all dose levels with microscopic evidence of hepatocellular hypertrophy and

vacuolisation in the highest dose only. Minimal histological lesions (hepatocellular cytoplasmic vacuolisation and/or hypertrophy) were recorded in the liver of males at all dose levels, and among females in the highest dose. Testicular atrophy was also observed in most male rats at the highest dose level (spermatocytes and developing spermatids were affected). The 2 lower doses were devoid of such effects. There was no evidence of neurotoxicity (Gill and Negley, 1990; Gill *et al*, 1998).

4.6.4.9 Immunotoxicity

No information available.

4.6.5 Human effects data

No data are available.

Table 4.6.1: Systemic toxicity of TEGME

Route / Species, strain, number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
Oral, diet				
Rat, SD, 10 M, 10 F	0, 5,000	*Ad libitum*, 2 wk	No effects	Cosse and Atkin, 1989
Oral, drinking water				
Rat, SD, 10 M, 10 F	0 5,000	*Ad libitum*, 2 wk	No effects ↓ food intake and bw, ↑ water intake	Cosse and Atkin, 1989
Rat, CD, 10 M	0,750, 1,600 3,900 8,000	*Ad libitum*, 2 wk	No effects Food intake, ↓ bw Water and food intake, ↓ bw, clinical signs, FOB changes	Gill and Hurley, 1990
Rat, CD, 15 M, 15 F	0 400 1,200 4,000	*Ad libitum*, 13 wk	No effects ↑ liver weight. Testicular atrophy. ↓ food intake and bw. ↑ liver weight ↓ food intake and bw. ↓ motor activity. ↑ liver weight	Gill and Negley, 1990; Gill *et al*, 1998
Dermal, occluded				
Rabbit, NZW, 5 M, 5 F	0 1,000	6 h/d, 21 d	No effects Slight local irritation	Leber *et al*, 1990
Rat, CD, 10 M, 10 F	0, 400, 1,200 4,000	6 h/d, 5 d/wk, 13 wk	No effect Local irritation of abraded skin	Corley *et al*, 1990; Gill *et al*, 1998

Table 4.6.2: Genotoxicity of TEGME

Endpoint / Organism	Strain or type / Target	Concentration	Result	Remark	Reference
In vitro					
Gene mutation					
Salmonella typhimurium	TA1535, TA100, TA1537, TA98	2,000 - 5,000 µg/plate	**–ve**	+/– S9	Samson and Gollapudi, 1990
CHO cells	HGPRT locus	2,000 - 5,000 µg/ml	**–ve**	+/– S9	Linscombe and Gollapudi, 1990
In vivo					
Micronucleus frequency					
CD-1 mouse	Bone marrow	0, 500, 1,667, 5,000 mg/kgbw	**–ve**	1 x oral gavage	McClintock and Gollapudi, 1990

Table 4.6.3: Reproductive and developmental toxicity of TEGME

Species, strain, number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
Oral, gavage				
Rat, Wistar, 10 F	0, 250, 1,000	1 x/d, g.d. 6 - 15	No effects (Chernoff-Kavlok screening assay)	Leber *et al*, 1990
Rat Crl:CD(SD)BR, 25 F	0, 625 1,250 2,500 5,000	1 x/d, g.d. 6 - 15	No effects ↓ food consumption, ↓ foetal bw, delayed foetal ossification ↑ foetal variations Maternal death and embryo-foetal lethality, ↑ resorptions	Hoberman *et al*, 1990a; 1996
Rabbit, NZW, 20 F	0, 250, 500 1,000 1,500	1 x/d, g.d. 6 - 18	No effects 1 death, no foetal effects Maternal death other signs of maternal toxicity. Abortions and 2 common skeletal variations	Hoberman *et al*, 1990a; 1996

4.7 Substance profile: TEGDME

4.7.1 Identity

Name:	Triethylene glycol dimethyl ether
IUPAC name:	2,5,8,11-Tetraoxadodecane
CAS registry No.:	112-49-2
Molecular formula:	$C_8H_{18}O_4$
Structural formula:	$CH_3–(O–CH_2–CH_2)_3–O–CH_3$
Molecular weight:	178.2
Other components:	Purity > 99%

4.7.2 Physico-chemical properties

Melting point:	–40°C
Boiling point:	210 - 230°C
Vapour pressure:	1.2 hPa
Solubility in water:	Completely soluble
Relative density:	$D_4^{20} = 0.986$

4.7.3 Conversion factors

1 ppm = 7.408 mg/m^3
1 mg/m^3 = 0.135 ppm

4.7.4 Toxicological data

4.7.4.1 Acute toxicity

Oral

Rat: LD_{50} 5,877 mg/kgbw (female) (Hofmann *et al*, 1992).

Dermal

No data are available.

Inhalation

No data are available.

4.7.4.2 Irritation and sensitisation

Skin irritation

No data are available.

Eye irritation

No data are available.

Sensitisation

No data are available.

4.7.4.3 Repeated-dose toxicity

Subacute toxicity

Wistar rats (5/sex/group) were administered oral doses of 0, 62.5, 250 or 1,000 mg TEGDME/kgbw/d for 28 days. Retardation in growth rate, reduced water consumption, reductions in testes and thymus weight and thrombocytopenia were reported for the male animals given 1,000 mg/kgbw. Histopathological examination showed degenerative changes in the seminiferous epithelium, and atrophy of the thymus similar to that observed in animals treated with MAA. Also females showed thymus reduction. At doses of 250 mg/kgbw, thymus reductions in females (not significant in males) were reported in the absence of any histopathological changes. No effects were seen at 62.5 mg/kgbw (Hofmann *et al*, 1992).

Subchronic toxicity

No data are available.

4.7.4.4 Genotoxicity

No data are available.

4.7.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.7.4.6 Reproductive and developmental toxicity (Table 4.7.1)

Administration of 3,500 mg/kgbw/d orally to time-mated, pregnant CD-1 mice on day 7 to 14 of gestation resulted in 4% maternal mortality and 100% resorption of implantations in the surviving animals (Schuler *et al*, 1984).

The reproductive performance of CD-1 mice was evaluated following administration of TEGDME in drinking water at levels of 0.25, 0.5 and 1.0% (estimated daily dose 440, 880 and 1,630 mg/kgbw) using a continuous breeding protocol. During the continuous breeding phase, there were statistically significant decreases in the mean number of litters and live pups per pair at the high dose and in the proportion of pups born alive and live pup weights at both the high and mid doses. A reduced fertility index was reported during the crossover mating trial, in which treated females (dosed at 1,850 mg/kgbw/d) were mated with control males. The proportion of live pups and the mean pup weight (live births) was also reduced (Morrissey *et al*, 1989).

Time mated CD-1 mice were given a single equimolar oral dose (4 mmol/kg) of either EGME, EGDME, DEGDME or TEGDME (713 mg/kgbw) on day 11 of gestation. Foetuses were examined on day 18 for gross, soft tissue and skeletal abnormalities. There were no signs of maternal toxicity or effects on intrauterine survival in any of the treated animals. Characteristic paw malformations were seen in all but the TEGDME groups (Hardin and Eisenmann, 1987).

TEGDME was administered to time mated CD-1 mice at doses of 0, 250, 500 or 1,000 mg/kgbw/d from day 6 to 15 of gestation. An increased incidence of malformed foetuses, mainly neural tube, craniofacial and axial skeletal defects, occurred at 1,000 mg/kgbw. A significant increase in maternal relative liver weight occurred at doses of 500 and 1,000 mg/kgbw but otherwise there were no signs of maternal toxicity (George *et al*, 1987).

Oral administration of TEGDME at doses of 175 or 250 mg/kgbw/d to NZW rabbits during organogenesis was reported to induce external and visceral malformations. Maternal and foetal

body weight was reduced at these doses. Dose levels of 75 and 125 mg/kgbw were without effects (George *et al*, 1990).

Bantle *et al* (1999) reported TEGDME to be teratogenic when tested with frog embryos *in vitro* in the frog embryo teratogenesis - *Xenopus* (FETAX) assay. In the presence of a metabolic activation system (rat liver microsomes), head, face, and eye malformations were severe in all embryos.

4.7.4.7 Kinetics and metabolism

No specific data are available.

The reported developmental effects and subchronic toxicity of this material are consistent with those of EGME and MAA suggesting that both dealkylation and subsequent oxidation occur (Sections 4.1 and 4.8).

4.7.4.8 Neurotoxicity

There was no evidence of any specific effects of TEGDME on either the central or peripheral nervous system of rats given daily oral doses up to 1,000 mg/kgbw for 28 days (Hofmann *et al*, 1992).

4.7.4.9 Immunotoxicity

Evidence of thymic involution was reported among rats given oral doses of 250 and 1,000 mg TEGDME/kgbw for 28 days (Hofmann *et al*, 1992). Thymic involution in this study occurred earlier than usual and may have been in response to stress.

4.7.5 Human effects data

No data are available.

Table 4.7.1: Reproductive and developmental toxicity of TEGDME

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**			
Mouse, CD-1, 20 F	0, 713	1 x, g.d. 11	No effects	Hardin and Eisenmann, 1987
Mouse, CD-1, 50 F	0 3,500	1 x/d, g.d. 7 - 14	No effects Maternal death (2/50); 100% resorption	Schuler *et al*, 1984
Mouse, CD-1, 29-30 F	0, 250 500 1,000	1 x/d, g.d. 6 - 15	No effects ↑ maternal liver weight, ↓ foetal bw ↑ maternal liver weight; ↓ foetal bw and malformations	George *et al*, 1987
Rabbit, NZW, 27-32 F	0, 75, 125 175 250	1 x/d, g.d. 6 - 19	No effects ↓ maternal pup bw; ↑ external and visceral malformations ↓ maternal pup bw; ↑ external and visceral malformations	George *et al*, 1990
Oral, drinking water	**% (mg/kgbw/d)**			
Mouse, CD-1, 20 M, 20 F	0, 0.25 (0, 440)	*Ad libitum*, Continuous breeding protocol with cross-over mating	No effects	Morrissey *et al*, 1989; Chapin and Sloane 1997
	0.5% (880) 1.0% (1,750)		↓ pup bw ↓ pup bw, live pups/litter and litters/pair	

Table 4.7.1: Reproductive and developmental toxicity of TEGDME (cont'd)

Route / Species, strain, number and sex/group	**Dose or concentration**	**Exposure regime**	**Result**	**Reference**
In vitro	**(mg/l)**			
Xenopus laevis, whole embryo	Range of concentrations including EC_{50}	96 h	Severe head, face and eye malformations at or near EC_{50}. Tails recurved upward and embryos stunted. Cyclopia present in some embryos. EC_{50} (malformation) 6.09 mg/ml, minimum concentration inhibiting growth 3.13 mg/ml	Bantle *et al*, 1999

4.8 Substance profile: MAA

4.8.1 Identity

Name:	Methoxyacetic acid
IUPAC name:	Methoxyacetic acid
CAS registry No.:	625-45-6
Molecular formula:	$C_3H_6O_3$
Structural formula:	$CH_3–O–CH_2–COOH$
Molecular weight:	90.1
Other components:	No data

4.8.2 Physico-chemical properties

Melting point:	Approximately 7°C
Boiling point:	202°C
Vapour pressure:	1.8 hPa
Solubility in water:	Completely soluble
Relative density:	$D_4^{20} = 1.18$

4.8.3 Conversion factors

1 ppm = 3.745 mg/m^3
1 mg/m^3 = 0.267 ppm

4.8.4 Toxicological data

4.8.4.1 Acute toxicity (Table 4.8.1)

Oral

Rat:	LD_{50} between 1,000 and 1,500 mg/kgbw (aqueous preparation). CNS symptoms (convulsions, paresis, atonia) were reported (BASF, 1980).

Single administration of MAA typically leads to specific testicular atrophy. This effect can also be shown *in vitro* in testicular cell cultures (Section 4.8.4.6) and in subacute studies (Section 4.8.4.3).

Gavage administration of 118, 296 or 592 mg MAA/kgbw to Wistar rats produced dose-dependent testicular atrophy at all dose levels. At the highest dose, after 1, 4 and 14 days of observation, lower relative testis weight and damage to germinative epithelium in pachytene and diplotene were reported, as well as secondary spermatocytes. (Under the same conditions 868 mg BAA/kgbw caused haematuria but no testicular damage). The lowest dose of 118 mg MAA/kgbw still showed some effect after 24 hours (Foster *et al*, 1987).

Testicular atrophy seen after single gavage of sodium-MAA to SD rats at a dose of 650 mg/kgbw was reversible by day 70 (Bartlett *et al*, 1988). It has been suggested that changes in the Sertoli cells were sequelae to the changes in the germ cell compartment (Sharpe, 1989) (Section 4.8.6).

Two days after oral administration of single doses of 325 or 650 mg MAA/kgbw to male Wistar rats a specific depletion of spermatocytes was evident. The lowest dose of 65 mg/kgbw was without effects (Suter *et al*, 1998).

In selectively destroyed pachytene spermatocytes of adult SD rats given 650 mg MAA/kgbw once, there was an accumulation of clusterin (sulphated glycoprotein-2) in the cytoplasm of pachytene spermatocytes after 6 hours. The clusterin m-RNA was localised in Sertoli cells. Apoptosis of pachytene spermatocytes (DNA fragmentation; karyopyknosis) was not visible until 12 hours after dosing (Clark *et al*, 1997).

Single doses of 400 and 600 mg MAA/kgbw administered by oral gavage to male $B6C3F_1$ mice revealed a reduction in urinary creatine and creatinine at 24 hours after dosing and an increase of creatine and creatinine levels at 48 and 72 hours; the creatine/creatinine ratio also increased after 24 hours. Thus, this parameter may be regarded as indicative of interference of MAA with the metabolism of creatine in the testes (Traina *et al*, 1997).

A single dose of 650 mg MAA/kgbw in adult SD rats caused a near complete depletion of IX - II spermatocytes by an apoptotic mechanism. The effects were similar to a gonadotropin depletion (Brinkworth *et al*, 1995).

MAA-induced testicular cell death in SD (Crl:CD) rats could be largely prevented by treatment with calcium antagonists, which were able to inhibit calcium movement through plasma membranes (Li *et al*, 1997).

Germ cell apoptosis occurred 24 hours after single i.p. injection of 650 or 1,300 mg MAA//kgbw to male C57/BL6 mice (Krishnamurthy *et al*, 1998).

Testicular effects seen in male Syrian golden hamsters following single (and repeated) oral administration are discussed in Section 4.8.4.3 (Peiris and Moore, 2001).

Dermal

No data are available.

Inhalation

Rat: No lethalities following exposure for 7 hours to a vapour saturated atmosphere at 20°C (Miller *et al*, 1982b).

4.8.4.2 Irritation/sensitisation

Skin irritation

MAA was corrosive to rabbit skin (BASF, 1980).

Eye irritation

No data are available.

Skin sensitisation

No data are available.

4.8.4.3 Repeated-dose toxicity (Table 4.8.1)

Subacute toxicity

Male SD rats received a dose of 592 mg MAA/kgbw/d by oral gavage for 4 consecutive days. Relative body, liver weight and testicular weights were significantly reduced. Histological examination of the testes showed degenerative effects in spermatocytes of the pachytene, diplotene and diakinesis. Sertoli cells, leptotenic and zygotean spermatocytes and spermatids were unaffected with the exception of early spermatocytes (Foster *et al*, 1983; Gray *et al*, 1985).

Male F344 rats received 8 daily gavage administrations of 0, 30, 100 or 300 mg MAA/kgbw over a 2-week period. At the two highest dose levels, absolute and relative thymus weights, RBC, Hb and Hct values were significantly decreased. In the 300 mg/kgbw group, there was a decrease of

body weight, absolute and relative spleen and testicular weights, and leukopenia. Cellularity of thymus core and germinal epithelium were affected from at 100 mg/kgbw and above. Testicular giant cells and reduced cellularity in bone marrow were observed at 300 mg/kgbw, whereas doses of 30 mg/kgbw produced no effects within the observation period (Miller *et al*, 1982b).

Male Syrian golden hamsters received either single doses of 0, 80, 160 or 650 mg MAA/kgbw or repeated daily doses of 0, 8, 32 or 64 mg/kgbw for 5 weeks. The animals were killed at weekly intervals and spermatozoa were recovered from epididymides and assessed for their fertilising capability *in vitro*. Decreased fertilisation capacity was observed from weeks 3 and 4 following single administration and in all subacutely treated animals. The authors assumed that sperm function may be decreased by the loss of several populations and a preponderance of immature sperm (Peiris and Moore, 2001).

Subchronic toxicity

No data are available.

4.8.4.4 Genotoxicity (Table 4.8.2)

MAA was not mutagenic in *S. typhimurium* strains without metabolic activation. Dose levels greater than 125 µg/plate were toxic to the bacteria (McGregor *et al*, 1983; McGregor, 1984).

MAA was not mutagenic at a bacterial gpt gene inserted in an autosome CHO-AS52 cell line as well as in the HGPRT gene on the X chromosome of the CHO-K1-BH4 cell line (Ma *et al*, 1993).

In V79 cells and human lymphocytes MAA did not induce chromosome aberrations while a micronucleus assay and a test for the induction of aneugenic effects, both in V79 cells, revealed weakly positive results (Elias *et al*, 1996).

MAA increased the SCE frequency in human lymphocytes at concentrations of 1 and 10 mmol/l but not at 0.1 mmol/l (Arashidani *et al*, 1998).

No inhibition of metabolic cooperation between V79 cells was seen (Elias *et al* 1996).

These data, together with results of a battery of *in vitro* and *in vivo* assays with the metabolic precursor EGME, suggest that MAA do not pose a genotoxic hazard (Section 4.1.4.4).

4.8.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.8.4.6 Reproductive and developmental toxicity (Table 4.8.3)

In vitro

In post-implantation rat embryonic cultures MAA (1 - 5 mmol/l; 90 - 450 mg/l) interfered with normal growth and development of the early neurula stage. The NOEC was 1 mmol/l (Yonemoto *et al*, 1984).

MAA (5 mmol/l; 450 mg/l) induced gross structural defects in cultured 9.5-day old rat embryos following treatment with MAA for 2 days. EAA produced similar defects, albeit to a lesser extent, whereas longer chain alkoxy acids (*n*-propoxyacetic acid, *n*-butoxyacetic acid, 3-MPA and 4-MPA) induced small defects (Rawlings *et al*, 1985).

In vivo

Pregnant Wistar rats (8/group) received undiluted oral doses of 0, 0.16 or 0.32 ml MAA/kgbw (about 190 - 380 mg/kgbw) on day 12 of gestation. On day 20 of gestation an increase in the number of resorptions (4, 15.1 and 53.8% for the respective doses) and of malformed foetuses (0, 53.1 and 98.9%) was noted. Hydronephrosis, cardiac and limb malformations and shortening of limbs and tails were reported (Ritter *et al*, 1985).

Following i.p. injection of 225 mg MAA/kgbw on day 8, 10, 12 or 14 of gestation, Wistar rats (8 - 10/group) showed a high foetal mortality on day 8 (93 versus 0% in controls) and day 10 (61%); significant foetal mortality was also seen after injection on days 12 (16%) and 14 (3%). Malformations occurred at all days with the highest rate on day 12 (92.5 versus 15.4%). The malformations mostly consisted of skeletal malformations and hydrocephalus. These could also be demonstrated in a second experiment (5/group) employing i.p. doses of 9 to 225 mg MAA/kgbw injected on day 10 or 12 of gestation, at all dose levels down to 9 mg/kgbw. A NOAEL was not obtained (Brown *et al*, 1984).

Single oral administration of MAA to pregnant CD-1 mice at dose levels ranging from 2 to 8 mmol/kgbw (180 - 720 mg/kgbw) on day 11 of gestation caused a dose-related increase in paw malformations, similar to the dose-response induced by EGME (Sleet *et al*, 1987).

Single oral gavage treatment of pregnant CD-1 mice on day 11 of gestation at 3.4 and 4.6 mmol/kg/bw (306 and 414 mg/kgbw) led to a high incidence (52 - 100%) of digit malformations. Concomitant treatment of 3.4 and 4.6 mmol MAA/kgbw and 43.3 mmol sodium acetate/kgbw reduced the incidence (expressed per foetus) significantly (Welsch *et al*, 1987).

Sleet *et al* (1987, 1988) compared the teratogenic effects of MAA in CD-1 mice after oral and i.v. administration of 2.9 or 3.8 mmol/kgbw (260 or 340 mg/kgbw). The incidence of digit malformations was dose-dependently increased after both routes with somewhat higher rates following gavage treatment. Intravenous dosing reduced the ratios of tissue to whole blood ^{14}C for maternal liver, intact conceptus, and embryo relative to those resulting after gavage.

When MAA (88 - 164 mg/kgbw) was administered i.p. to pregnant C57BL/6 mice on day 10 of gestation, a localisation in the forelimb bud could be made visible. MAA was neither bound to maternal plasma nor to embryonic proteins. Medium and higher doses caused a dose-dependent transient depression in tissue pH. MAA concentrations were not increased in the distal postaxial sector, which is the site of the precursor cells of the missing digits. The internal exposure levels (AUC) appeared to supra-proportionally increase with the administered dose (O'Flaherty *et al*, 1995).

MAA was administered in the liquid diet of pregnant SD rats at levels of 0, 0.03 or 0.014% (equivalent to doses of 79 or 39 mg MAA/kgbw/d) on day 7 to 18 of gestation. MAA produced clear teratogenicity (cardiovascular malformations in 15% of the animals) at the low dose and complete resorption at the high dose (Nelson *et al*, 1989).

In a 2-generation continuous breeding study, CD-1 mice (20/sex/group) received MAA via the drinking water for 98 days after 7 days pre-treatment. Concentrations were 0, 0.1, 0.2 or 0.4% (approximate doses 0, 140, 240 or 390 mg MAA/kgbw/d). During the continuous breeding phase body weight was slightly (9%) reduced in males at 0.4% and markedly in all female groups (19 - 32%). These were related to dose-dependent reductions (19 - 44%) in water consumption. The fertility index of the F_1 generation in the 0.2% group was 95%; in the 0.4% group no animal became pregnant. At the two lower dose levels, the number of litters per pair and the number of viable animals per litter were severely reduced: pup lethality was 10.3% at 0.1% and 75.4 at 0.2% during lactation. Pups at 0.2% died by day 4. In a subsequent cross-mating experiment the proportion of detected matings was not adversely affected, however, the fertility index at 0.4% MAA was reduced. In the surviving F_1 generation continuously exposed to 0.1%, there was no mating and the fertility index was zero. In males at 0.4%, testis, epididymis and seminal vesicle weight was reduced, as was sperm motility. Sperm abnormalities were markedly elevated at this level (NTP, 1986; Chapin and Sloane, 1997).

In conclusion, MAA shows a pronounced selective foetal toxicity after single and repeated administration. A NOAEL has so far not been established.

4.8.4.7 Kinetics and metabolism (Table 4.8.4)

MAA is the major metabolite of EGME and appears to mediate the systemic effects of EGME (Miller *et al*, 1983b; Gargas *et al*, 2000a). The *in vivo* toxicity of EGME roughly correlates with that of MAA. It may be employed as a biomonitoring parameter relevant for a biological action level (Laitinen, 1998).

There are indications that MAA is incorporated into the intermediary metabolism. Some of its activity is attenuated by small carboxylic acids (Coakley *et al*, 1986; Welsch *et al*, 1987; Stedman and Welsch, 1989).

MAA disposition in maternal and conceptus compartments was determined comparatively after single oral and i.v. administration of 3.8 mmol/kgbw to pregnant CD-1 mice on day 11 of gestation. The elimination phase kinetics of ^{14}C and the acid solubility of radioactivity accumulated by the liver or embryo were unaffected by the route of administration. I.v. dosing reduced the ratios of tissues to whole-blood ^{14}C for maternal liver, intact conceptus, and embryo relative to those after gavage treatment (Sleet *et al*, 187, 1988). The concentration of MAA in 12-day old mice embryos and in extra-embryonic fluid after EGME (3.3 mmol/kgbw; 250 mg/kgbw) gavage was 20% higher than in maternal serum (Sleet *et al*, 1988).

Following administration of EGME, MAA was excreted renally, partially in a conjugated form. The biological half-life appeared to be moderate (9 - 13 h) in rats (Aasmoe *et al*, 1999).

There were signs of slow accumulation of MAA in non-human primates (*Macaca fascicularis*) (Scott *et al*, 1989) and humans (Welch *et al*, 1988). The average half-life time in humans exposed to EGME by inhalation was 77.1 hours and there was an apparent increase during the working week (Groeseneken *et al*, 1989a). Shih *et al* (2000c, 2001) also observed a long half-life time for MAA in humans but dermal exposure towards EGME may have confounded the data.

4.8.4.8 Neurotoxicity

No data are available.

The precursor compound EGME was shown to exhibit behavioural and CNS toxicity in rats and signs of behavioural and neurotoxicity in humans (Section 4.1.4.8), but this cannot be attributed to MAA with certainty.

4.8.4.9 Immunotoxicity

F344 rats received 10 consecutive daily oral doses of MAA ranging from 25 to 400 mg/kgbw in several experiments with somewhat diverging dosing regimens. At 100 and 200 mg/kgbw, thymic involution was observed in the absence of body weight reduction; there was also a reduction of lympho-proliferative responses to mitogens (Con A, PHA and PWM). At 200 mg/kgbw, the *in vitro* generated cytotoxic T-lymphocyte response was reduced, whereas mixed lymphocyte reaction and NKA were unaffected. The PFC response to TNP-LPS was suppressed throughout all dose levels, while increased to SRBC at 50 mg/kgbw. TNP-LPS- and SRBC-immunised rats dosed with MAA showed suppression of PFC responses at 100 or 200 mg/kgbw and 200 or 400 mg/kgbw, respectively. Phenotypic analysis of splenocytes revealed a small (3%) reduction in the percentage of W3/25-positive cells (i.e. CD4, helper/inducer cells). Spleen cellularity appeared to be unaffected. Interleukin-2 production was decreased in the 150mg/kgbw group (Smialowicz *et al*, 1991a,b).

Treatment of female F344 rats and C57BL/6J mice with MAA doses ranging from 50 to 400 mg/kgbw/d for 10 consecutive days by oral gavage revealed sensitivity differences between these species. Rats dosed at 50 – 400 mg/kgbw had decreased thymus weights, at 100 to 400 mg/kgbw suppressed PFC response to TNP-LPS, reduced LP response to Con A (at all dose levels), PHA (at all dose levels), PWM (at 100 mg/kgbw and above), and STM (at 100 mg/kgbw and above). No thymic involution or suppression of LP responses, or PFC to TNP-LPS were observed in mice at these dose levels (Smialowicz *et al*, 1992a).

In an experiment designed to compare the potential immunosuppressive activity of various glycol ethers MAA was administered to male F344 rats at dosages ranging from 50 to 400 mg/kgbw on 2 consecutive days. MAA (also EGME and EGMEA) suppressed PFC response to TNP-LPS (Smialowicz *et al*, 1992b).

Riddle *et al* (1992) compared the immunosuppressive activity of MAA on the TNP-LPS plaque forming response using several strains of rats and mice dosed, respectively, with 0.33 to 2.64 mmol/kg/d (30 - 238 mg/kgbw/d) and 0.66 to 5.28 mmol/kg/d (59.5 - 476 mg/kgbw/d) for 10 days. There were no effects in mice, but the (inbred) Lewis rat, being the most sensitive strain, showed suppression starting at 0.66 mmol/kg. EGME was equally active.

Riddle *et al* (1996) further investigated whether the much lower immunotoxicity of MAA in mice is due to its more rapid clearance from mice than from rats. Female $B6C3F_1$ mice and F344 rats were dosed orally twice a day with MAA at total doses ranging from 240 to 1,920 mg/kgbw/d or 40 to 320 mg/kgbw/d for 4 days. Mice were also infused subcutaneously via osmotic mini-pumps containing MAA, which was delivered at 840 mg/kgbw/d over a 7-day period. Humoral immunity was evaluated using the PFC response to SRBC or TNP-LPS. In contrast to rats, thymus weights of mice were reduced only at the top doses (960 and 1,920 mg/kgbw) with PFC response remaining unaffected; upon continuous infusion of 840 mg/kgbw over 7 days the PFC response to TNP-LPS was even enhanced. These data indicate that the lower sensitivity of mice is unrelated to the bioavailability of MAA to target lymphoid tissue.

In all, the studies clearly demonstrate that MAA exerts a significant immunotoxic potential in rats. Mice appear to be less sensitive.

4.8.5 Human effects data

No data are available on the toxicological effects of MAA exposure on humans. However, extensive data exist for its metabolic precursor EGME (Section 4.1.5).

MAA induced apoptosis in spermatocyte cultures from human testes (Li *et al*, 1996).

MAA excretion should be monitored wherever there is possible occupational exposure to EGME or other glycol ethers (EGMEA, DEGDME) that may form MAA.

4.8.6 *In vitro* investigations

In mitochondria from rat liver and testes, 300 µg MAA/ml inhibited oxidative phosphorylation and cytochrome C oxidase activity (Beatti and Brabec, 1986).

Germ cell detachment in Sertoli-germ cell co-cultures was increased at 2 to 10 mmol/l. These concentrations were overtly toxic to the cultures (Gray, 1986).

MAA was used as a positive control for *in vitro* embryotoxicity tests (Genschow *et al*, 2000). It was also employed in the validation of an embryo-neural retina cell culture screen for developmental toxicants (Daston *et al*, 1995).

In NZW rabbit whole embryo cell cultures, MAA (5 mmol/l; 450 mg/l) induced a 90% rate of foetal malformation (Pitt and Carney, 1999).

In mouse whole embryo cultures, 5 mmol MAA/l serum-free medium reduced ^{3}H-incorporation; this could be antagonised by some small carboxylic acids (Stedman and Welsch, 1989; Mebus and Welsch, 1989). Concentrations of as high as 5 to 10 mmol/l (450 - 900 mg/l) may occur *in vivo* in mouse embryos or extra-embryonic fluid after a single treatment with a teratogenic dose of EGME (Scott *et al*, 1987; Stedman and Welsch, 1989).

In rat foetuses (10.5 d old), 5.0 mmol MAA/l did not decrease lactate production, in contrast to the effects seen with 0.1 mmol iodoacetate/l (Coakley *et al*, 1986).

MAA increased ovarian luteal cell progesterone production in cultured rat luteal cells but also in cells from human oocyte donors (25,000 cells per well), treated with HCG and with MAA for 6 to 48 hours. At 1 - 10 mmol/l, the progesterone production into the culture medium was significantly increased after 24 and 48 hours in rat luteal cells, while ATP was decreased, but only at 48 hours and at 2.5 mmol/l or greater concentration. In human granulosa cells progesterone production was dose-dependently increased at 0.1 to 5 mmol (significant at 1 - 5 mmol). Thus, MAA (and EGME) may have the potential to alter ovarian luteal function in women (Almekinder *et al*, 1997; Davis *et al*, 1997).

Ovarian luteal cells (recovered from 23-day old, human chorionic gonadotropin-primed SD rats) treated *in vitro* with 0 to 10 mmol MAA/l (0 - 900 mg/l) showed some specific damage. Whereas the progesterone production in untreated cells declined after 24 and 48 hours of culture, it remained intact with MAA present, independent from LH-stimulated cAMP level (Almekinder *et al*, 1997; Davis *et al*, 1997).

In Sertoli cells incubated with MAA at 3 or 10 mmol/l (270 - 900 mg/l) for 6, 9 and 12 hours, there was a decrease in lactate production. The authors pointed to earlier evidence that lactate may be needed by surrounding spermatogenic cells depending as a nutritional source (Beatti *et al*, 1984; Williams and Foster, 1988).

Several investigators reported MAA having detrimental effects on spermatocytes; pachytenic spermatocytes were most sensitive including leakage of LDH (Foster *et al*, 1983; Gray *et al*, 1985; Blackburn *et al*, 1985; Foster *et al*, 1986, 1987). BAA at 5 mmol/l (14,000 mg/l) was without effect (Foster *et al*, 1987).

Among other alkoxy acids, MAA had the least haemolytic activity *in vitro* (Ghanayem *et al*, 1989).

MAA (≥ 1 mmol/l; ≥ 90 mg/l) induced spermatocyte apoptosis in human testicular tissue culture (24 hours exposure) and spermatocyte degeneration/apoptosis in rat seminiferous tubule culture

(19 hours exposure). These effects could be significantly attenuated by calcium channel blockers (Li *et al*, 1996).

These *in vitro* studies show that human testes are equally sensitive to MAA than rat testes and that MAA induces germ cell apoptosis in humans and rats through a similar calcium-dependent mechanism. MAA concentrations of 1 to 100 µmol/l did decrease thymocyte viability and proliferation but significantly inhibited proliferation of foetal liver cells enriched for lymphoid precursors (Holladay *et al*, 1994).

Table 4.8.1: Systemic toxicity of MAA by oral gavage

Species, strain, number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
Rat, Wistar, 6 M	0 118, 296, 592	1 x, 1 - 14 d observation	No effects Testicular damage (pachytene spermatids)	Foster *et al*, 1987
Rat, SD, 88 M, 27 controls	0	1 x, 70 d observation (with several interim kills)	No effects	Bartlett *et al*, 1988
	650		↓ serum testosterone and serum FSH. After 3 d, depletion of spermatocytes at all stages other than VIII - XI and beginning with pachytene, testicular atrophy	
Rat, SD, 6 M	0 592	1 x/d, 4 d	No effects Testicular damage (pachytene, diplotene, diakinesis)	Foster *et al*, 1983; Gray *et al*, 1985
Rat, F344, 5 M	0 30 100 300	1 x/d, 8 d in 2 wk	No effects NOEL ↓ testicular weight, cellularity, thymus weight and cortical cellularity , ↓ RBC, WBC, Hb, Hct ↓ body and thymus weight, severe degeneration of germinal epithelium, ↓ bone marrow cellularity and RBC, WBC, Hb, Hct , depletion of thymic cortical lymphoid elements	Miller *et al*, 1982b
Rat, F344, 10 M	0, 25 50, 100, 150, 200, 400	1 x/d, 10 d	No effects Thymic involution, immunosuppression	Smialowicz *et al*, 1991a,b
Rat, Wistar, 10 M	0, 65 325, 650	1 x	No effects ↓ Testes weight; damage to spermatocytes, germ cell necrosis	Suter *et al*, 1998

Table 4.8.1: Systemic toxicity of MAA by oral gavage (cont'd)

Species, strain, number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
Mouse, B6C3F$_1$, 10 F	0 25 50, 100	1 x/d, 5d/wk, 2 wk	No effects ↓ Spleen, bone marrow and thymus cellularity No effects on NK cell activity	House *et al*, 1985
Hamster, Syrian golden, 36 M	0 80, 160, 650	1 x	No effects Dose-related decrease of fertilising capacity *in vitro*	Peiris and Moore, 2001
Hamster, Syrian golden, 36 M	0 8, 32, 64	1 x/d, 5 wk	No effects Dose-related decrease of fertilising capacity *in vitro*	Peiris and Moore, 2001

Table 4.8.2: Genotoxicity of MAA in vitro

Endpoint / Organism	Strain or type / Target	Concentration	Result	Remark	Reference
Gene mutation					
Salmonella typhimurium	TA98, TA100, TA1535, TA1537, 1538	Up to 125 µg /plate	**–ve**	+/– S9	McGregor et al, 1983; McGregor, 1984
CHO-AS52 cell	Bacterial gpt gene	5 - 100 mmol/l	**–ve**	– S9	Ma *et al*, 1993
CHO-K1-BH4 cell	HGPRT	5 - 200 mmol/l	**–ve**	– S9	Ma *et al*, 1993
CHO-K1-BH4 cell	HGPRT	100 mmol/l	**–ve**	+ S9	Ma *et al*, 1993
Chromosome aberration					
V79 cells		1.6 - 6.4 mmol/l	**–ve**	– S9	Elias *et al*, 1996
Human lymphocytes		NS	**–ve**	– S9	Elias *et al*, 1996
Sister chromatid exchange					
Human lymphocytes		0.1, 1, 10 mmol/l	**+ve**	– S9, 72 h	Arashidani *et al*, 1998
Micronucleus induction					
V79 cells		1.6 - 6.4 mmol/l	**±ve**	– S9	Elias *et al*, 1996
Aneuploidy					
V79 cells		1.6 - 6.4 mmol/l	**±ve**	– S9	Elias *et al*, 1996

Table 4.8.3: Reproductive and developmental toxicity of MAA

Route / Species, strain, number and sex/group	Dose	Exposure regime	Result	Reference
Oral	**(mg/kgbw)**			
Rat, Wistar, 8 F	0 190 380	g.d. 12	No effects ↑ resorptions (15.1%), ↑ malformations (53.1%) ↑ resorptions (53.8%), ↑ malformations (98.9%)	Ritter *et al*, 1985
Mouse, CD-1, NS F	0 180 - 720	g.d. 11	No effects ↑ paw malformations	Sleet *et al*, 1987, 1988
Mouse, CD-1, 10 - 15 F	260, 340	g.d. 11	↑ digit malformations	Sleet *et al*, 1987, 1988
Mouse, CD-1, 11 - 18 F	0 306 - 414	g.d. 11	No effects ↑ paw malformations	Welsch *et al*, 1987, 1988
Intravenous				
Mouse, CD-1, 15 - 16 F	260, 340	g.d. 11	↑ digit malformations	Sleet *et al*, 1987, 1988
Intraperitoneal				
Rat, Wistar, 8 - 10 F	0 225	g.d. 8, 10, 12 or 14	No effects ↑ foetal mortality, ↑ skeletal malformations and hydrocephalus	Brown *et al*, 1984
Rat, Wistar, 5 F	0 9 - 225	g.d. 10 or 12	No effects ↑ foetal mortality, ↑ skeletal malformations and hydrocephalus	Brown *et al*, 1984

Table 4.8.4: Absorption (uptake), distribution, metabolism and elimination of MAA

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral	**(mmol/kgbw)**	**(mg/kgbw)**			
Mouse, CD-1, 4 F	2.9, 3.8	261, 342	1 x on g.d. 11	Radioactivity in maternal blood measured 5 to 360 min after dosing reached peak levels of 302.1 nmol equivalents/100 µl whole blood after 120 min at 3.8 mmol/kgbw (data for 2.9 mmol/kgbw not presented)	Sleet *et al*, 1987, 1988
Mouse, CD-1, 6 F	2.9, 3.8	261, 342	1 x on g.d. 11	Ratio of tissue to whole blood radioactivity measured 6 h after treatment with 3.8 mmol/kg was: in maternal liver (0.88) and kidney (0.83); in intact conceptus 1.76, in embryo 1.79, in placenta/membranes 1.10 (data for 2.9 mmol/kgbw not presented)	Sleet *et al*, 1987, 1988
Injection *i.v.*					
Rat, Wistar, 5 M, 5 F		100	1 x	The plasma-concentration data fitted well to a one-compartment model. Elimination half-life from plasma data was higher in F (18.6 h) than in M (13.2 h). There was no difference in the elimination half-lives from urine data (21.8 to 21.4 h). Renal clearance was only 25 % of total clearance	Aasmoe *et al*, 1999
Mouse, CD-1, 3 F	2.9, 3.8	261, 342	1 x on g.d. 11	Radioactivity in maternal blood measured 30 to 360 min after dosing reached peak levels of 410.2 nmol equivalents/100 µl whole blood after 30 min at 3.8 mmol/kgbw (data for 2.9 mmol/kgbw not presented)	Sleet *et al*, 1987, 1988

Table 4.8.4: Absorption (uptake), distribution, metabolism and elimination of MAA (cont'd)

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Injection *i.v.*	**(mmol/kgbw)**	**(mg/kgbw)**			
Mouse, CD-1, 4 F	2.9, 3.8	261, 342	1 x on g.d. 11	Ratio of tissue to whole-blood radioactivity measured 6 h after treatment with 3.8 mmol/kgbw was: in maternal liver (0.74) and kidney (0.78); in conceptus compartment (n = 16): in intact conceptus 1.56, in embryo 1.58, in placenta/membranes 0.97 (data for 2.9 mmol/kg not presented)	Sleet *et al*, 1987, 1988

4.9 Substance profile: EGEE

4.9.1 Identity

Name:	Ethylene glycol ethyl ether
IUPAC name:	2-Ethoxyethanol
CAS registry No.:	110-80-5
Molecular formula:	$C_4H_{10}O_2$
Structural formula:	C_2H_5–O–CH_2–CH_2–OH
Molecular weight:	90.1
Other components:	No data

4.9.2 Physico-chemical properties

Melting point:	< –80°C
Boiling point:	135 - 137°C
Vapour pressure:	5 hPa
Solubility in water:	No data
Relative density:	D_4^{20} = 0.931

4.9.3 Conversion factors

1 ppm = 3.745 mg/m^3
1 mg/m^3 = 0.267 ppm

4.9.4 Toxicological data

4.9.4.1 Acute toxicity

Oral

Rat:	LD_{50} 2,125 - 5,487 mg/kgbw (Laug *et al*, 1939; Smyth *et al*, 1941; Carpenter *et al*, 1956; Stenger *et al*, 1971; Cheever *et al*, 1984; Pis'ko and Werbilow, 1988; Gingell *et al*, 1994). Male animals appeared to be more sensitive than females. Main signs of toxicity were dyspnoea, ataxia, and loss of righting reflex.

Mouse: LD_{50} 2,451 - 4,831 mg/kgbw (Laug *et al*, 1939; Stenger *et al* 1971; Eastman Kodak, 1982 cited by RTECS, 1991; Pis'ko and Werbilow, 1988).

Rabbit: LD_{50} 3,100 mg/kgbw (Carpenter *et al*, 1956) and 1,486 mg/kgbw (Stenger *et al*, 1971).

Guinea pig: LD_{50} 2,137 mg/kgbw (Stenger *et al*, 1971); 1,400 mg/kgbw (Smyth *et al*, 1941) and 2,595 mg/kgbw (Laug *et al*, 1939).

Dermal

Rabbit: 24-h LD_{50} 3,311 mg/kgbw (Carpenter *et al*, 1956) or 3,900 mg/kgbw (Daughtrey *et al*, 1984). The studies used undiluted EGEE under occlusive patch.

Inhalation

Rat: 4-h LC_{50} 16 mg/l (4,270 ppm); 8-h LC_{50} 8 mg/l (2,140 ppm) (Carpenter *et al*, 1956). Female rats tolerated several 4-hour whole-body exposures of 4,000 ppm (14,980 mg/m^3), while a single 4-hour exposure of 5,656 ppm (21,180 mg/m^3) resulted in over 60% mortality (Goldberg *et al,* 1962). Single 3-hour whole-body exposure of male Alpk/Ap rats to 17 mg/l (4,500 ppm) caused reduction in testes weight and haematuria at terminal kill after 14 days (Doe, 1984b).

Intraperitoneal

Rat: LD_{50} 2,140 mg/kgbw (Carpenter *et al*, 1956).

Mouse: LD_{50} 1,709 mg/kgbw (Karel *et al,* 1947).

Intravenous

Rat: LD_{50} 2,380 mg/kgbw (undiluted), 3,250 mg/kgbw (in NaCl) (Carpenter *et al*, 1956).

4.9.4.2 Irritation and sensitisation

Skin irritation

EGEE was non-irritant (4-h occluded exposure) (Zissu, 1995) or slightly irritant to rabbit skin (4-h non-occluded exposure) (Kodak, 1982 cited by RTECS, 1991).

Eye irritation

EGEE was slightly irritant to the eye of rabbits (Carpenter and Smyth, 1946; Sanderson, 1959; Kodak, 1982 cited by RTECS, 1991).

Sensitisation

No data are available.

4.9.4.3 Repeated-dose toxicity (Table 4.9.1)

Various studies have been conducted with EGEE following oral or dermal application in rat and mouse. The main systemic findings were histopathological changes in liver, kidney, spleen and testis. Main haematological findings included changes in Hb and Hct.

Subacute toxicity

Male Long Evans rats were orally administered 0, 150 or 300 mg EGEE/kgbw/d for 6 weeks. Effects at 300 mg/kgbw were reduced testes weights and numbers of spermatocytes, as well as increased sperm abnormalities. The NOAEL was 150 mg/kgbw (Hürtt and Zenick, 1986). Daily oral gavage with 500 or 1,000 mg EGEE/kgbw for 11 days produced testicular changes in SD rats. The NOAEL was 250 mg/kgbw/d (Foster *et al*, 1983, 1984). Long Evans rats orally dosed with 936 mg EGEE/kgbw/d for 6 weeks showed abnormal spermatocytes and decreased Hb and Hct counts (Oudiz and Zenick, 1986).

Following a 10-day dermal application to female SD rats (undiluted material), 4,428 mg/kgbw was the NOAEL, while 6,200 and 8,857 mg/kgbw caused ataxia (Hardin *et al*, 1982).

In the ICL-ICR mouse a NOAEL of 500 mg EGEE/kgbw/d was determined following 5 weeks of oral treatment. Testicular atrophy was diagnosed at 1,000 to 4,000 mg/kgbw (Nagano *et al*, 1979).

Male and female F344 rats received EGEE in the drinking water at estimated dose levels ranging from 200 to 1,600 mg/kgbw/d for 2 weeks. Water consumption and body weight gain were decreased and thymus and testes were atrophic (NTP, 1983). When male and female $B6C3F_1$ rats received EGEE in the drinking water at estimated dose levels ranging from 400 to 2,800 mg/kgbw/d for 2 weeks, there were no adverse effects (NTP 1983).

Subchronic toxicity

The results of subchronic studies revealed that EGEE produced histopathological changes in liver, kidney, spleen and testis as well as haematological changes as the main findings in rats, mice, rabbits and dogs.

In two 90-day drinking water studies in rats, the NOAELs were 210 mg EGEE/kgbw/d (Smyth *et al*, 1951) or 600 mg/kgbw/d (NTP, 1993). In a 13-week inhalation study in Wistar rats the NOAEL was 100 ppm EGEE vapour. The same NOAEL was reported in a 13-week inhalation study in rabbits, where at the higher concentration levels essentially degenerative changes in testes, thymus, and haematopoietic tissues (spleen, bone marrow, and liver) were observed (Biodynamics, 1983a,b; Barbee *et al*, 1984).

4.9.4.4 Genotoxicity and cell transformation (Table 4.9.2)

In vitro

EGEE showed no genotoxic activity in point mutation assays including the Ames test *in Salmonella typhimurium* as well as the mammalian cell test systems of mouse lymphoma and the HGPRT-test in CHO cells (Ong, 1980; Shimizu *et al*, 1985; Zeiger *et al*, 1985; Guzzie *et al*, 1986; Myhr and Bowers, 1986).

Some *in vitro* test systems indicated clastogenic activity since EGEE enhanced the formation of SCE and chromosomal aberrations in CHO cells. However, the applied concentrations were exceedingly high (up to 9,510 μg/ml). In most cases the response was eliminated after addition of a metabolic activation system, which is in good accordance with the below-mentioned *in vivo* studies (Galloway *et al*, 1987; Guzzie *et al*, 1986). In V79 cells EGEE caused a small, statistically significant SCE increase at high concentrations but did not induce chromosome

aberrations in these cells as well as in human lymphocytes. These tests were carried out without metabolic activation (Elias *et al*, 1996).

In a micronucleus assay and a test for measuring aneugenic effects, both in V79 cells, EGEE was weakly positive at high concentrations (Elias *et al*, 1996).

At non-cytotoxic concentrations of 55 to 166 mmol/l, EGEE inhibited metabolic cooperation between V79 cells but was inactive in a cell transformation assay with SHE cells (Elias *et al*, 1996).

In vivo

EGEE was tested for induction of sex-linked recessive lethal mutations in *Drosophila melanogaster* and micronucleated PCEs in mouse peripheral blood. The results of these *in vivo* studies did not suggest a genotoxic activity of EGEE (McGregor, 1984; Valencia *et al*, 1985; Guzzie *et al*, 1986; Elias *et al*, 1996).

Taking into account that the mostly weakly positive results were observed only *in vitro* and at high concentrations with partly poorly defined genetic endpoints (i.e. SCE), and that both *in vivo* tests and a number of *in vitro* tests with a high specificity and sensitivity were negative, the overall conclusion is that EGEE has no relevant genotoxic potential.

4.9.4.5 Chronic toxicity and carcinogenicity (Table 4.9.1)

Inbred male albino rats were fed diets containing 0 or 1.45% EGEE (corresponding to 0 or 725 mg/kgbw/d) for 24 months. Growth and mortality were not affected. Two-thirds of the animals showed enlarged oedematous testes with tubular atrophy and a slight increase in chronic kidney damage (Morris *et al*, 1942).

In a life-time gavage study, F344 rats and $B6C3F_1$ mice received oral doses of 0, 500, 1,000 or 2,000 mg EGEE/kgbw/d for 103 weeks. Because of high mortality in the high-dose group all animals were killed following week 18 of treatment. At 1,000 mg/kgbw, survival of rats was reduced. In the rat, a dose-dependent decrease in body weight gain was observed, whereas in mice, no decrease was seen. Administration of EGEE at 2,000 mg/kgbw was lethal to rats and mice. Early mortality in the high-dose groups of rats and mice appeared to be due to stomach ulceration. EGEE caused testicular atrophy in both male rats and mice. This effect was apparent in high dose male rats, which died early, and in the medium and high dose male mice. Gross observations indicated that chronic treatment with EGEE at dose levels of 500 or 1,000 mg/kgbw

caused an apparent enlargement of the adrenal gland in male rats and reduced the occurrence of spontaneous gross lesions of the spleen, pituitary gland and testes that commonly occur in the aging male F344 rat. Chronic treatment with EGEE also caused a decrease in the incidences of enlarged spleens and pituitaries and of s.c. masses in the mammary gland region of the aging female F344 rat (further histopathological details were not reported) (Melnick, 1984).

4.9.4.6 Reproductive and developmental toxicity (Table 4.9.3)

Reproductive, teratogenic, embryotoxic, and foetotoxic effects of EGEE have been reported in the rat, the mouse and the rabbit following oral, dermal and inhalation exposure. At high paternally toxic doses/concentrations testicular atrophy in conjunction with histopathological changes in the testis of males of all species tested were observed. Female fertility was not affected (references and study details are given in Table 4.9.3). Co-administration of toluene and xylene with EGEE to male rats attenuated the testicular atrophy induced by EGEE, as well as reduced the plasma level of EAA. The data suggest that atrophy is related to peak plasma levels of EAA rather than AUC (Chung *et al*, 1999).

Embryotoxicity, such as mortality and post-implantation losses and foetotoxicity (decreased foetal weight), were observed in rodents. Teratogenic effects, such as increased skeletal and cardiovascular malformations, were seen predominantly in rat, whereas exencephaly and cleft palate were only seen in the mouse. Only minor skeletal variations were noted in rabbits (references and study details are given in Table 4.9.3).

In several studies, an increased post-implantation and postnatal mortality was observed (Stenger *et al*, 1971; Nelson *et al*, 1981; Hardin *et al*, 1982; Tinston *et al*, 1983a,b; Andrew and Hardin, 1984; Doe, 1984a; Goad and Cranmer, 1984; Hardin *et al*, 1984; Schuler *et al*, 1984; Chester *et al*, 1986; Wier *et al*, 1987). Some of these studies were designed to determine NOAELs for the different species tested. For teratogenic and foetotoxic effects, the NOAELs were 50 ppm in rats and rabbits and 23 mg/kgbw following oral administration in the rat or s.c. injection in the rabbit (Stenger *et al*, 1971; Tinston *et al*, 1983a,b; Doe, 1984a).

The developmental toxicity of EGEE was also assessed in *Drosophila melanogaster* after exposure to dose levels ranging from 54 to 78 mg/culture throughout development. The incidence of wing notches was significantly increased at all concentrations, that of bent humeral bristles at 71 mg/culture (Lynch and Toraason, 1996).

4.9.4.7 Kinetics and metabolism (Table 4.9.4)

Uptake *in vitro*

EGEE is absorbed through human skin *in vitro* at a rate of 0.796 mg/cm^2/h (permeability constant 8.42 cm/h x 10^4); the damage ratio (of final ^3H_2O permeability constant after 8 hours to initial value) was 2.74 (Dugard *et al*, 1984).

Skin permeation was calculated using the Franz cell method with human skin. EGEE was tested in pure form and with 70% acetone. In pure form the lag time was 59 minutes, flux at steady state was 0.820 mg/cm^2/h, and permeation 0.882 cm/h x 10^{-3}. In mixture with acetone the respective values were 43 minutes, 0.833 mg/cm^2/h and 2.980 cm/h x 10^{-3} (Larese *et al*, 1999).

The permeation rate of EGEE through non-occluded rat split skin was 20% greater than through rat whole skin (11%). Absorption through human split skin (8%) was lower than in the rat skin. First pass metabolism was not detected during percutaneous penetration through viable human or rat skin. The *in vitro* system provided a reasonable estimate of dermal absorption for the rat *in vivo* and comparison of human and rat skin *in vitro* indicated EGEE absorption in humans is about one-third of that in the rat (Lockley *et al*, 2002).

Percutaneous absorption of EGEE, in aqueous solution or undiluted, through full thickness or dermatomed human breast skin was measured for 24 hours using flow-through diffusion cells. In aqueous solution, steady-state flux was 143 nmol/cm^2/h, time to steady state 1.67 h, and the final level of absorption 0.34 μmol in dermatomed skin. In full-thickness skin time to steady state was nearly doubled (3.33 h), while the steady-state flux was not significantly affected, and the final level of absorption was slightly decreased (Wilkinson and Williams, 2002).

In vivo

Following oral or inhalation exposure of F344 rats, 60 to 80% of the administered dose of EGEE was excreted in the urine. The main urinary metabolites identified were EAA (25 - 40%) and ethylene glycol (18%). Approximately 20% was exhaled as CO_2 whereas 1 to 3% was exhaled unchanged, and 0.5 to 5% of the dose was excreted by the faeces (Medinsky *et al*, 1990). Following dermal application, EAA was also determined as the main urinary metabolite. The half-life for the elimination of EAA was approximately 7.2 hours (Cheever *et al*, 1984; Groeseneken *et al*, 1988; Medinsky *et al*, 1990; Sabourin *et al*, 1992b).

The effect of dose on the absorption, metabolism, and excretion of ^{14}C-labeled EGEE by F344/N rats was investigated after inhalation exposure to either 5 ppm for 5 hours and 40 minutes, or

46 ppm for 6 hours. The uptake and metabolism were linear. Significant percentages of the retained doses were exhaled during (22%) and after exposure (16%) as CO_2, 46% excreted in the urine. Approximately 10% of the retained dose was detected in the carcass 66 hours after exposure. The major urinary metabolite, EAA, was linearly related to exposure concentration (Kennedy *et al*, 1993).

As shown in experimental studies, the main metabolite following inhalation of EGEE by human volunteers was determined to be EAA (Groeseneken *et al*, 1986a,b; 1987a). Urinary excretion of EAA has been used to determine the dermal absorption of EGEE in liquid and vapour forms in human volunteers. Values of 19 cm/h for 990 ppm EGEE vapour and 0.7 mg/cm^2/h for undiluted liquid were reported (Kežić *et al*, 1997) (Section 3.1.1).

Topical application of ^{14}C-EGEE to occluded rat skin *in vivo* resulted in 25% of the dose being absorbed after 24 hours. The major routes of excretion included the urine (15%), expiration as CO_2 (6%), and faeces (1.2%). Free EGEE, EAA and glycine conjugate were detected in urine (Lockley *et al*, 2002).

Ethanol pretreatment (20 mmol/kgbw i.p.) efficiently blocked oxidation of EGEE (420 ppm, 2 h inhalation), and this led to higher plasma levels of EGEE in female SD rats than expected from the original clearance rates. After single i.p. co-administration of 10 mmol/kgbw (901 mg/kgbw) with ethanol (20 mmol/kgbw) the blood levels of EGEE remained nearly constant as long as ethanol blood levels were above 3 mmol/l, whereas repeated i.p. dosing plus ethanol resulted in an almost complete accumulation in the blood (Römer *et al*, 1985). This finding is indicative for the involvement of the liver ADH-enzyme system.

4.9.4.8 Neurotoxicity

Trained female CFE rats were exposed (whole-body, 4 h/d, 5 d/wk) for 2 weeks to concentrations of 0, 500, 1,000, 2,000 or 4,000 ppm EGEE (0, 1,870, 3,750, 7,500 or 15,000 mg/m^3). Tests included conditioned avoidance-escape behaviour by a modification of the pole-climb method. Besides a transient body weight retardation at 4,000 ppm, no other effect was observed (Goldberg *et al*, 1964).

4.9.4.9 Immunotoxicity

In an experiment designed to compare the potential immunosuppressive activity of various glycol ethers EGEE was administered orally to male F344 rats at dosages ranging from 50 to

400 mg/kgbw on 2 consecutive days. EGEE (in contrast with EGME, EGMEA, and MAA) did not suppress PFC response to TNP-LPS (Smialowicz *et al*, 1992b).

4.9.5 Human effects data

A case of human poisoning by accidental ingestion of approximately 40 ml EGEE (37 g) has been reported. Main symptoms were cyanosis, oedema of the lung and convulsion. The subject recovered after approximately 40 days (Fucik, 1969 cited by Rowe and Wolf, 1982).

The concentration of the main metabolite EAA in the urine of exposed workers has been used as a qualitative measure in workplace monitoring studies (Veulemans *et al*, 1987b; Ratcliffe *et al*, 1989; Angerer *et al*, 1990). Some of these occupational studies included investigations of sperm samples. The incidence of oligospermia was increased in some cases, whereas sperm morphology and sperm motility were not affected (Sparer *et al*, 1988; Welch *et al*, 1988; Welch and Cuflen, 1988; Ratcliffe *et al*, 1989, Schrader *et al*, 1996). In one investigation of painters a significant proportion of the examined individuals were anaemic (10%) and granulocytopenic (5%), compared to none of the controls (Welch and Cullen, 1988).

Veulemans *et al* (1993) carried out a case controlled study of 1,019 first-time patients of a clinic for reproduction disorders, defined as patients diagnosed as infertile or sub-fertile on the basis of a spermiogram. The investigation suggested a correlation between exposure to EGEE, the occurrence of urinary metabolites (EAA) and impaired sperm morphology. Firm conclusions could not be drawn because of the unknown latency period between exposure and sampling time.

Table 4.9.1: Systemic toxicity of EGEE

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, feeding				
Rat, inbred albino, 20 M	0 1.45% corresponding to about 725 mg/kgbw	24 months	No effects ↑ enlarged oedematous testes with atrophic tubules and slight chronic kidney damage	Morris *et al*, 1942
Oral, gavage	**(mg/kgbw)**			
Rat, Wistar, 5 M, 5 F	0, 46 93 (372 from d 59) 186 (743 from d 59)	1 x/d, 5 d/wk, 13 wk	No effects NOAEL Histopathological changes in testes and spleen. ↓ Hb and Hct	Stenger *et al*, 1971
Rat, SD, 36 M	0 250 500, 1,000	1 x/d, 5 d/wk, 11 d	No effects NOAEL Histopathological changes in testes	Foster *et al*, 1983, 1984
Rat, Long Evans, 9 M	0 936	1 x/d, 5 d/wk, 6 wk	No effects Abnormal spermatocyte morphology, ↓ Hb and Hct	Oudiz and Zenick, 1986
Rat, Long Evans, 11 - 13 M	0 150 300	1 x/d, 5 d/wk, 6 wk	No effects NOAEL Abnormal spermatocyte morphology, ↓ testes weight and number of spermatocytes	Hürtt and Zenick, 1986

Table 4.9.1: Systemic toxicity of EGEE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage (cont'd)	**(mg/kgbw)**			
Rat, F344/N, 50 M, 50 F	0 500 1,000 2,000	1 x/d, 5 d/wk, 103 wk	No effects Enlargement of adrenal gland (M), ↓ bw ↓ bw and survival, enlargement of adrenal gland (M) High mortality due to stomach ulcers (termination wk 18), testicular atrophy	Melnick, 1984
Mouse, B6C3F$_1$, 50 M, 50 F	0 500 1,000 2,000	1 x/d, 5 d/wk, 103 wk	No effects NOAEL Testicular atrophy High mortality due to stomach ulcers (termination wk 18)	Melnick, 1984
Mouse, ICL-ICR, 5 M	0 500 1,000, 2,000, 4,000	1 x/d, 5 d/wk, 5 wk	No effects NOAEL Testicular atrophy	Nagano *et al*, 1979
Dog, 3 M, 3 F	0, 46 93 186	6 h/d, 5d/wk, 13 wk	No effects NOAEL Histopathological changes of the germinal epithelium in testes	Stenger *et al*, 1971
Oral, drinking water	**(mg/kgbw)**			
Rat, 10 M, 10 F	0, 52 210 740, 1,890	*Ad libitum*, 90 d	No effects NOAEL LOAEL. ↓ bw and food consumption; histopathological changes in liver, kidney, spleen, and testis	Smyth *et al*, 1951

Table 4.9.1: Systemic toxicity of EGEE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, drinking water (cont'd)	**(mg/kgbw)**				
Rat, 10 M, 10 F	0, 52 210 740, 1,890		*Ad libitum*, 90 d	No effects NOAEL LOAEL. ↓ bw and food consumption; histopathological changes in liver, kidney, spleen, and testis	Smyth *et al*, 1951
Rat, F344/N, 5 M, 5 F	0 200, 600, 900 1,500 2,500		*Ad libitum*, 14 d	No effects ↓ bw (M) ↓ bw (F), testis degeneration (mild) Testis degeneration (marked)	NTP, 1992
	(mg/l)	**(mg/kgbw)**			
Rat, F344/N, 10 M, 10 F	0 1,250 2,500 5,000, 10,000 20,000	(0 109 205 400, 792 2,240)	*Ad libitum*, 90 d	No effects NOAEL Anaemia, ↓ thymus wt (M and F) ↓ bw (M and F), testis degeneration Survival: 5/10 (M), 3/10 (F)	NTP, 1992
	(mg/kgbw)				
Mouse, B6C3F_1, 5 M, 5 F	0 300, 600, 900, 1,500 2,500		*Ad libitum*, 14 d	No effects No microscopic examination of the tissues was performed ↓ relative testis weight	NTP (1992)

Table 4.9.1: Systemic toxicity of EGEE (cont'd)

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Mouse, B6C3F_1, 10 M, 10 F	0 2,500, 5,000 10,000 20,000, 40,000		*Ad libitum*, 90 d	No effects Hypertrophy of F adrenal gland in all dose groups Haematopoiesis in spleen of F from 10,000 ppm upwards ↓ bw, testicular degeneration	NTP, 1992
Dermal	**(mg/kgbw)**				
Rat, SD, 5 F	0, 1,552, 2,214, 3,100 4,428 6,200, 8,857		1 x/d, 5 d/wk,10 d	No effects NOAEL Ataxia	Hardin *et al*, 1982
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, Wistar, 15 M, 15 F	0 25 100 400	(0 94 375 1,500)	6 h/d (whole-body), 5 d/wk, 13 wk	No effects ↑ lachrymation ↓ lachrymation ↑ lachrymation, ↓ pituitary wt (M), no histopathological correlations	Biodynamics, 1983a; Barbee *et al*, 1984
Rat, Wistar Alderley Park	(4,540)	17,000	1 x	Testicular atrophy, haematuria	Doe, 1984b

Table 4.9.1: Systemic toxicity of EGEE (cont'd)

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Inhalation	**(ppm)**	**(mg/m^3)**			
Rabbit, NZW, 10 M, 10 F		(0	6 h/d (whole-body), 5 d/wk, 13 wk	No effects	Biodynamics, 1983b; Barbee *et al,* 1984
	25	94		↑ lachrymation	
	100	375		↑ lachrymation	
	400	1,500)		↑ lachrymation, ↓ testes weight, histopathological changes in testes	
Dog, 2 M, 2 F	(839)	3,142	7 h/d (whole-body), 5 d/wk, 12 wk	↓ Hb and Hct	Werner *et al,* 1943b

Table 4.9.2: Genotoxicity of EGEE

Endpoint / Organism	Strain or type / Target	Dose / concentration	Result	Remark	Reference
In vitro					
Gene mutation					
Salmonella typhimurium	TA1535, TA100, TA1537, TA98	0 - 23 mg/plate	**–ve**	+/– S9	Ong, 1980
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	5 - 5,000 µg/plate	**–ve**	+/– S9	Shimizu *et al*, 1985
Salmonella typhimurium	TA1535, TA100, TA1537, TA98	100 - 1,000 µg/plate	**–ve**	+/– S9	Zeiger *et al*, 1985
Salmonella typhimurium	NS	0 - 93.3 mg/plate	**–ve**	+/– S9	Guzzie *et al*, 1986
Mouse lymphoma	L51784 TK+/–	NS	**–ve**	+/– S9	Myhr *et al*, 1986
CHO cells	HGPRT	0 - 42 mg/ml	**–ve**	+/– S9	Guzzie *et al*, 1986
Chromosome aberration					
Human lymphocyte		NS	**–ve**	48 h	Villalobos-Pietrini *et al*, 1989
CHO cells		4,780 - 9,510 µg/ml	**+ve**	+/– S9	Galloway *et al*, 1987
CHO cells		NS	**+ve**	– S9	Guzzie *et al*, 1986
CHO cells		NS	**–ve**	+S9	Guzzie *et al*, 1986
V79 cells		Up to 166 mmol/l	**–ve**	– S9	Elias *et al*, 1996
Human lymphocytes		NS	**–ve**	– S9	Elias *et al*, 1996
Sister chromatid exchange					
Human lymphocyte		NS	**+ve**	48 h	Villalobos-Pietrini *et al*, 1989
CHO cells		951 - 9,510 µg/ml	**+ve**	+/– S9	Galloway *et al*, 1987
CHO cells		NS	**+ve**	+/– S9	Guzzie *et al*, 1986

Table 4.9.2: Genotoxicity of EGEE (cont'd)

Endpoint / Organism	Strain or type / Target	Dose / concentration	Result	Remark	Reference
Sister chromatid exchange (cont'd)					
V79 cells		NS	**Weakly +ve**	– S9	Elias *et al*, 1996.
Micronucleus frequency					
V79 cells		27.74 - 55.50 mmol/l 111.00 mmol/l	**–ve** **Weakly +ve**	– S9	Elias *et al*, 1996.
In vivo					
Micronucleus frequency		**(mg/kgbw)**			
Mouse	Swiss Webster, sex NS, peripheral blood	1 x 647, 1,295 or 2,071 i.p. at 30, 48, 72 h	**–ve**		Guzzie *et al*, 1986
Mouse	CD-1, 4 M, 4 F, bone marrow	1 x 1,368, 1,710, 2,500 or 3,000 i.p. at 24 h	**–ve**		Elias *et al*, 1996.
Sex-linked recessive lethal mutations		**(mg/l)**			
Drosophila melanogaster	ORK m; M-5f	Injection, NS	**–ve**		McGregor, 1984
Drosophila melanogaster	NS	Injection, 50,000	**±**		Valencia *et al*, 1985
Drosophila melanogaster	NS	Oral feed, 20,000	**–ve**		Valencia *et al*, 1985
Drosophila melanogaster	ORK m; M-5f	Oral feed, NS	**–ve**		McGregor, 1984

Table 4.9.3: Reproductive and developmental toxicity of EGEE

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage				
Rat, Wistar, 20 - 39 F	0, 12	1 x/d, g.d. 1 - 21	No effects	Stenger *et al*, 1971
	23		NOAEL	
	46		↓ foetuses and implantations	
	93		↑ skeletal variations (21%)	
	186, 372		↑ skeletal variations (90%)	
Rat, SD, NS	200	1 x/d, g.d. 7 - 9	5% cardiovascular abnormalities, ↓ foetus bw	Goad and Cranmer, 1984
		1 x/d, g.d. 10 - 12	11% cardiovascular abnormalities, ↓ foetus bw	
		1 x/d, g.d. 13 - 15	1% cardiovascular abnormalities, ↓ foetus bw	
		1 x/d, g.d. 7 - 15	24% cardiovascular abnormalities, ↑ prenatal mortality, ↓ maternal bw gain	
Rat, Long Evans, 4 M	0	1 x/d, 5 d; 4 M/group mated with ovari-ectomised, hormonally primed F on several occasions over 2 wk	No effects	Oudiz *et al*, 1984
	936		↓ sperm counts, ↑ abnormal sperm morphology	
	1,872		↓ sperm counts; azoospermia	
	2,808		↓ sperm counts; azoospermia	

Table 4.9.3: Reproductive and developmental toxicity of EGEE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage (cont'd)	**(mg/kgbw)**			
Rat, SD, 20 M	0	1 x/d, 5 or 7 wk; 10 M/group mated	No effects	Horimoto *et al*, 2000
(19 M control)	250		↓ testis and epididymis weight; ↓ sperm parameters	
	500		↓ testis and epididymis weight; ↓ sperm parameters; ↓ fertility	
Oral, drinking water				
Rat, NS	0	*Ad libitum*, g.d. 1 - 21	No effects	Chester *et al*, 1986
	210		No maternal toxicity	
	270		31% embryo-mortality	
	400		69% embryo-mortality; ↓ foetus bw	
	550		Delayed foetal development; embryo-mortality	
Mouse, CD-1, 50 F	0	*Ad libitum*, g.d. 7 - 14	No effects	Schuler *et al*, 1984
	3,605		100% post-implantation loss	
Mouse, CD-1, 6 F	0	*Ad libitum*, g.d. 8 - 14	No effects	Wier *et al*, 1987
	1,000		↑ post-implantation losses	
	1,800, 2,600, 3,400		↑ exencephaly, cleft palate	
	4,200		100% embryo lethality	

Table 4.9.3: Reproductive and developmental toxicity of EGEE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, drinking water (cont'd)					
Mouse, CD-1, 20 F	0 800 1,200		*Ad libitum*, g.d. 8 - 14	No effects ↓ number of live pups; ↓ pup bw ↑ kinked tail	Wier *et al*, 1987
Mouse, CD-1, 40 M, 40 F	0 800, 1,500, 2,600		*Ad libitum*, g.d. 8 - 14	No effects Dose-related ↑ testicular atrophy. No effects on female fertility	Wier *et al*, 1987
	(%)	**(mg/kgbw)**			
Mouse, CD-1, 20 M, 20 F	0	0	*Ad libitum*, continuous breeding	No effects	Morissey *et al*, 1989; Chapin and Sloane, 1997
	0.5	760		NOAEL	
	1	1,500		F_0 ↓ litter/pair, ↓ live pups/litter, ↓ pup bw; ↑ gestation length; ↓ weight testis, ↑ sperm abnormalities	
	2	2,600		F_0 no live litters; ↓ weight testis, epididymis, seminal vesicle; ↓ sperm number, ↑ sperm abnormalities, ↑ vaginal cycle length	
Dermal	**(mg/kgbw)**				
Rat, SD, 25 F	0 3,445 6,889		4 x/d, g.d. 7 - 16	No effects ↓ live foetuses; ↓ foetal bw ↑ skeletal variations and cardiovascular malformations	Hardin *et al*, 1982

Table 4.9.3: Reproductive and developmental toxicity of EGEE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Dermal (cont'd)	**(mg/kgbw)**				
Rat, 18 F	0 3,875		4 x/d, g.d. 7 - 16	No effects Skeletal, cardiovascular, renal malformations	Hardin *et al*, 1984
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, 24 F	0, 10 50 250	(0, 37 190 940)	6 h/d (whole-body), g.d. 6 - 15;	No effects NOAEL ↓ Hb and Hct; ↑ skeletal variations	Tinston *et al*, 1983b; Doe *et al*, 1984a
Rat, SD, 2 - 6 F	0 300 600 900, 1,200	(0 1,120 2,250 3,370, 4,500)	7 h/d, g.d. 7 - 13 (whole-body)	No effects NOAEL ↑ resorptions; ↓ live pups/dam 100% post-implantation loss	Nelson *et al*, 1981
Rat, SD, 2 - 6 F	0 200, 300 600, 900, 1,200	(0 750, 1,120 2,250, 3,370, 4,500)	7 h/d, g.d. 14 - 20 (whole-body)	No effects ↑ postnatal mortality 100% postnatal mortality	Nelson *et al*, 1981
Rat, SD, 35-38 F	0	(0	7 h/d, 3 wk pre-exposure and/or g.d.1 - 19 (whole-body)	No effects	Andrew and Hardin, 1984
	200 770	750 2,880)		No effect on female fertility. No effect on female fertility. ↑ skeletal variations and cardiovascular malformations	

Table 4.9.3: Reproductive and developmental toxicity of EGEE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Rabbit, NZW, 29 F	0 160 620	(0 600 2,320)	6 h/d, g.d. 6 - 18 (whole-body)	No effects ↑ skeletal variations and cardiovascular malformations 100% embryo mortality	Andrew and Hardin, 1984
Rabbit, NZW, 24 F	0, 10 50 170	(0, 37 190 640)	6 h/d, g.d. 6 - 18 (whole-body)	No effects NOAEL ↑ skeletal variations	Doe *et al*, 1984a; Tinston *et al*, 1983b
Injection s.c.	**(mg/kgbw)**				
Rat, Wistar, 20 F	0, 23 46 93		1 x/d, g.d. 1 - 21	No effects NOAEL ↑ skeletal variations	Stenger *et al*, 1971
Mouse, Swiss, 22 F	0 46		1 x/d, g.d. 1 - 18	No effects NOAEL	Stenger *et al*, 1971
Rabbit, Silver Yellow, 15 F	0 23		1 x/d, g.d. 7 - 18	No effects NOAEL	Stenger *et al*, 1971
	mg/culture vial				
Drosophila melanogaster	0 54, 59, 65, 71, 78 71		Throughout development	No effects ↑ wing blade notches ↑ bent humeral bristles	Lynch and Toraason, 1996

Table 4.9.4: Absorption (uptake), distribution, metabolism and elimination of EGEE

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime, sampling time	Result	Reference
Oral	**(mg/kgbw)**			
Rat, SD, (a) 4 M (b) 5 M	230	1 x, 96 h	(a) [1,2-^{14}C]-2-ethoxyethanol (b) [ethoxy-1-^{14}C]-2-ethoxyethanol Recovery (a) 99.3%, (b) 99.4%. Urinary excretion 76 - 80% ^{14}C; major urinary metabolites ethoxy acetic acid and *n*-ethoxy-acetyl-glycine. $^{14}CO_2$ expiration (a) 4.61%, (b) 11.7%. Biological $t_{½}$ (a) 12.5h, (b) 9.9h	Cheever *et al,* 1984
Rat, Wistar, 5 M	0.5, 1, 5, 10, 50, 100	1 x, 60 h	Recovery increased with dose 13.4 - 36.8%. Major urinary metabolites ethoxy acetic acid and *n*-ethoxy-acetyl-glycine. Biological $t_{½}$ 7.2 h	Groeseneken *et al,* 1988
Rat, F344/N, 4 M	10, 20, 120	24 h, 72 h	Recovery: 27%, 59%, 32% for corresponding doses. Major urinary metabolites ethoxy acetic acid (25 - 40%) and MEG (18%). CO_2 expiration 20%	Medinsky *et al,* 1990
Dermal, non-occluded	**(mg/kgbw)**			
Rat, F344/N, 4 M	12, 24, 60	1 x, 72 h	Dermal absorption of administered dose 20 - 25%. Major urinary metabolites ethoxy acetic acid (50%) and MEG (18%). CO_2 expiration 10%	Sabourin *et al,* 1992b
	(ppm) **(mg/m^3)**			
Human, volunteer, 2 M, 3 F	(990) / 1,000 cm^2 3,700 / 1,000 cm^2	45 min vapour	Absorption rate of vapour: 19 cm/h, of liquid: 0.7 mg/cm^2/h	Kežić *et al,* 1997

Table 4.9.4: Absorption (uptake), distribution, metabolism and elimination of EGEE

Route / Species, strain, number and sex/group	**Dose or concentration**	**Exposure regime, sampling time**	**Result**	**Reference**
Dermal, occluded				
Human, volunteer, 2 M, 3 F	Undiluted / 27 cm^2	15 min in glass chamber	Absorption rate 0.7 $mg/cm^2/h$	Kežić *et al*, 1997
In vitro				
Human, skin	Undiluted / 3.14 cm^2	4 h	Lag time 59 min. Penetration rate 820 $\mu g/cm^2/h$	Larese *et al*, 1999
Human, skin	Undiluted (10.5 µl) or diluted (3 or 6 mg/ml, 100 or 200 µl)	24 h	Steady-state flux: 143 $nmol/cm^2/h$ in aqueous solution, 6,481 $nmol/cm^2/h$ when undulited. Time to steady-state: 0.60 h (undiluted), 1.67 (aqueous solution)	Wilkinson and Williams, 2002
Inhalation	**(mg/m^3)**			
Human, volunteer, 5 M	10, 20, 40	4 h	Absorption 70% of inhaled EGEE. Major urinary metabolite EAA (23% of absorbed dose). Biological $t_{½}$ (EAA) 21 - 24 h	Groeseneken *et al*, 1986a, 1987a

4.10 Substance profile: EGEEA

4.10.1 Identity

Name:	Ethylene glycol (mono) ethyl ether acetate
IUPAC name:	2-Ethoxyethyl acetate
CAS registry No.:	111-15-9
Molecular formula:	$C_6H_{12}O_3$
Structural formula:	C_2H_5–O–CH_2–CH_2–O–CO–CH_3
Molecular weight:	132.2
Other components:	No data

4.10.2 Physico-chemical properties

Melting point:	–62°C
Boiling point:	153 - 159°C
Vapour pressure:	2 hPa
Solubility in water:	235 g/l
Relative density:	D_4^{20} = 0.9740

4.10.3 Conversion factors

1 ppm = 5.496 mg/m^3
1 mg/m^3 = 0.182 ppm

4.10.4 Toxicological data

4.10.4.1 Acute toxicity

Oral

Rat:	LD_{50} 2,900 - 7,500 mg/kgbw (Smyth *et al*, 1941; Pozzani *et al*, 1959; Truhaut *et al*, 1979). Main signs of toxicity were ataxia and transient haematuria.
Guinea pig:	LD_{50} 1,910 mg/kgbw (Smyth *et al*, 1941). Main signs of toxicity were ataxia and transient haematuria.
Rabbit:	LD_{50} 1,950 mg/kgbw (Carpenter, 1947). Main signs of toxicity were ataxia and transient haematuria.

Dermal

Guinea pig:	LD_{50} 1,818 mg/kgbw, occluded application (Carpenter, 1947); > 19,500 mg/kgbw, non-occluded application (Eastman, 1982).
Rabbit:	LD_{50} 10,300 - 10,500 mg/kgbw, occluded exposure (Carpenter, 1947; Truhaut *et al*, 1979).

Inhalation

Rat:	8-h LC_{50} 8.25 - 12.1 mg/l (1,500 - 2,200 ppm) (Pozzani *et al*, 1959; TyI *et al*, 1988).

4.10.4.2 Irritation and sensitisation

Skin irritation

EGEEA was not irritant to rabbit skin following the standard EEC study protocol upon 4 hours under occlusive patch (Zissu, 1995), and slightly irritant to rabbit skin according to the Draize protocol after 24 hours occluded exposure (Truhaut *et al*, 1979; Zissu, 1995). Similar results have been reported in the guinea pig (Eastman, 1982).

Eye irritation

Undiluted EGEEA (0.1 ml) was slightly irritant to the eye of rabbits (Von Oettingen and Jirouch, 1931; Carpenter and Smyth, 1946; Truhaut *et al*, 1979; Eastman, 1982; Kennah *et al*, 1989).

Sensitisation

EGEEA was not sensitising in guinea pigs after a cutaneous challenge concentration of 10% (Magnusson-Kligman test) (Zissu, 1995).

4.10.4.3 Repeated-dose toxicity (Table 4.10.1)

Subacute toxicity

ICL-ICR mice received daily oral doses of 0, 500, 1,000, 2,000 or 4,000 mg EGEEA/kgbw for 5 weeks. No compound related changes in the haematological parameters (except reduced leukocytes) were observed. At 1,000 mg/kgbw and above a dose-dependent increase of the incidence of testicular atrophy in male animals was reported (Nagano *et al*, 1979).

Subchronic toxicity

No data are available.

4.10.4.4 Genotoxicity (Table 4.10.2)

In vitro

EGEEA was not genotoxic when tested in *Salmonella typhimurium* (standard Ames test) up to 5,000 µg/plate (Hüls, 1989; Slesinski *et al*, 1988), the HGPRT-test and SCE-tests in CHO-cells (Slesinski *et al*, 1988).

In vivo

A mouse micronucleus assay (bone marrow; i.p. injection) revealed no genotoxic activity of EGEEA (Slesinski *et al*, 1988).

4.10.4.5 Chronic toxicity and carcinogenicity (Table 4.10.1)

In a comparative study with Wistar rats and NZW rabbits exposed (whole-body) by inhalation to EGEEA at a concentration of 200 ppm for 10 months no changes of any haematological parameter were observed in either species. Body weight gain was also in the range of the control. Histopathological investigations revealed no increase of incidences of testicular atrophy in the male rabbit and rat. However, in the rabbit and the male rat nephrotoxicity was observed characterised by tubular nephritis and degeneration of the epithelium with hyaline and granular tubular casts. No appreciable alterations were seen in the females (Truhaut *et al*, 1979).

In a 6-month study in the dog, whole-body inhalation of 600 ppm induced no effects on haematological parameters. No compound-related macroscopic or histopathological variations were observed either (Carpenter, 1947).

4.10.4.6 Reproductive and developmental toxicity (Table 4.10.3)

Oral

A multi-generation drinking water study in CD-1 mice was conducted following the continuous breeding protocol at doses of 0, 930, 1,860 or 3,000 mg EGEEA/kgbw. EGEEA was a reproductive toxicant as evidenced by significant decreases in the number of litters per fertile pair, decreased life pups per litter and decreased proportion of pups born live especially at the medium and highest dose levels. The females appeared to be more sensitive to the effects of EGEEA as judged by a nearly 50% drop of the fertility index and a decreased number of live pups per litter following cross-over mating. Decreased testes weight and increased incidences of abnormal sperms at 3,000 mg/kgbw did suggest modest effects on male mice. EGEEA treatment at 1% (1,860 mg/kgbw) also affected fertility and certain reproductive parameters in the second-generation mice but the observed response was not statistically significant. A significant finding was that 3,000 mg EGEEA/kgbw resulted in 30% reduction in caudal epididymal sperm density at 3,000 mg/kgbw of second-generation mice (Chapin and Sloane, 1997).

Dermal

In a dermal developmental toxicity study in pregnant SD rats, a dose of 5,923 mg EGEEA/kgbw applied between day 7 and 16 of gestation also induced an increased number of post-implantation losses as well as an increased incidence of cardiovascular and skeletal malformations (Hardin *et al*, 1984).

Inhalation

In a number of inhalation studies with EGEEA in pregnant F344 or SD rats and NZW or Dutch rabbits various embryotoxic, foetotoxic, and teratogenic effects were observed following exposure to high concentrations. Embryotoxicity was expressed as increased resorptions per litter and reduced foetal body weights. Teratogenic effects were skeletal and cardiovascular malformations (Tinston, 1983; Nelson *et al*, 1984b; Doe, 1984a; Tyl *et al*, 1988). A clear NOAEL for rabbits was 50 ppm (Tyl *et al*, 1988).

4.10.4.7 Kinetics and metabolism (Table 4.10.4)

EGEEA is rapidly absorbed after inhalation. Hydrolysis of the ester group occurs more or less quantitatively and the main route of excretion is the urine. In a study comparing percutaneous absorption in rat and human skin *in vitro* the following mean values were obtained: absorption rat skin 2.41 mg/cm^2/h, human skin 1.41 mg/cm^2/h; permeability constant rat skin 2.47 cm/h, human skin 1.45 cm/h (Barber *et al*, 1992).

The half-life time for hydrolysis of EGEEA in rat plasma (37°C) *in vitro* was determined to be 9.92 minutes, with subsequent formation of EGEE (Hoffmann and Jäckh, 1985).

Following i.v. application, approximately 60% of the applied EGEEA dose was excreted in the urine after 24 hours. Only minor amounts (1.6%) were exhaled as CO_2 (Guest *et al*, 1984). The penetration rate through human skin *in vitro* was determined to be 0.8 mg/cm^2/h (Dugard *et al*, 1984) and the absorption rate through dog skin *in vivo* 29 or 14.5 µg/min (Guest *et al*, 1984). The half-life of ^{14}C-EGEEA in the blood of dogs following i.v. administration was determined to be 7.9 hours (Guest *et al*, 1984).

Following inhalation of EGEEA and absorption via the respiratory system, the main metabolite in humans was identified as EAA (similar to results with EGEE, Section 4.9.4.7). Approximately 24% of the absorbed EGEEA dose was excreted as EAA with a half-life of 23.6 hours. Only 0.5% of the absorbed EGEEA was exhaled unchanged (Groeseneken *et al*, 1987a,b).

Similar to findings with EGEE, ethanol inhibited the oxidation of EGEE to EAA following inhalation exposure to EGEEA. This indicated an involvement of the liver ADH enzyme system in the oxidation of EGEE (Römer *et al*, 1985).

4.10.4.8 Neurotoxicity

No data are available.

4.10.4.9 Immunotoxicity

In an experiment designed to compare the potential immunosuppressive activity of various glycol ethers EGEEA was administered orally to male F344 rats at dosages ranging from 50 to 400 mg/kgbw on 2 consecutive days. EGEEA (in contrast with EGME, EGMEA and MAA) did not suppress PFC response to TNP-LPS (Smialowicz *et al*, 1992b).

4.10.5 Human effects data

A study of 52 female workers exposed to EGEEA in the liquid crystal display industry (mean 0.51 ppm, range 0.15 - 3.03 ppm, 8-h TWA; 2.8 mg/m^3, 0.8 - 16.65 mg/m^3), and 55 unexposed workers from the same factory, showed no apparent effect of exposure upon duration of the menstrual cycle, duration of the menses, or amount of flow (Chia *et al*, 1997).

The effects of EGEEA upon haematological parameters were studied in male shipyard workers using EGEEA-based paints. Exposed workers were assigned to high (n = 30) or low (n = 27) exposure groups based on work practice, and compared to a control group consisting primarily of office workers (n = 41). Personal monitoring was conducted on a subset of each exposure group, but the control group was not monitored to confirm the level of exposure to EGEEA or other substances. The mean 8-h TWA level of EGEEA exposure in the high exposure group was 3.03 ppm (16.65 mg/m^3) (peaks up to 18.27 ppm; 100.4 mg/m^3) and in the low exposure group 1.76 ppm (9.6 mg/m^3) (up to 8.12 ppm; 44.6 mg/m^3). The concentration of WBC and granulocytes were decreased and MCV was increased in the high exposure group compared to control. However, workers in both groups experienced co-exposure to high levels of other solvents (toluene, xylene, methyl isobutyl ketone), which were not accounted for in the analysis (Lee *et al*, 1999).

The identification of EAA as the main urinary metabolite after EGEEA exposure (Section 4.10.4) was used in several studies to monitor exposed workers. The studies showed that even low concentrations of EGEEA such as 1 ppm (5.5 mg/m^3) were sufficient to show an increased level of EAA in the urine of the workers (Veulemans *et al*, 1987b; Johanson *et al*, 1989; Angerer *et al*, 1990).

Table 4.10.1: Systemic toxicity of EGEEA

Route / Species, strain, number and sex/group	**Dose or concentration**	**Exposure regime**	**Result**	**Reference**
Oral, gavage	**(mg/kgbw)**			
Mouse, ICL-ICR, 5 M	0 500 1,000, 2,000, 4,000	1 x/d, 5 wk	No effects NOAEL Testicular atrophy; ↓ leukocytes	Nagano *et al*, 1979
Inhalation	**(ppm)** **(mg/m^3)**			
Rat, Wistar, 10 M, 10 F	0 (0 200 1,100)	4 h/d, 5d/wk, 10 months	Renal lesions). No testicular or haematological effects	Truhaut *et al*, 1979
Rabbit, NZW, 2 M, 2 F	0 (0 200 1,100)	4 h/d, 5d/wk, 10 months	Renal lesions. No testicular or haematological effects	Truhaut *et al*, 1979
Dog, 3 M, 3 F	0, 600 (0, 3,300)	7 h/d, 5d/wk, 6 months	NOAEL	Carpenter, 1947

Table 4.10.2: Genotoxicity of EGEEA

Endpoint / Organism	Strain or type / Target	Dose / concentration	Result	Remark	Reference
In vitro					
Gene mutation					
Salmonella typhimurium	TA1535, TA100, TA1538, TA98	5 - 5,000 µg/plate	–ve	+/– S9	Hüls, 1989
Salmonella typhimurium	NS	NS	–ve	+/– S9	Slesinski *et al*, 1988
CHO cells	HGPRT locus	NS	–ve	+/– S9	Slesinski *et al*, 1988
Sister chromatid exchange					
CHO cells		NS	**–ve**	+/– S9	Slesinski *et al*, 1988
In vivo					
Micronucleus frequency					
Mouse,	Swiss Webster, Sex NS, Bone marrow	NS, i.p.	**–ve**		Slesinski *et al*, 1988

Table 4.10.3: Reproductive and developmental toxicity of EGEEA

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, drinking water	**(ppm)**	**(mg/kgbw)**			
Mouse, CD-1, NS	0 0.5% 1% 2%	0 930 1,860 3,000	Continuous breeding	No effects NOAEL reproductive toxicity F_0: ↓ litter/pair, ↓ live pup/litter, ↓ pup bw; F_1: ↓ weight epididymis; ↓ sperm number F_0: ↓ litter/pair, ↓ live pup/litter, ↓ pup bw; ↓ weight testis; ↑ weight seminal vesicle, ↑ abnormal sperms, ↓ caudal epididymal sperm density and weight in F_1	Chapin and Sloane, 1997
Dermal	**(mg/kgbw)**				
Rat, SD, 18 F	0 5,923		4 x/d, g.d. 7 - 16	No effects ↑ post-implantation loss; ↑ cardiovascular and skeletal malformations	Hardin *et al*, 1984
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, SD, 9 - 20 F	0 130 390, 600	(0 710 2,140, 3,300)	7 h/d (whole-body), g.d. 7 - 15	No effects Skeletal variations ↑ resorptions/litter; ↑ skeletal and visceral malformations 100% resorptions	Nelson *et al*, 1984b
Rat, F344, 30 F	0 50 100, 200, 300	(0 275 550, 1,100, 1,650)	6 h/d (whole-body), g.d. 6 - 15	No effects ↑ resorptions/litter; ↑ skeletal variations ↓ foetal bw; ↑ skeletal and visceral malformations	Tyl *et al*, 1988

Table 4.10.3: Reproductive and developmental toxicity of EGEEA (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Rabbit, NZW, 8 F	0 100, 250, 450	(0 550, 1,370, 2,470)	6 h/d (whole-body), g.d. 6 - 18	No effects ↑ pre- and post-implantation loss; ↓ foetal bw at all dose levels	Tinston, 1983
Rabbit, NZW, 24 F	0 50 100 200, 300	(0 275 550 1,100, 1,650)	6 h/d, g.d. 6 - 18 (whole-body)	No effects NOAEL Skeletal and visceral malformations ↓ live pups; ↑ skeletal and visceral malformations	Tyl *et al*, 1988
Rabbit, Dutch, 24 F	0 25 100, 400	(0 140 550, 2,200)	6 h/d, g.d. 6 - 18 (whole-body)	No effects NOAEL ↓ foetal bw; ↑ post-implantation loss, skeletal and visceral malformations	Doe, 1984a

Table 4.10.4: Absorption (uptake), distribution, metabolism and elimination of EGEEA

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime, sampling time**	**Result**	**Reference**
Oral, drinking water	**(mg/l)**	**(mg/kgbw)**			
Rat, Wistar, 20 M	5,400	NS	*Ad libitum*, 2 wk	Excretion of oxalic acid approximately 10% that of EAA	Liesivouri *et al*, 1999
Dermal					
Dog, Beagle, 3	14.6 g		1 x 30 - 60 min, 24 h	Absorption rate: 29 or 14.5 µg/cm^2/min	Guest *et al*, 1984
Dog, Beagle, skin, *in vitro*	0.3 ml / 0.9 cm^2		7 h, diffusion cell	Absorption rate: 1.5 mg/cm^2/h	Guest *et al*, 1984
Human, skin, *in vitro*	5 ml / 1.2 cm^2		8 h, diffusion cell	Penetration rate 0.8 mg/cm^2/h	Dugard *et al*, 1984
Inhalation	**(ppm)**	**(mg/m^3)**			
Dog, Beagle, 4 M	50	275	1 x 5 h (whole-body)	Rapid absorption through lungs and elimination from blood	Guest *et al*, 1984
Human, 5 volunteers	2.5, 5, 9, 0.9, 2.5, 5	14, 28, 50 (at rest) or 5, 14, 28 (at exercise)	4 h	Major urinary metabolite EAA (22% of absorbed compound). Biological $t_{½}$ (EAA) 23.6 h	Groeseneken *et al*, 1987a, b
Intravenous	**(mg/kgbw)**				
Dog, Beagle, 3	1		1 x, 24 h	Approximately 60% dose excreted in urine within 24 h; 1.6% as CO_2. Biological $t_{½}$ (EGEEA in blood) 7.9 h	Guest *et al*, 1984

4.11 Substance profile: EGDEE

4.11.1 Identity

Name:	Ethylene glycol diethyl ether
IUPAC name:	1,2-diethoxyethanol
CAS registry No.:	629-14-1
Molecular formula:	$C_6H_{14}O_2$
Structural formula:	C_2H_5–O–CH_2–CH_2–O–C_2H_5
Molecular weight:	118.2
Other components:	Ethanol, EGEE

4.11.2 Physico-chemical properties

Melting point:	–74°C
Boiling point:	121°C
Vapour pressure:	12.5 hPa
Solubility in water:	204 g/l
Relative density:	D_4^{20} = 0.8484

4.11.3 Conversion factors

1 ppm = 4.914 mg/m^3
1 mg/m^3 = 0.204 ppm

4.11.4 Toxicological data

4.11.4.1 Acute toxicity

Oral

Rat:	LD_{50} > 4,390 mg/kgbw (10% aqueous solution) (Smyth *et al*, 1941).
Guinea pig:	LD_{50} 2,440 mg/kgbw (10% aqueous solution) (Smyth *et al*, 1941).

Dermal

No data are available.

Inhalation

Exposure of rabbits, guinea pigs, cats and dogs to 10,000 ppm EGDEE (49,000 mg/m^3) for 1 hour resulted in irritation of mucous membranes, possible narcosis, but no lethality. The cat was more sensitive than the rabbit, guinea pig or dog (Lehmann and Flury, 1938).

4.11.4.2 Irritation and sensitisation

Skin irritation

EGDEE was slightly irritant to rabbit skin following 4-hour application (OECD test) (Shell, 1991).

Eye irritation

EGDEE was slightly irritant to the eye causing an initial pain response. Conjunctival irritation and slight transitory injury of the cornea (Carpenter and Smyth, 1946).

Sensitisation

No data are available.

4.11.4.3 Repeated-dose toxicity (Table 4.11.1)

Subacute toxicity

Preliminary reports of studies conducted in a variety of animal species following EGDEE administration (oral, inhalation or by s.c. injection) indicated kidney damage and some mortality in selected species. In some species and via some treatment routes, however, no abnormalities were noted (Lehmann and Flury, 1938; Wiley *et al*, 1938).

Subchronic toxicity

No data are available.

4.11.4.4 Genotoxicity

No data are available.

4.11.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.11.4.6 Reproductive and developmental toxicity (Table 4.11.2)

Developmental toxicity screening assays indicated potential adverse developmental effects in mice and rats exposed to EGDEE (Schuler *et al*, 1984; Shell, 1988 cited by Tyl *et al*, 1988).

EGDEE was selectively toxic to the offspring of CD-1 mice dosed at levels not causing maternal toxicity. The NOAEL values for developmental and maternal toxicity in the mice were 50 and 500 mg/kgbw/d respectively. At 150 mg/kgbw and above a dose-related increase in malformations (a variety of external and skeletal) was observed (George *et al*, 1992). A similar pattern of malformations but, in addition, many visceral ones, were seen in NZW rabbits, with developmental and maternal NOAEL values of 25 and 100 mg/kgbw/d (George *et al*, 1992).

No data are available on which to assess the reproductive toxicity of EGDEE.

4.11.4.7 Kinetics and metabolism

The skin penetration of undiluted EGDEE, as well as in mixture with 70% acetone, was measured *in vitro* using full thickness human abdominal skin (3.14 cm^2). In pure form the lag time for penetration was 27 min, with a steady-state flux of 166 µg/cm^2/h. The permeation rate was 0.198 cm/h x 10^{-3}. The respective values in mixture with acetone were 7 min, 141 µg/cm^2/h, and 0.207 cm/h x 10^{-3} (Larese *et al*, 1999).

4.11.4.8 Neurotoxicity

No data are available.

4.11.4.9 Immunotoxicity

No data are available.

4.11.5 Human effects data

No data are available.

Table 4.11.1: Systemic toxicity of EGDEE

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral	**(mg/kgbw)**				
Dog, NS,1	900		6 x, 1 wk	No signs of toxicity noted.	Lehmann and Flury, 1938
Cat, NS,1	900		4 x	Animal died within 2 d of last dose. Serious intoxication noted after each dose	Lehmann and Flury, 1938
Rabbit, 1	900		6 x, 1 wk	No signs of toxicity noted.	Lehmann and Flury, 1938
Inhalation	**(ppm)**	**(mg/m^3)**			
Mouse, NS, 5	500	(2,460)	8 h/d, 12 d	No abnormalities reported	Lehmann and Flury, 1938
Rabbit, NS, 2	500	(2,460)	8 h/d, 12 d	1 animal died, 10 d after final exposure	Lehmann and Flury, 1938
Cat, NS, 2	500	(2,460)	8 h/d, 12 d	Both animals died within 2 d of last exposure. Microscopic kidney damage and, in one, purulent inflammation of the trachea	Lehmann and Flury, 1938
Guinea pig, NS, 1	500	(2,460)	8 h/d, 12 d	No abnormalities reported	Lehmann and Flury, 1938
Subcutaneous	**(mg/kgbw)**				
Guinea pig, NS	400		7 x	Marked weight loss; kidney injury (perenchymatous and interstitial nephritis)	Lehmann and Flury, 1938
	800			Death after 7 injections, preceded by narcosis and prostration	
Dog, NS, 2	7,600		1 x/d, 7 d	No adverse clinical signs. At necropsy: injury to vasculature, liver, brain, testes and particularly kidney	Wiley *et al*, 1938

Table 4.11.2: Reproductive and developmental toxicity of EGDEE

Route / Species, strain, number and sex/group	**Dose**	**Exposure regime**	**Result**	**Reference**
Oral	**(mg/kgbw)**			
Mouse, CD-1, 22 - 24 F	0	g.d. 6 - 15, kill g.d. 17	No effects	George *et al*, 1992
	50		NOAEL (developmental)	
	150		Exencephaly, and others	
	500		NOAEL (maternal); exencephaly, fused ribs and several others	
	1,000		↓ bw; exencephaly, fused ribs and many others	
Rabbit, NZW, 26 - 32 F	0	g.d. 6 - 19, kill g.d. 30	No effects	George *et al*, 1992
	25		NOAEL (developmental)	
	50		↑ malformations	
	100		NOAEL (maternal); ↑ malformations and resorptions	

4.12 Substance profile: DEGEE

4.12.1 Identity

Name:	Diethylene glycol (mono) ethyl ether
IUPAC name:	2-(2-Ethoxyethoxy)ethanol
CAS registry No.:	111-90-0
Molecular formula:	$C_6H_{14}O_3$
Structural formula:	$C_2H_5–(O–CH_2–CH_2)_2–OH$
Molecular weight:	134.2
Other components:	Ethylene glycol; diethylene glycol; triethylene glycol

4.12.2 Physico-chemical properties

Melting point:	–76°C
Boiling point:	197 - 205°C
Vapour pressure:	0.19 hPa
Solubility in water:	Miscible
Relative density:	$D_4^{20} = 0.988$

4.12.3 Conversion factors

1 ppm = 5.579 mg/m^3
1 mg/m^3 = 0.179 ppm

4.12.4 Toxicological data

4.12.4.1 Acute toxicity

Oral

Rat:	LD_{50} 5,400 - 5,500 mg/kgbw. Signs of toxicity were CNS depression, ataxia, coma and death (Laug *et al*, 1939); LD_{50} about 6,000 mg/kgbw (Hanzlik *et el*, 1947a); LD_{50} 6,310 mg/kgbw (Weil, 1972); LD_{50} 7,410 mg/kgbw (Berté *et al*, 1986).
Mouse:	LD_{50} 7,410 mg/kgbw (Berté *et al*, 1986).

Guinea pig: LD_{50} 3,900 mg/kgbw (Laug *et al*, 1939).

Rabbit: LD_{50} (50% aqueous solution) 3,600 mg/kgbw (Smyth *et al*, 1941).

Dermal

Rat: LD_{50} 6,000 mg/kgbw. Signs of toxicity were depressed activity, ataxia and coma (Hanzlik *et al*, 1947b).

Rabbit: LD_{50} 8,300 mg/kgbw (Hanzlik *et al*, 1947b).

Inhalation

No data are available.

Subcutaneous

Rat: LD_{50} about 6,000 mg/kgbw (Hanzlik *et al,* 1947a).

Mouse: LD_{50} about 6,000 mg/kgbw (Hanzlik *et al,* 1947a).

Intraperitoneal

Rat: LD_{50} 5,325 mg/kgbw (Berté *et al*, 1986).

Mouse: LD_{50} about 2,000 mg/kgbw (Hanzlik *et al,* 1947a); LD_{50} 2,300 mg/kgbw (Budden *et al*, 1978); LD_{50} 5,325 mg/kgbw (Berté *et al*, 1986).

Intravenous

Rat: LD_{50} about 5,000 mg/kgbw (Hanzlik *et al,* 1947a); LD_{50} 2,300 mg/kgbw (Budden *et al*, 1978).

Cat: $LD_{50} > 1,900$ mg/kgbw (Budden *et al*, 1978).

Dog: $LD_{50} > 1,900$ mg/kgbw (Budden *et al*, 1978).

4.12.4.2 Irritation and sensitisation

Skin irritation

DEGEE (500 mg) was slightly irritant to rabbit skin after 24 hour application (Draize *et al*, 1944).

Eye irritation

DEGEE was slightly irritant to the rabbit eye. Slight pain response, conjunctival redness, thickening of cornea were noted (Conquet *et al*, 1977; Jacobs and Martens, 1989).

Sensitisation

No data are available.

4.12.4.3 Repeated-dose toxicity (Table 4.12.1)

Subacute toxicity

Kidney damage and treatment-related mortality were reported in cats treated orally with DEGEE for up to 52 days (Walther, 1942) and rabbits following dermal application for 30 days (Hanzlik *et al*, 1947b). Rats receiving DEGEE in drinking water for 30 days showed reductions in food intake, growth and unspecified micro-pathological changes at all dose levels above approximately 490 mg/kgbw (Smyth and Carpenter, 1948).

The effect of three vapour concentrations of DEGEE (16, 50 or 200 ppm) on SD rats was investigated following nose-only exposure for 28 days. There were no signs of systemic intoxication, but there were histopathological changes indicative of mild non-specific irritation in the upper respiratory tract at the mid- and high-exposure levels (Hardy *et al*, 1997).

Daily exposure of mice, rabbits, cats and guinea pigs to an atmosphere saturated with DEGEE for 12 days was reported not to cause adverse effects (Lehmann and Flury, 1943).

Subchronic toxicity

Dietary feeding of Wistar or CFE rats with DEGEE at doses of up to 2,500 mg/kgbw/d for 90 days caused treatment related kidney (CFE and Wistar rats) and liver and testes (Wistar rats)

damage in the highest dose group (Hall *et al*, 1966; Gaunt *et al*, 1968). Similar effects on the kidney and liver were seen following subchronic feeding of DEGEE to CD-1 mice and large white pigs (Gaunt *et al*, 1968).

Rabbits receiving dermal treatments (not further specified) of re-purified DEGEE for 90 days showed no effects on growth, mortality, haematology, clinical chemistry or gross pathology at dose levels up to 300 mg/kgbw. A treatment related histopathological effect was seen in the kidneys of animals at 1,000 and 3,000 mg/kgbw (Drill, 1950 cited by Gingell *et al*, 1994).

Continuous DEGEE inhalation exposure of rats at 0.27 or 4.5 ppm for 4 months followed by a recovery period resulted in changes in blood cell (anaemia) and chemistry profiles as well as CNS effects (Krotov *et al*, 1981). It is difficult to draw meaningful conclusions from this study given the imprecise reporting of methodology and results.

4.12.4.4 Genotoxicity (Table 4.12.2)

In vitro

DEGEE displayed a weak mutagenic activity at high concentrations in some of the tested *Salmonella typhimurium* strains (Ames test) and in *Saccharomyces cerevisiae* (Berté *et al*, 1986).

In vivo

DEGEE did not induce micronuclei in CD-1 mouse bone marrow following 2 daily i.p. injections at 2 ml/kgbw (1,980 mg/kgbw) (Berté *et al*, 1986).

Taking the available knowledge on the genotoxicity of glycol ethers into account including the negative Ames test results on DEGEEA with metabolic activation (Hüls, 1990d, see below) it is concluded that DEGEE does not possess a relevant genotoxic potential.

4.12.4.5 Chronic toxicity and carcinogenicity (Table 4.12.1)

Several chronic toxicity studies have been performed with DEGEE in the rat.

In a 2-year dietary study in the rat, employing limited pathological examination, DEGEE-related effects included testicular atrophy and slight liver damage in the treatment group (Morris *et al*, 1942). Rats receiving DEGEE at concentrations up to 1% in drinking water and exposed for 2 years showed treatment related kidney damage (Smyth *et al*, 1964). In another, incomplete study,

DEGEE caused no apparent adverse effects when presented at 1% concentration in the drinking water to rats or mice for up to 23 months (Hanzlik *et al*, 1947c).

Ferrets showed no adverse treatment related effects following dietary feeding with DEGEE at concentrations ranging from 490 to 2,960 mg/kgbw/d for 9 months (Butterworth *et al*, 1975).

4.12.4.6 Reproductive and developmental toxicity (Table 4.12.3)

In a screening assay and a short-term test performed with DEGEE in SD rats and CD-1 mice, no selective developmental toxicity was seen under treatment conditions not also causing maternal toxicity. Thus, DEGEE was not considered a developmental toxicant in these laboratory animals exposed orally (Schuler *et al*, 1984; Hardin *et al*, 1987), dermally (Hardin *et al*, 1984) or by inhalation at the maximum achievable vapour concentration of 100 ppm (Nelson *et al*, 1984b).

One study has employed a continuous breeding protocol to examine the effects of DEGEE in CD-1 mice. The animals received DEGEE in drinking water at concentrations of 0, 0.25, 1.25 or 2.5% (corresponding to an uptake of 540, 2,600 or 5,600 mg/kgbw/d). DEGEE had no effect on fertility or reproductive performance. A significant decrease in sperm motility was observed in the males exposed at the highest dose (as well as an increased liver weight in F_1 mice (Williams *et al*, 1990; Chapin and Sloane, 1997).

4.12.4.7 Kinetics and metabolism (Table 4.12.4)

An anecdotal report of rabbits treated orally or by s.c. injection indicated degradation of DEGEE and elimination in the urine as glucuronic acid conjugates (Fellows *et al*, 1947). DEGEE (1.5 g) given orally to an adult human (hospital patient) at a dose of about 20 mg/kgbw resulted in formation of 2-(2-ethoxyethoxy)acetic acid as a major (68% of dose) metabolite in the urine (Kamerling *et al*, 1977). This appears to be similar to the biotransformation of DEGBE in rats (Section 4.23.4.7) where the major metabolite in the urine is 2-(2-butoxyethoxy)acetic acid.

DEGEE penetrates animal and human skin. Dugard *et al* (1984) determined a rate of penetration through excised human skin for DEGEE of 0.125 mg/cm^2/h, which was some 6-fold less than EGEE.

4.12.4.8 Neurotoxicity

DEGEE, in common with many other organic solvents, causes depression of the CNS at high acute doses (Section 4.12.4.1).

4.12.4.9 Immunotoxicity

No data are available.

4.12.5 Human effects data

In an isolated case report, an alcoholic male (aged 44) drank a liquid containing approximately 300 ml DEGEE (296 g). Severe symptoms of central nervous and respiratory injury (dyspnoea), thirst and acidosis occurred. The urine contained albumin. The subject recovered following symptomatic treatment (Browning, 1965). In describing the fate of a single oral dose of DEGEE in a human subject (Section 4.12.4.7), Kamerling *et al* (1977) provided no information on clinical signs or outcome.

DEGEE is reported to be neither a primary irritant nor a skin sensitiser in humans (Cranch *et al*, 1942; Grandolfo *et al*, 1996; Meininger, 1948; Opdyke, 1974).

Table 4.12.1: Systemic toxicity of DEGEE

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**				
Cat, 8	300 500, 1,000 4,900		1 x/d, 2 - 52 d	NOAEL for 8 d Albuminuria, kidney damage Mortalities after 2 d	Walther, 1942
Oral, diet	**(%)**	**(mg/kgbw)**			
Rat, Wistar, 12 M, 12 F	0, 0.25 1.0 5.0	0, 125 500 2,500	90 d, DEGEE, 0.4% MEG	No effects NOAEL ↓ weight gain; damage to liver, kidney and testes	Hall *et al*, 1966
Rat, 20 M, 20 F	2.16	NS	2 y, DEGEE, 0.4% MEG	Damage to testis and liver	Morris *et al*, 1942
Rat, CFE, 15 M, 25 F	0 0.5 5.0	0 250 2,500	90 d, DEGEE, 0.4% MEG	No effects NOAEL ↓ weight gain; anaemia; kidney damage	Gaunt *et al*, 1968
Mouse, CD-1, 20 M, 20 F	0, 0.2 0.6 1.8, 5.4	0, 300 900 2,800, 8,000	90 d	No effects NOAEL ↓ RBC count; liver and kidney damage	Gaunt *et al*, 1968
	(ml/kg)	**(mg/kgbw)**			
Ferret, 3 M, 2 F	0, 0.5, 2.0 3.0	0, 490, 1,970 2,960	9 months	No effects NOAEL	Butterworth *et al*, 1975

Table 4.12.1: Systemic toxicity of DEGEE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, diet (cont'd)	**(ml/kg)**	**(mg/kgbw)**			
Pig, large white, 3 M, 3 F (DEGEE, 0.4% MEG)	NS	0 167 500, 1,500 (reduced to 1,000 after 3 wk)	90 d	No effects NOAEL ↓ CNS, kidney and liver effects	Gaunt *et al*, 1968
Oral, drinking water	**(%)**	**(mg/kgbw)**			
Rat, 5 or 10 M (13 controls), 5 or 9 F (8 controls)	1	NS	*Ad libitum*, 23 months	No apparent effects noted. Incomplete assessment.	Hanzlik *et al*, 1947c
Rat, 5, sex NS	NS	210 - 3,880	*Ad libitum*, 30 d	NOAEL about 490 mg/kgbw. No mortalities, ↓ food intake and growth	Smyth and Carpenter, 1948
Mouse, presumably 5 - 20/sex	5	NS	21 months	No apparent effects noted. Incomplete assessment	Hanzlik *et al*, 1947c
Dermal, non-occluded	**(mg/kgbw)**				
Rabbit, 3 (sex NS)	20 - 490		1 h/d, 30 d on 100 cm^2 clipped skin, 30 d recovery	NOAEL about 40 - 80 mg/kgbw. Weight loss, CNS depression, kidney damage	Hanzlik *et al*, 1947b
Rabbit, NS	50% or 70%		23 d, continuous contact with open wound	No systemic injury, no effect on wound healing	Cranch *et al*, 1942

Table 4.12.1: Systemic toxicity of DEGEE (cont'd)

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Dermal, occluded	**(mg/kgbw)**				
Rabbit, NS	0, 100		5 d/wk, 90 d, re-purified DEGEE	No effects	Drill, 1950 cited by Gingell *et al*, 1994
	300			NOAEL	
	1,000			Kidney damage	
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, SD, 5 M, 5 F	(0	0	6 h/d (nose-only), 5 d/wk, 28 d	No effects	Hardy *et al*, 1997
	16	90		NOAEL (irritation)	
	50	270		Local irritation	
	200)	1,100		Local irritation; NOAEL (systemic)	
Rat, presumably 15 M, 15 F	(0.27, 4.5)	1.5, 25	4 months continuous whole body, followed by recovery period	CNS effects, anaemia (not in text), changes in blood/clinical chemistry	Krotov *et al*, 1981

Table 4.12.2: Genotoxicity of DEGEE

Endpoint / Species	**Strain or type / Target**	**Concentration**		**Result**	**Remark**	**Reference**
In vitro						
Gene mutation		**(ml/plate)**				
Salmonella typhimurium	TA97, TA100, TA102 TA1535, TA1537, TA1538	0.01, 0.1, 1.0		**–ve** **±**	+/– S9	Berté *et al*, 1986
		(%)	**(µg/ml)**			
Saccharomyces cerevisiae	D7	1, 10 10	(10, 100 100)	**–ve** **±**	4 h	Berté *et al*, 1986
In vivo						
Micronucleus frequency		**(ml/kgbw)**	**(mg/kgbw)**			
Mouse, 5 M	CD-1, bone marrow	2 x 2, i.p.	(2 x 1,980)	**–ve**		Berté *et al*, 1986

Table 4.12.3: Reproductive and developmental toxicity of DEGEE

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Oral, drinking water	**(%)**	**(mg/kgbw)**			
Rat, 8 M, 8 F	0, 0.01, 0.04, 0.2, 1%	(0, 10, 40, 200, 950 mg/kgbw)	3 generations, up to 2 y	Early cessation of breeding at 1%. Animals displayed severe kidney damage	Smyth *et al,* 1964
Mouse, CD-1, 20 M, 20 F (40 M, 40 F control)	0, 0.25	0, 440	Continuous breeding. Breeding pairs exposed over 14 wk	No effect on reproduction in F_0 and F_1 generations	Williams *et al,* 1990
	1.25, 2.5	2,200, 4,400		↓ sperm motility	
Mouse, CD-1, NS	0, 0.25 1.25 2.5	0, 540 2,600 5,400	Continuous breeding	No effects NOAEL F_1 ↑ liver weight (M, F); ↓ number epididymal sperm	Chapin and Slaone, 1997
Dermal	**(ml)**	**(mg/kgbw)**			
Rat, SD, 13 F (17 control)	0	0	4 x/d, g.d. 7 - 16, necropsy g.d. 21	No effects	Hardin *et al,* 1984
	4 x 0.35	6,000		NOAEL (development). ↓ maternal bw	
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, SD, 15 F	0	(0	7 h/d, g.d. 7 - 15, necropsy g.d. 20	No effects	Nelson *et al,* 1984b
	100 (highest achievable)	560)		NOAEL (maternal), NOAEL (developmental)	

Table 4.12.4: Absorption (uptake), distribution, metabolism and elimination of DEGEE

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral				
Human, 1, NS	20 mg/kgbw approximately	NS	12 h urine contained metabolite (2-ethoxy-ethoxy)acetic acid (68% dose)	Kamerling *et al*, 1977
Dermal				
Human, skin, *in vitro*	1 or 5 ml/1.8 cm^2	8 h, diffusion cell	Permeability constant 1.32 cm/h × 10^4. Penetration rate 0.125 $mg/cm^2/h$	Dugard *et al*, 1984

4.13 Substance profile: DEGEEA

4.13.1 Identity

Name:	Diethylene glycol ethyl ether acetate
IUPAC name:	Ethanol, 2-(2-ethoxyethoxy)-, acetate
CAS registry No.:	112-15-2
Molecular formula:	$C_8H_{16}O_4$
Structural formula:	$C_2H_5–(O–CH_2–CH_2)_2–O–CO–CH_3$
Molecular weight:	176.2
Other components:	No data

4.13.2 Physico-chemical properties

Melting point:	< –25°C
Boiling point:	210 - 220°C
Vapour pressure:	0.13 hPa
Solubility in water:	Soluble
Relative density:	D_4^{20} = 1.0096

4.13.3 Conversion factors

1 ppm = 7.325 mg/m^3
1 mg/m^3 = 0.137 ppm

4.13.4 Toxicological data

4.13.4.1 Acute toxicity

Oral

Rat:	LD_{50} 11,000 mg/kgbw (50% aqueous solution) (Smyth *et al*, 1948).
Rabbit:	LD_{50} 4,400 mg/kgbw (50% aqueous solution) (Smyth *et al*, 1948).
Guinea pig:	LD_{50} 3,930 mg/kgbw (50% aqueous solution) (Smyth *et al*, 1948).

Dermal

Rabbit: LD_{50} 15,000 mg/kgbw (Smyth *et al*, 1948).

Inhalation

All rats and guinea pigs exposed to saturated DEGEEA atmospheres for 8 hours survived. Gross pathological examination at necropsy revealed injury to the lungs and kidneys (Smyth *et al*, 1948).

4.13.4.2 Irritation and sensitisation

Skin irritation

DEGEEA caused slight irritation to the rabbit skin (Smyth *et al*, 1948; Hüls, 1990a).

Eye irritation

Undiluted DEGEEA (0.1 ml) was slightly to moderately irritant to the rabbit eye (Smyth *et al*, 1948; Hüls, 1990b).

Sensitisation

DEGEEA was not sensitising in guinea pigs in a Magnusson-Kligman maximisation test (Hüls, 1990c).

4.13.4.3 Repeated-dose toxicity

Subacute toxicity

No data are available.

Subchronic toxicity

No data are available.

4.13.4.4 Genotoxicity

In vitro

In a standard Ames assay using *Salmonella typhimurium* strains TA1535, TA100, TA1538 and TA98, DEGEEA showed no mutagenic activity up to 5,000 µg/plate (Hüls, 1990d).

4.13.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.13.4.6 Reproductive and developmental toxicity

No data are available.

4.13.4.7 Kinetics and metabolism

No data are available.

4.13.4.8 Neurotoxicity

No data are available.

4.13.4.9 Immunotoxicity

No data are available.

4.13.5 Human effects data

A human patch test using undiluted DEGEEA resulted in a limited number of subjects developing mild skin irritation. No further details were reported (Gingell *et al*, 1994).

4.14 Substance profile: DEGDEE

4.14.1 Identity

Name:	Diethylene glycol diethyl ether
IUPAC name:	Bis (2-ethoxyethyl)ether
CAS registry No.:	112-36-7
Molecular formula:	$C_8H_{18}O_3$
Structural formula:	$C_2H_5–(O–CH_2–CH_2)_2–O–C_2H_5$
Molecular weight:	162.2
Other components:	No data

4.14.2 Physico-chemical properties

Melting point:	No data
Boiling point:	189°C
Vapour pressure:	67 hPa at 25°C
Solubility in water:	Miscible
Relative density:	$D_4^{20} = 0.9063$

4.14.3 Conversion factors

1 ppm = 6.743 mg/m^3
1 mg/m^3 = 0.148 ppm

4.14.4 Toxicological data

4.14.4.1 Acute toxicity

Oral

Rat:	LD_{50} 4,970 mg/kgbw (Union Carbide, 1984).
Guinea pig:	LD_{50} 1,850 mg/kgbw (Smyth *et al*, 1941).
Mouse:	LD_{50} 3,670 mg/kgbw (Plasterer *et al*, 1985).

Dermal

No data are available.

Inhalation

No data are available.

4.14.4.2 Irritation and sensitisation

Skin irritation

No data are available.

Eye irritation

DEGDEE caused moderate irritation to the rabbit eye following instillation of 50 mg (Union Carbide, 1984).

Sensitisation

No data are available.

4.14.4.3 Repeated-dose toxicity (Table 4.14.1)

Subacute toxicity

Repeated exposure of Wistar rats to saturated DEGDEE vapour (400 ppm) resulted in restlessness but no remarkable findings at necropsy. No further details were provided (Gage, 1970).

Subchronic toxicity

No data are available.

4.14.4.4 Genotoxicity

No data are available.

4.14.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.14.4.6 Reproductive and developmental toxicity (Table 4.14.2)

Results from developmental toxicity screening assays suggested possible adverse effects of DEGDEE, warranting further study (Schuler *et al*, 1984; Plasterer *et al*, 1985). Follow up teratology studies in mice and rabbits (NTP, 1987a,b) failed to show any adverse effects on embryonic or foetal development, even at maternally toxic doses.

4.14.4.7 Kinetics and metabolism

No data are available.

4.14.4.8 Neurotoxicity

No data are available.

4.14.4.9 Immunotoxicity

No data are available.

4.14.5 Human effects data

No data are available.

Table 4.14.1: Systemic toxicity of DEGDEE in Wistar Ald/Pk rats exposed by inhalation

Number and sex/group	Concentration		Exposure regime	Result	Reference
	(ppm)	(mg/m^3)			
4 M	400 (saturated atmosphere)	2,700	17 x 17 h	Restlessness, no abnormalities noted at necropsy	Gage, 1970

Table 4.14.2: Reproductive and developmental toxicity of DEGDEE following oral (gavage) exposure

Species, strain, number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
Mouse, CD-1, 7 F	0	g.d. 6 - 15, kill g.d. 17	No effects	NTP, 1987a
	300		NOAEL (maternal)	
	1,500		NOAEL (developmental)	
	3,000, 4,500		Maternal CNS effects and mortality	
Rabbit, NZW, 27-31 F	0, 50	g.d. 6 - 19, kill g.d. 30	No effects	NTP, 1987b
	200		NOAEL (maternal)	
	400		NOAEL (developmental) (Maternal CNS effects and mortality)	

4.15 Substance profile: TEGEE

4.15.1 Identity

Name:	Triethylene glycol (mono) ethyl ether
IUPAC name:	2-[2-(2-Ethoxyethoxy)ethoxy]ethanol
CAS registry No.:	112-50-5
Molecular formula:	$C_8H_{18}O_4$
Structural formula:	$C_2H_5–(O–CH_2–CH_2)_3–OH$
Molecular weight:	178.2
Other components:	No data

4.15.2 Physico-chemical properties

Melting point:	–30°C
Boiling point:	235 - 280°C
Vapour pressure:	< 0.01 hPa
Solubility in water:	Soluble

4.15.3 Conversion factors

1 ppm = 7.408 mg/m^3
1 mg/m^3 = 0.135 ppm

4.15.4 Toxicological data

4.15.4.1 Acute toxicity

Oral

Rat:	LD_{50} 6,500 mg/kgbw. Signs of toxicity were lethargy, ataxia and piloerection (US-EPA, 1982a).
	LD_{50} 10,610 mg/kgbw. Signs of toxicity not specified. (Smyth and Carpenter, 1948).

Dermal

Rabbit: LD_{50} 8,200 mg/kgbw, no details given (Smyth *et al*, 1951). At a single dose of 2,000 mg/kgbw, no signs of toxicity were observed (US-EPA, 1982b).

Inhalation

Rat: No signs of toxicity or mortality were observed following 1 hour exposure to a nominal concentration of 200 mg/l (27,000 ppm) (US-EPA, 1982c).

4.15.4.2 Irritation and sensitisation

Skin irritation

Undiluted TEGEE (0.5 ml) was not irritant to rabbit skin following 24-hour application under semi-occlusive patch (Smyth and Carpenter, 1948; US-EPA, 1982d).

Eye irritation

Undiluted TEGEE (0.1 ml) was not irritant to the rabbit eye (US-EPA, 1982e; Smyth and Carpenter, 1948).

Sensitisation

No data are available.

4.15.4.3 Repeated-dose toxicity (Table 4.15.1)

Subacute toxicity

Rats were given TEGEE in drinking water at actual dose levels of 0, 180, 750, 3,300 or 13,290 mg/kgbw/d for 30 days. The rats with the highest dose received only 25% of the control value and all these animals died in 6 to 24 days due mainly to liver and kidney injury. There was no mortality at 3,300 mg/kgbw/d, but decreased body weight gain and liver and kidney lesions

were observed occasionally. At 180 and 750 mg/kgbw/d, no animal showed any abnormalities (US-EPA, 1985).

In a dermal study in NZW rabbits a limit dose of 1,000 mg/kgbw TEGEE was applied for 21 days. TEGEE did not produce any systemic toxicity in male or female animals. TEGEE produced slight erythema and oedema starting on day 6 or 7 of treatment. Erythema continued throughout the study whereas oedema was not observed after day 18 (Leber *et al*, 1990).

Subchronic toxicity

No data are available.

4.15.4.4 Genotoxicity

No data are available.

Studies with TEGME (Ames, HGPRT and micronucleus test) showed no mutagenic activity (Section 4.6.4.4).

4.15.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.15.4.6 Reproductive and developmental toxicity (Table 4.15.2)

TEGEE was screened in rats for potential developmental toxicity following a modified Chernoff-Kavlock protocol. Mated female Wistar rats were administered daily doses of 0, 250 or 1,000 mg/kgbw by gavage on day 6 to 15 of gestation. No significant changes in clinical conditions or body weights were seen in maternal rats following exposure to TEGEE. All rats in the control and treated groups were pregnant and delivered live foetuses. The number of live pups on day 1 and 5 post partum were also comparable in these groups, indicating no chemically-induced developmental effects (Leber *et al*, 1990).

4.15.4.7 Kinetics and metabolism

Human abdominal whole skin (2.54 cm^2) was mounted in a glass diffusion apparatus and the penetration of TEGEE from the donor into the receiver chamber was monitored during a 12-hour period using GC analysis. The diffusion rate of TEGEE was determined to be 0.125 μmol/cm^2/h (22.3 mg/cm^2/h) indicating a low skin permeability of the compound (Leber *et al*, 1990).

4.15.4.8 Neurotoxicity

No data are available.

4.15.4.9 Immunotoxicity

No data are available.

4.15.5 Human effects data

Undiluted TEGEE (0.033 ml) was slightly irritant to the skin of human volunteers following 2 hour application under semi-occlusive patch. Very slight erythema occurred (US-EPA, 1982f).

Table 4.15.1: Systemic toxicity of TEGEE

Route / Species, strain, number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
Oral, drinking water				
Rat, NS, 10, sex NS	0, 180 750 3,300 13,290	30 d	No effects NOAEL ↓ bw; liver and kidney damage ↓ bw; liver and kidney damage lethality	US-EPA, 1985
Dermal				
Rabbit, NZW, 5 M, 5 F	0 1,000	6 h/d, 5 d/wk, 21 d	No effects Slight oedema and erythema at site of application. No systemic effects	Leber *et al,* 1990

Table 4.15.2: Reproductive and developmental toxicity of TEGEE in Wistar Ald/Pk rats dosed by oral gavage

Number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
10 F	0, 250 1,000	g.d. 6 - 15, Chernoff-Kavlock screening	No effects NOAEL	Leber *et al,* 1990

4.16 Substance profile: EGiPE

4.16.1 Identity

Name:	Ethylene glycol (mono) isopropyl ether
IUPAC name:	2-Isopropoxyethanol
CAS registry No.:	109-59-1
Molecular formula:	$C_5H_{12}O_2$
Structural formula:	$(CH_3)_2CH–O–CH_2–CH_2–OH$
Molecular weight:	104.2
Other components:	No data

4.16.2 Physico-chemical properties

Melting point:	< 60°C
Boiling point:	144°C
Vapour pressure:	6.9 hPa at 25°C
Solubility in water:	Completely soluble
Relative density:	D_4^{20} = 0.9030

4.16.3 Conversion factors

1 ppm = 4.332 mg/m^3
1 mg/m3 = 0.231 ppm

4.16.4 Toxicological data

4.16.4.1 Acute toxicity

Oral

Rat:	LD_{50} between 500 and 1,000 mg/kgbw. Signs of toxicity were CNS depression, dyspnoea and haemoglobinuria with kidney damage, liver and spleen alterations (Boatman and Knaak, 2001).
	LD_{50} 5,600 mg/kgbw (Smyth *et al*, 1969).

Mouse: LD_{50} 2,300 mg/kgbw (administration in oil) 2,180 g/kgbw (administration in water) (Saparmamedov, 1974 cited by Boatman and Knaak, 2001).

Dermal

Rabbit: LD_{50} 1,600 mg/kgbw (undiluted material) (Smyth *et al*, 1969).

Inhalation

Rat: 4-h LC_{50} 4,000 ppm (17,300 mg/m^3) (Smyth *et al*, 1969).

Mouse: 7-h LC_{50} 1,930 ppm (8,360 mg/m^3) (Smyth *et al*, 1969).

Haemolytic effects were seen in rats after a single inhalation exposure to 62 ppm (270 mg/m^3), but not to 32 ppm (140 mg/m^3) (Carpenter *et al*, 1956). In another study, 80 ppm (347 mg/m^3) did not produce haemolysis (Saparmamedov, 1974 cited by Boatman, 2001).

4.16.4.2 Irritation and sensitisation

Skin irritation

EGiPE was moderately irritant to the uncovered rabbit skin following 24-hour exposure (Smyth *et al*, 1969)**.**

EGiPE was irritant to rabbit skin according to EEC criteria (4 h, semi-occlusive patch) and moderately irritant following the primary irritation index = 4.8 (24 h, occluded exposure) (Zissu, 1995).

Eye irritation

EGiPE was irritant to rabbit eyes, causing iritis and corneal damage, which reversed after 7 days (Smyth *et al*, 1969)

Sensitisation

EGiPE was not a sensitiser in guinea pigs (Magnusson-Kligman test) when challenged with a cutaneous concentration of 1% (Zissu, 1995).

4.16.4.3 Repeated-dose toxicity (Table 4.16.1)

Subacute toxicity

Haemolytic effects were reported in Wistar rats exposed by inhalation to 390 ppm EGiPE for 5 weeks. The number of reticulocytes was increased. After the fourth week of exposure haematological parameters were near control values indicting a lower sensitivity of young RBC (Werner *et al*, 1943c).

Male Wistar rats exposed to 600 or 1,000 ppm EGiPE for 9 days within a 14-day experimental period showed haemolytic effects and haematuria after initial exposures. No effects were seen at 300 ppm and there were no testicular changes at any dose (Doe *et al*, 1984b).

Haemolytic effects and signs of haemolytic anaemia were observed in Wistar rats exposed to 150, 450 or 900 ppm EGiPE for 28 days. In the two highest dose groups, urinary pH was lower, possibly indicative of metabolic acidosis, while spleen weights were increased. Body weight was unchanged and no testicular effects were observed. A subsequent study with exposure to 10, 30 or 100 ppm demonstrated a NOAEL of 30 ppm for haemolysis (Reuzel *et al*, 1987; Arts *et al*, 1992).

Wistar rats exposed to 100, 300 or 1,000 ppm EGiPE for 3 weeks initially showed haemoglobinuria and decreased Hb at the top dose. The effects decreased with time, due probably to compensatory erythropoiesis. Respiratory tract irritation and increased lung weight were also observed at this top dose. Transient reductions in Hb were seen at 300 ppm. The NOAEL was 100 ppm (Gage, 1970).

Subchronic toxicity

No data are available.

4.16.4.4 Genotoxicity

In vitro

EGiPE was not mutagenic when tested in *Salmonella typhimurium* strains TA1535, TA100, TA1537 and TA98 (Ames test) or in *Escherichia coli* strain WP2*uvr*A up to 5,000 µg/plate, the maximum concentration tested, both with and without metabolic activation (Wagner, 1996).

4.16.4.5 Chronic toxicity and carcinogenicity (Table 4.16.1)

Rats, guinea pigs, rabbits and dogs were exposed by inhalation to 25, 50 or 200 ppm EGiPE for 26 weeks to compare cross species differences in response. In rats at 200 ppm, Hb levels were decreased throughout the exposure period in both males and females. Liver Kupffer cell and splenic haemosiderosis were observed at 200 ppm. Splenic haemosiderosis also occurred at 50 ppm. Erythrocyte osmotic fragility was decreased down to 25 ppm, but not consistently throughout the exposure period. No organ weight, clinical chemistry or other histopathological changes were observed. Haematological effects were not observed in the other species (Moffett *et al*, 1976).

4.16.4.6 Reproductive and developmental toxicity (Table 4.16.2)

Pregnant Wistar rats were exposed by inhalation to 0, 50, 150 or 450 ppm EGiPE from day 6 to 15 of gestation. Haemoglobinuria was observed in dams at the top two concentrations. Some evidence of delayed foetal development was observed at the highest concentration (Koeter *et al*, 1987).

Pregnant CD(SD) rats were exposed to vapours of 100, 300 or 600 ppm EGiPE from day 6 to 15 of gestation. Maternal body weights and food consumption were decreased and spleen weights increased and haemoglobinuria observed in the top two dose groups. There were no adverse effects on gestational parameters; the incidence of adversely affected implants/litter was increased at 600 ppm but there was no evidence of teratogenicity at any dose level. The maternal NOAEL was 100 ppm and the developmental NOAEL 300 ppm (Tyl *et al*, 1999).

Pregnant NZW rabbits exposed by inhalation to 0, 20, 90 or 490 ppm EGiPE from day 6 to 18 of gestation showed reduced food consumption and body weight gain at the highest concentration. Haematological parameters indicated maternal haemolytic anaemia and there were an increased number of foetuses showing reduced foetal weight and signs of delayed maturation in the top dose group. No maternal or foetal effects were seen at 90 or 20 ppm (Koeter *et al*, 1988).

4.16.4.7 Kinetics and metabolism

Carworth Farms-Elias (CFE) rats were injected i.p. with EGiPE (1 mg/animal), ^{14}C-labelled in both C-atoms of the ethylene group. Most (87%) of the radioactivity was recovered within 2 hours (73% in the urine and 14% in expired air). Urinary metabolites were mainly isopropoxyacetic acid and its glycine conjugate, *n*-isopropyoxyacetyl glycine, with lesser amounts of acetone and MEG. A similar metabolic profile of urinary isopropoxyacetic acid and its glycine conjugate was observed in dogs dosed with 75 mg/kgbw i.p. (Hutson and Pickering, 1971).

4.16.4.8 Neurotoxicity

No data are available.

4.16.4.9 Immunotoxicity

No data are available.

4.16.5 Human effects data

No data are available.

Table 4.16.1: Systemic inhalation toxicity of EGiPE in rats

Route / Strain, number and sex/group	Concentration (ppm)	Concentration (mg/m^3)	Exposure regime	Result	Reference
Wistar, 23 M, 23 F	390	(1,700)	7 h/d, 5 d/wk, 5 wk	Haemolytic effects; ↑ reticulocytes. Reticulocytes less sensitive to haemolysis	Werner *et al*, 1943c
Wistar Alderley Park, 10 M	0, 300 600, 1,000	(0, 1,300 2,600, 4,300)	6 h/d, 9 d	No effects; 300 ppm NOAEL Haemolytic effects	Doe, 1984b
Wistar, 10 M, 10 F	0, 10	(0, 43	6 h/d, 28 d	No effects	Reuzel *et al*, 1987; Arts *et al*, 1992
	30	130		NOAEL	
	100	430		Haemolytic effects in F	
	150, 450, 900	650, 1,950, 3,900)		Haemolytic effects; ↑ spleen weights	
Wistar Alderley Park, 4 M, 4 F	0	(0	6 h/d, 5 d/wk, 3 wk	No effects	Gage, 1970
	100	430		NOAEL	
	300	1,300		Transient haemolytic effects	
	1,000	4,300)		Haemolytic effects; ↑ reticulocytes and lung weights	
Rat, CFE (40 M, 40 F), guinea pig (30 M, 30 F), rabbit, Banded Dutch (2 M, 2 F) and dog, Beagle (2 M, 2 F)	0, 25, 50, 200	(0, 110, 220, 870)	6 h/d, 5 d/wk, 26 wk	Haemosiderosis, ↑ osmotic fragility at 50 and 200 ppm in both sexes. Anaemia at 200 ppm, both M and F. Changes only seen in rats	Moffett *et al*, 1976

Table 4.16.2: Reproductive and developmental toxicity of EGiPE by inhalation

Species, strain, number and sex/group	Concentration		Exposure regime	Result	Reference
	(ppm)	(mg/m^3)			
Rat, Wistar, 21 - 23 F	0, 50 150 450	(0, 220 650 1,950)	6 h/d on g.d. 6 - 15	No effects; 50 ppm NOAEL Haemolytic effects in the dams ↑ incidences in delayed development, immature foetuses No teratogenicity	Koeter *et al*, 1987
Rat, CD(SD), 25 F	0, 100 300 600	(0, 430 1,300 2,600)	6h/d on g.d. 6-15	No effects; 100 ppm NOAEL Small ↓ maternal bw, haematuria Maternal bw ↓, haematuria, piloerection Overall ↑ % affected implants/litter, but no specific parameter changed	Tyl *et al* 1999
Rabbit, NZW, 18 F	0, 20 90 490	(0, 90 390 2,100)	6 h/d on g.d. 6-18	No effects NOAEL ↓ haemolytic effects in the dams; ↓ foetal weight; ↓ maturation	Koeter *et al*, 1988

4.17 Substance profile: EGiPEA

4.17.1 Identity

Name:	Ethylene glycol (mono) isopropyl ether acetate
IUPAC name:	2-Isopropyl acetoxyethanol
CAS registry No.:	91598-97-9
Molecular formula:	$C_7H_{14}O_3$
Structural formula:	$(CH_3)_2CH–O–CH_2–CH_2–O–CO–CH_3$
Molecular weight:	146.2
Other components:	No data

4.17.2 Physico-chemical properties

Melting point:	No data
Boiling point:	No data
Vapour pressure:	No data
Solubility in water:	No data

4.17.3 Conversion factors

1 ppm = 6.078 mg/m^3
1 mg/m^3 = 0.165 ppm

4.17.4 Toxicological data

No data are available.

4.17.5 Human effects data

No data available.

4.18 Substance Profile: EGnPE

4.18.1 Identity

Name:	Ethylene glycol (mono) *n*-propyl ether
IUPAC name:	*n*-Propoxyethanol
CAS registry No.:	2807-30-9
Molecular formula:	$C_5H_{12}O_2$
Structural formula:	C_3H_7-O-CH_2-CH_2-OH
Molecular weight:	104.2
Other components:	No data

4.18.2 Physico-chemical properties

Melting point:	–60°C approximately
Boiling point:	150 - 152°C
Vapour pressure:	1.3 hPa at 25°C
Solubility in water:	Completely soluble
Relative density:	D_4^{20} = 0.9112

4.18.3 Conversion factors

1 ppm = 4.332 mg/m^3
1 mg/m^3 = 0.231 ppm

4.18.4 Toxicological data

4.18.4.1 Acute toxicity

Oral

Rat:	LD_{50} 3,090 mg/kgbw. Signs of toxicity were weakness, abnormal respiration, haemoglobinuria and tremors (Katz *et al*, 1984).

Dermal

Guinea pig: 24-h LD_{50} between 1 and 5 ml/kgbw (910 - 4,600 mg/kgbw), occluded exposure (Katz *et al*, 1984).

Rabbit: 6-h LD_{50} 1,340 mg/kgbw, occluded application (Boatman and Knaak, 2001).

Inhalation

Rat: 6-h LC_{50} > 2,132 ppm (9,200 mg/m^3) (males), the highest (saturated vapour) concentration tested. No deaths. Haemoglobinuria was observed at 1,121 ppm (4,860 mg/m^3) and 2,132 ppm (Katz *et al*, 1984).

Pregnant rats: 1,600 ppm (6,930 mg/m^3) killed 8 out of 10 dams after 2 exposures (Krasavage and Katz, 1985).

Pregnant rabbits: LC_{50} > 500 ppm (> 2,170 mg/m^3); 800 ppm (3,500 mg/m^3) was lethal after 2 to 5 exposures (Krasavage *et al*, 1990).

4.18.4.2 Irritation and sensitisation

Skin irritation

Undiluted EGnPE was slightly irritant to guinea pig skin following occluded exposure for 24 hours (Katz *et al*, 1984).

Eye irritation

EGnPE (0.1 ml undiluted) was moderately to markedly irritant to the rabbit eye. Severe erythema, oedema of the conjunctiva, iritis and some corneal opacity were observed which reversed within 14 days (Katz *et al*, 1984).

Sensitisation

A weak response was observed in 1/5 guinea pigs following induction by footpad injection of 0.05 ml Freund's adjuvant with 1% EGnPE and challenge 1 week later by 0.3 ml of a 1% solution dosed topically to the depilated dorsal skin. The authors concluded that EgnPE had, at most, a low sensitisation potential in this study; the acetate ester was negative (Katz *et al*, 1984).

EGnPE was not a sensitiser when tested by the Buehler method or the alternative footpad method (Shepard, 1988a,b).

In the light of the overall lack of sensitisation activity within the glycol ether family, the balance of experimental results suggests that EGnPE is not a skin sensitiser.

4.18.4.3 Repeated-dose toxicity (Table 4.18.1)

Subacute toxicity

COBS/CD rats were dosed by oral gavage with 195, 390, 780 or 1,560 mg EGnPE/kgbw/d for 6 weeks. There was little effect on feed consumption and body weight gain. Weakness, laboured breathing and prostration were observed at 780 or 1,560 mg/kgbw. There was a dose-dependant decrease in Hb and RBC count, as well as increases in platelet count, anisocytosis, macrocytosis, and poly- and hypochromasia; haemoglobinuria was seen at least for 1 day at all dose levels. Histopathological changes in kidney (all dose levels), spleen (at 390 mg/kgbw and above) and liver (at 1,560 mg/kgbw), secondary to red cell haemolysis, were observed; no testicular changes were seen. At all dose levels absolute and relative spleen weights were increased (Katz *et al*, 1984).

In a range-finding developmental toxicity study, pregnant NZW rabbits exposed by inhalation to 400, 800 or 1,000 ppm EGnPE showed haemoglobinuria following the first exposures. Doses of 800 and 1000 ppm were lethal. Body weights were decreased at 400 ppm (Krasavage *et al*, 1990) (Table 4.18.2).

COBS/CD rats were exposed (whole-body) by inhalation to 0, 100, 200, 400 or 800 ppm for a total of 11 exposures. Initial haemoglobinuria occurred in the males at 400 and 800 ppm and in the females exposed to 800 ppm. Blood changes indicative of haemolysis and increased spleen weight were observed at 400 and 800 ppm. Congestion, lymphoid hyperplasia, extra-medullary haematopoiesis, and haemosiderin were seen in the spleen of rats exposed to 400 and 800 ppm (Katz *et al*, 1984).

In a range-finding developmental toxicity study, 2 out of 10 pregnant NZW rats exposed to 1,600 ppm EGnPE died after 2 exposures. Exposure at 800 ppm produced anaemia, loss in body weight and reduced liver weight (Krasavage *et al*, 1990). At concentrations of 200, 300 and 400 pm, haematuria, increased spleen weight and anaemia were seen (Krasavage and Katz, 1984a) (Table 4.18.2).

Subchronic toxicity

COBS/CD rats were exposed to vapour concentrations of 0, 100, 200 or 400 ppm EGnPE for 14 weeks. Haematuria and haematological changes, indicative of a compensatory haemolytic anaemia were seen at 200 and 400 ppm. Haemosiderin deposition was observed in kidneys, liver and spleen and spleen weights were increased at 200 and 400 ppm. No changes were seen at 100 ppm (Katz, 1987).

4.18.4.4 Genotoxicity

No data are available.

4.18.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.18.4.6 Reproductive and developmental toxicity (Table 4.18.2)

In a range-finding teratology study with SD rats with concentrations of 0, 400, 800 or 1,600 ppm by inhalation, 800 and 1,600 ppm EGnPE caused severe maternal toxicity (body weight loss, reduced food intake, at 1,600 ppm also 80% mortality) and foetal resorptions (Krasavage and Katz, 1983). In the following main study, SD rats were exposed to concentrations of 0, 100, 200, 300 or 400 ppm on day 6 to 15 of gestation. No teratogenicity or significant foetal toxicity was reported. Minor variations of skeletal ossification and supra-numerary ribs, probably related to maternal toxicity, were observed in all exposed groups at 200 ppm or higher (Krasavage and Katz, 1984a, 1985).

Pregnant NZW rabbits were exposed to 0, 125, 250, or 500 ppm EGnPE on day 6 to 18 of gestation. No developmental toxicity was observed. Exposures at 500 ppm produced decreases in maternal body weight gain (Krasavage *et al*, 1990).

No testicular effects were reported in a subchronic inhalation study in COBS/CD rats (Katz, 1987) (Section 4.18.4.3). No testicular toxicity was observed in ICL-ICR mice receiving up to 2,000 mg EGnPE/kgbw/d by oral gavage for 5 weeks (Nagano *et al*, 1984).

A NIOSH co-ordinated study of 60 chemicals using the Chernoff-Kavlock protocol did not report any evidence of adverse findings when pregnant mice were dosed with 2,000 mg EGnPE/kgbw/d (Hardin *et al*, 1987).

4.18.4.7 Kinetics and metabolism

In a study comparing percutaneous absorption in rat and human skin *in vitro* the following mean values were obtained: absorption rat skin 2.30 mg/cm^2/h, human skin 0.584 mg/cm^2/h; permeability constant rat skin 2.52 cm/h, human skin 6.43 cm/h (Barber and Kolberg, 1987; Barber *et al*,1992).

Studies in rats show that elimination of EGnPE is rapid with approximately 80% being eliminated in urine within 12 hours as 2-propoxyacetic acid and its glycine conjugate. Smaller amounts (14%) of MEG were also detected in urine (Boatman and Knaak, 2001).

4.18.4.8 Neurotoxicity

Male and female F344 rats were exposed to vapour concentrations of 100, 200 or 400 ppm (6 h/d, 14w). No effects were noted in a functional observational battery (FOB), on grip strength or in histopathology of central and peripheral nerves (Boatman and Knaak, 2001).

4.18.4.9 Immunotoxicity

No data are available.

4.18.5 Human effects data

No data are available.

Table 4.18.1: Systemic toxicity of EGnPE in COBS/CD rats

Route / Number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**				
10 M	0, 195, 390, 780, 1560		1 x/d, 5 d/wk, 6 wk	↓ Hb, RBC; ↑ haemoglobinuria; ↑ spleen weight; histopathological changes in kidneys, spleen, liver	Katz *et al*, 1984
Inhalation	**(ppm)**	**(mg/m^3)**			
5 M, 5 F	0, 100 200 400, 800	(0, 430 870 1,730, 3,500)	6 h/d, 5 d/wk, 11 d	No effects NOAEL Initial haemoglobinuria; blood changes indicative of haemolysis; congestion, lymphoid hyperplasia, extra-medullary haematopoiesis, haemosiderin in spleen	Katz *et al*, 1984
15 M, 15 F	0 100 200 400	(0 430 870 1,730)	6 h/d, 5 d/wk, 14 wk	No effects NOAEL F ↓ RBC, Hb, reticulocytes, MCHb concentration, F ↑ platelets, spleen weight, haemosiderosis; M ↑ kidney weight; haematuria As for 200 ppm plus: F ↑ kidney weight, M ↑ spleen weight, haemosiderosis, M ↓ Hct, M ↑ MCHb concentration	Katz, 1987

Table 4.18.2: Reproductive and developmental toxicity of EGnPE

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Oral, gavage	**(mg/kgbw)**				
Mouse, ICL-ICR, 5 M	Up to 2,000		1 x/d, 5 wk	No testicular toxicity	Nagano *et al*, 1984
Mouse, CD-1, 50 F	2,000		g.d. 6 - 13	No effects. Chernoff-Kavlock protocol	Hardin *et al*, 1987
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, SD, 10 F,	0 100 200, 300, 400	(0 430 870, 1,730)	6 h/d, g.d. 6 - 15	No effects Haemolysis, haematuria at all doses Minor skeletal variations associated with maternal toxicity. Spleen wt ↑ at 200 and 400 ppm	Krasavage and Katz, 1985
Rabbit, NZW, 15 F	0 125, 250, 500	(0 540, 1,080, 2,170)	6 h/d, g.d. 6 - 18	No effects Slight ↓ maternal bw gain; no foetal effects at any concentration	Krasavage *et al*, 1990

4.19 Substance profile: EGnPEA

4.19.1 Identity

Name:	Ethylene glycol (mono) *n*-propyl ether acetate
IUPAC name:	2-Propoxyethyl acetate
CAS registry No.:	20706-25-6
Molecular formula:	$C_7H_{14}O_3$
Structural formula:	C_3H_7–O–CH_2–CH_2–O–CO–CH_3
Molecular weight:	146.2
Other components:	No data

4.19.2 Physico-chemical properties

Melting point:	No data
Boiling point:	173°C
Vapour pressure:	0.67 hPa
Solubility in water:	50 g/l

4.19.3 Conversion factors

1 ppm = 6.078 mg/m^3
1 mg/m^3 = 0.165 ppm

4.19.4 Toxicological data

4.19.4.1 Acute toxicity

Oral

Rat: LD_{50} 9,456 mg/kgbw. Signs of toxicity were abnormal respiration, haemoglobinuria and prostration (Katz *et al*, 1984).

Dermal

Guinea pig: 24-h LD_{50} > 20,000 mg/kgbw (semi-occlusive patch) (Katz *et al*, 1984).

Inhalation

Rat: 6-h LC_{50} > 934 ppm (5,680 mg/m^3), the highest (saturated vapour) concentration tested (Katz *et al* 1984).

4.19.4.2 Irritation and sensitisation

Skin irritation

Undiluted EGnPEA was slightly irritant to guinea pig skin following occluded exposure for 24 hours (Katz *et al*, 1984).

Eye irritation

EGnPEA (0.1 ml undiluted) was slightly irritant to rabbit eyes, causing slight erythema and oedema of the conjunctivae and nictitating membrane, which reversed within 2 days (Katz *et al*, 1984).

Sensitisation

EGnPEA was not a sensitiser in guinea pigs (10/group) following induction by footpad injection of 0.05 ml of Freund's complete adjuvant with (control group without) 1% EGnPEA and challenge 1 week later by 0.3 ml of a 1% solution dropped to the depilated back skin (Katz *et al*, 1984).

4.19.4.3 Repeated-dose toxicity (Table 4.19.1)

Subacute toxicity

COBS/CD rats were dosed by oral gavage with 0, 1,100, 2,200 or 4,400 mg/kgbw/d for 6 weeks. All animals died at 4,400 mg/kgbw. Haemoglobinuria and increased spleen weights were seen in at least one animal at all dose levels. Body weights were reduced at the two highest doses and feed intake was reduced at all levels. Haematological effects occurred at all dose levels. Histopathological effects were noted in spleen (congestion at 2,200 mg/kgbw), kidney (brown pigment at 1,100 and 2,200 mg/kgbw), and testes (atrophy or cytoplasmic vacuolisation of seminiferous tubules at 2,200 mg/kgbw). Similar testicular effects were not observed when an equimolar dose of EGnPE (the presumed major metabolite, Section 4.18.4.7) was administered.

Histopathology on 4,400 mg/kgbw animals, died or killed after 2 or 3 doses, revealed mostly degenerative changes in testes, thymus, liver, kidneys, spleen, and mesenteric lymph nodes. (Katz *et al,* 1984).

COBS/CD rats were exposed (whole-body) by inhalation to 0, 100, 200, 400 or 800 ppm EGnPEA for 11 days. Initial haemoglobinuria was seen in females at 200, 400 and 800 ppm, and in males at 800 ppm. Haematological changes indicative of red cell haemolysis occurred at exposures of 200 ppm and above. The NOAEL was 100 ppm, based on the occurrence of haemoglobinuria (Katz *et al,* 1984).

Subchronic toxicity

No data are available.

4.19.4.4 Genotoxicity

No data are available.

4.19.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.19.4.6 Reproductive and developmental toxicity (Table 4.19.2)

No teratogenicity was seen in COBS/CD rats exposed to vapour concentrations of 0, 100, 200, 400 or 800 ppm from day 6 to 15 of gestation. At 400 and 800 ppm, the dams showed haemoglobinuria and at 800 ppm reduced body weights. The incidence of resorptions was significantly increased at 800 ppm. Minor rib anomalies were slightly increased at 400 and 800 ppm and at 200 ppm a slight increase of common skeletal variants was seen (Krasavage and Katz, 1984a).

The presumed metabolic cleavage product, EGnPE, was not a developmental toxicant either in rabbits or in rats (Section 4.18.4.6).

Effects on testes in the subacute study (see above) were restricted to dose levels of general sever etoxicity and are therefore not interpreted as a primary hazard to male fertility.

It is concluded that EGnPE is not a primary developmental toxicant.

4.19.4.7 Kinetics and metabolism

After single dermal exposure of male Beagle dogs (n = 3) for 30 and 60 minutes to 97.9 mmol of [^{14}C]EGnPEA radioactivity was detected in the urine at 4, 8 and 24 hours thereafter. Peak levels occurred after 4 hours, which declined rapidly afterwards. No measurable concentrations of radioactivity were detected in the blood of dogs exposed for 60 minutes and only small amounts in the expired air (Guest *et al*, 1984).

Male Beagle dogs (n = 4) were whole-body exposed via inhalation to 50 ppm EGnPEA (304 mg/m^3) for 5 hours. Breath samples taken during and up to 3 hours post exposure indicated that between 74 and 90% of inhaled vapour was absorbed within 10 minutes. Post exposure samples showed a rapid, exponential decline (Guest *et al*, 1984).

After single i.v. treatment of male Beagle dogs (n = 3) with 1 mg/kgbw [ethyl-1,2-^{14}C]EGnPEA the urine contained 61% and 88% of the dose after 4 and 24 hours, respectively, and trace amounts (< 1%) were detectable in the expired air (Guest *et al*, 1984).

Percutaneous absorption rates through the skin from Beagle dogs *in vitro* were between 160 and 170 nmol/cm^2/min (1.5 mg/cm^2/h). Absorption lag time before reaching a steady-state was 1.2 hours (Guest *et al*, 1984).

By analogy to other glycol ether acetates, metabolic cleavage of the ester to form the alcohol, EGnPE, is expected to occur with subsequent oxidation to 2-propoxyacetic acid.

4.19.4.8 Neurotoxicity

No data are available. The parent alcohol, EGnPE, is not a neurotoxicant.

4.19.4.9 Immunotoxicity

No data are available.

4.19.5 Human effects data

No data are available.

Table 4.19.1: Systemic toxicity of EGnPEA in COBS/CD rats

Route / Number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, gavage	**(mmol/kgbw)**	**(mg/kgbw)**			
10 M	0 7.5 15 30	(0 1,100 2,200 4,400)	1 x/d, 5 d/wk, 6 wk	No effects Spleen and kidney affected, anaemia Spleen, kidney, liver and testes affected, bw ↓, anaemia Mortality in all animals. Spleen, kidney, liver, thymus, mesenteric lymph nodes and testes affected	Katz *et al*, 1984
Inhalation	**(ppm)**	**(mg/m^3)**			
5 M, 5 F	0, 100 200, 400 800	(0 610, 1,220, 2,430 4,860)	6 h/d, 5/wk, 11 d	No effects; 100 ppm NOAEL Haemoglobinuria in F; haemolysis Haemoglobinuria in M and F; haemolysis; extra-medullary haematopoiesis in spleen, kidneys; haemosiderin in liver (F only)	Katz *et al*, 1984

Table 4.19.2: Reproductive and developmental toxicity of EGnPEA in COBS/CD rats exposed by inhalation

Number and sex/group	Concentration		Exposure regime	Result	Reference
	(ppm)	**(mg/m^3)**			
20 - 23 F	0, 100 200 400 800	(0, 610 1,220 2,430 4,860)	6 h/d on g.d. 6 - 18	No effects Small ↑ in common variations. NOAEL Slight embryo/foetotoxicity, maternal bw ↓, anaemia Foetotoxicity, resorptions, maternal bw ↓, anaemia	Krasavage and Katz, 1984b

4.20 Substance profile: EGPhE

4.20.1 Identity

Name:	Ethylene glycol (mono) phenyl ether
IUPAC name:	2-Phenoxyethanol
CAS registry No.:	122-99-6
Molecular formula:	$C_8H_{10}O_2$
Structural formula:	$C_6H_5–O–CH_2–CH_2–OH$
Molecular weight:	138.2
Other components:	Diethylene glycol (mono) phenyl ether (DEGPhE, 8% maximum)

4.20.2 Physico-chemical properties

Melting point:	13°C
Boiling point:	246°C
Vapour pressure:	0.04 hPa at 25°C
Solubility in water:	27 g/l
Relative density:	$D_4^{22} = 1.102$

4.20.3 Conversion factors

1 ppm = 5.745 mg/m^3
1 mg/m^3 = 0.174 ppm

4.20.4 Toxicological data

A number of comprehensive toxicological reviews of EGPhE have been published including ECETOC special report No. 7 (ECETOC, 1994); Section 6 of Chapter 86 of "Patty's Toxicology (Boatman and Knaak, 2001), the US Cosmetic Ingredient Review expert panel (CIR, 1990) and BIBRA toxicity profile (BIBRA, 1988). A collection of available toxicological safety data has been published in the IUCLID Data Set of the European Chemicals Bureau (IUCLID, 2000). In the USA, the Environmental Protection Agency requested additional testing on EGPhE from the chemical industry, the results of which were submitted during 1985 to 1987, and made available in summarised form (US-EPA, 2002).

4.20.4.1 Acute toxicity

Oral

Rat: LD_{50} 1,300 to 4,000 mg/kgbw (Smyth *et al*, 1941; Grote and Woods, 1955; Unilever, 1981a cited by CIR, 1990)or 2,740 mg/kgbw (BASF, 1982b). LD_{50} of cosmetic grade EGPhE (≥ 92% EGPhE, ≤ 8% DEGPhE) was 1.26 ml/kgbw in male and 2.33 ml/kgbw in female rats (approximately 1,000 and 2,000 mg/kgbw, respectively) (CIR, 1990; Boatman and Knaak, 2001). Clinical signs included a dose-related decrease in spontaneous activity, reflexes, dyspnoea and laboured respiration.

Rabbit: Three females were treated with 800 mg/kgbw and observed for 24 hours. One animal exhibited darkened urine, red nasal discharge, and lethargy. Gross and histopathology revealed lesions consistent with haemolytic anaemia (enlarged, congested spleen, haemoglobin in renal tubules, red urine) (Breslin *et al*, 1991).

Dermal

Rat: LD_{50} 2,300 to 14,300 mg/kgbw (American Cyanamid, 1982; Union Carbide, 1982; Nipa, 1987; all cited by BIBRA, 1988).

Rabbit: LD_{50} 5,000 mg/kgbw (Union Carbide, 1982 cited by BIBRA, 1988).

Inhalation

Rat: No signs of toxicity were observed in rats after 8-hour exposure to saturated vapour (American Cyanamid, 1982; Union Carbide, 1982; both cited by BIBRA, 1988).

4.20.4.2 Irritation and sensitisation

Skin irritation

EGPhE (500 mg) was slightly irritant to rabbit skin (Union Carbide, 1958 cited by BIBRA, 1988). In a series of studies on intact rabbit skin, EGPhE was non-irritant to slightly irritant (BASF, 1963, 1983a).

Eye irritation

Undiluted EGPhE caused moderate to severe irritation in the rabbit eye (several studies cited by BIBRA, 1988). In another study, EGPhE was a severe irritant to the rabbit eye (BASF, 1963, 1983b).

Sensitisation

EGPhE was non-sensitising in a series of studies in guinea pigs, following the Magnusson-Kligman or similar maximisation protocols (Unilever, 1981b; Bruze *et al*, 1988; Hausen, 1993; BASF, 2002).

4.20.4.3 Repeated-dose toxicity (Table 4.20.1)

Subacute toxicity

Oral administration of EGPhE to female NZW rabbits at doses of 0, 100, 300, 600 or 1,000 mg/kgbw/d for 10 consecutive days resulted in death of 1 animal at 300, 3 animals at 600 and 4 animals at 1,000 mg/kgbw, and a dose-dependent (severity and time to onset) intravascular haemolytic anaemia, characterised by decreased RBC parameters, haemoglobinuria, splenic congestion, renal tubular damage, and regenerative erythroid response in the bone marrow and spleen (Breslin *et al*, 1991).

Dermal application to rabbits at 1,000 mg/kgbw for 14 days produced similar results (Dow, 1985; Scortichini *et al*, 1987 [Section 4.20.4.6]).

Female rabbits were exposed to 14 daily dermal applications of 1,000 mg/kg/d with no obvious adverse effects which could be related to treatment. No haemolytic anaemia was observed (Phillips *et al*, 1985).

Rabbits were more susceptible to haemolytic effects than F344 rats exposed orally to up to 2,500 mg EGPhE/kgbw for 2 weeks (Dow, 1986a; Breslin *et al*, 1991) or Wistar rats following oral administration of up to 500 mg EGPhE/kgbw for 4 weeks (Unilever, 1991a).

In a 5-week study in ICL-ICR mice at oral doses of up to 2,000 mg/kgbw/d no testicular effects were observed. At 2,000 mg/kgbw, all animals died (Nagano, 1979, 1984).

Subchronic toxicity

No systemic adverse treatment-related effects were reported following oral administration of up to 400 mg EGPhE/kgbw/d for 13 weeks to rats and mice (Nipa, 1977; Unilever, 1991b) and repeated dermal application of 500 mg/kgbw/d to rabbits (Dow, 1986b; Breslin *et al*, 1991). High oral doses of 2,000 mg/kgbw/d produced renal toxicity and death of 4 animals, and increased organ weights (liver, kidney, thyroid) (Nipa, 1977). No signs of testicular toxicity were seen.

EGPhE caused some lethality in a continuous breeding study with mice at high oral doses of 1,875 and 3,700 mg/kgbw (NTP, 1984; Heindel *et al*, 1990; Chapin and Sloane, 1997) (Section 4.20.4.6).

4.20.4.4 Genotoxicity (Table 4.20.2)

In vitro

EGPhE was not mutagenic in the standard *Salmonella typhimurium* assay (Ames test) up to 5,000 µg /plate (Nipa, 1982a) and the HGPRT assay in CHO cells up to 3,500 µg/ml (Dow, 1987). In a cytogenetic assay in CHO cells, EGPhE did not cause an increase in chromosome aberrations rate (Unilever, 1985). These tests were conducted with and without the addition of a metabolic activation system.

In vivo

EGPhE did not produce an increase in chromosome aberrations in a bone marrow cytogenetic assay in SD rats dosed up to 2,800 mg EGPhE/kgbw (Dow, 1988), nor did it have an effect on the incidence of micronucleated PCE in the bone marrow of CD-1 mice dosed up to 1,200 mg/kgbw) (Nipa, 1982b).

In all, no evidence for a genotoxic potential of EGPhE was seen in any test.

4.20.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.20.4.6 Reproductive and developmental toxicity (Table 4.20.3)

Effects of EGPhE on fertility were measured in a multigeneration, continuous breeding study in CD-1 mice using dietary administration of 0.25, 1.25 or 2.5% (corresponding approximately to375, 1875 or 3,700 mg/kgbw/d). Mice were treated for 7 days before mating, during mating and for a subsequent 98-day cohabitation period. Fertility was reduced (reduction in litter size by 19%) at the highest dose level, in the presence of decreased paternal body weight and severe liver weight increase. Reproductive organs were unaffected. The results of a cross-over mating (treated with untreated animals) attributed this effect to impaired female fertility. In the F_1 generation, there was severe neonatal mortality in the mid- and high-dose pups, whereas fertility was not affected. EGPhE produced reproductive and developmental toxicity only at doses that were maternally toxic (NTP, 1984b; Heindel *et al*, 1990; Chapin and Sloane, 1997).

Dermal application of EGPhE at 300, 600 or 1,000 mg/kgbw/d to pregnant NZW rabbits resulted in haemolysis and mortality at the highest dose. No effect was observed in the foetuses of the surviving high-dose animals (5/25) and no adverse maternal or foetal effects were observed at the mid- or low dose level (Scortichini *et al*, 1987).

No embryotoxic, foetotoxic or teratogenic effects were seen in Wistar rats dosed up to 175 mg EGPhE/kgbw s.c. (Unilever, 1984f).

4.20.4.7 Kinetics and metabolism (Table 4.20.4)

Following oral or dermal administration of up to 800 mg radiolabelled EGPhE/kgbw to Wistar rats and NZW rabbits, EGPhE was primarily excreted in the urine. The major urinary and blood metabolite was PhAA, as judged by radioactivity detection (Howes, 1988; Breslin *et al*, 1991). In skin post-mitochondrial fractions, EGPhE was metabolised to phenoxyacetic acid at 5% of the rate for liver. The responsible enzyme is likely to be ADH (Roper *et al*, 1997, 1998). In human volunteers, orally or topically administered EGPhE was excreted in the urine as free or conjugated phenoxyacetic acid (Howes, 1988).

The dermal penetration of EGPhE was studied *in vitro* using rat and human skin. The total absorption rates were 43 to 64%, respectively, in open systems, and nearly quantitative (85 to 94%) when tested under occlusion (Roper *et al*, 1997, 1998). In volunteers, 9 to 48% of topically

applied EGPhE (skin cream, containing 1.2% EGPhE) was recovered in the urine as PhAA (Howes, 1988).

4.20.4.8 Neurotoxicity

No data are available.

4.20.4.9 Immunotoxicity

No data are available.

4.20.5 Human effects data

EGPhE did not cause skin irritation, sensitisation or phototoxicity when tested in human clinical trials. The material is regarded as safe when used as a cosmetic ingredient at a rate of 1% (CIR, 1990).

No irritation was reported in human patch tests with 1, 5 or 10% EGPhE in petrolatum. Patch testing of 2,736 subjects with 1% EGPhE in petrolatum elicited no irritant or allergic reactions at 2 or 4 days. Further patch testing of 130 individuals with EGPhE at 1, 5 or 10% produced no irritant or allergic reaction. A single adverse reaction to a cream containing 1% EGPhE was reported in a patient with hand eczema (Lovell *et al*, 1984).

De Groot *et al* (1986) patch tested 501 individuals with suspected contact dermatitis with 5% EGPhE in petrolatum; one patient gave a positive patch test.

Several human patch tests confirmed that EGPhE is non-sensitising in man (Unilever, 1984 cited and reviewed by CIR, 1990).

Retrospectively, diminished sensation and strength of hands and fingers were reported in 3 women employed at salmon hatchery following exposure to EGPhE, primarily by skin contact. Each worker used about 500 ml/d (438 mg/d) to anaesthetise the fish for tagging (there is no further information on exposure level or dose in the publication). Other effects reported included headache, light-headedness, slurred speech, euphoria, grogginess and "feeling drunk". After 1 to 2 years of exposure, a gradual onset of cognitive impairment that persisted up to at least 3 years (confirmed by neuropsychological tests) with an inability to work developed. Persistent neuropathy did not develop. It was concluded that immediate and delayed effects of EGPhE on

the CNS resemble those of other organic solvents (Morton, 1990) (Section 3.2.2). Many possible confounders of this finding, as discussed by Schmuck *et al* (2000), cast serious doubts about whether there is a causal relationship between exposure to EGPhE and the reported effects.

Human erythrocyte osmotic resistance to EGPhE and PhAA, the major metabolite of EGPhE, was measured *in vitro*. EGPhE had no haemolytic effect up to 1.0% in human blood (Nipa, 1985).

4.20.6 Other information

The haemolytic activity of EGPhE was compared with that of PhAA and EGBE in erythrocytes from female rabbits after incubation for 1 hour at concentrations from 1 to 20 mg/ml. Starting at 5 mg/ml, EGPhE exhibited cell lysis which was nearly complete at 10 mg/ml, and was stronger than that of EGBE. PhAA failed to produce haemolysis (Breslin *et al*, 1991).

In experimental leukaemia models, EGPhE, in contrast to EGBE, was an ineffective anti-tumorigenic agent (Dieter *et al*, 1990) (Section 4.1.6).

Table 4.20.1: Systemic toxicity of EGPhE

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Results	Reference
Oral, gavage	**(mg/kgbw)**			
Rat, F344, 3 F	1,250 2,500	7 d/wk, 2 wk	No red cell haemolysis, ↓ Hct in one animal No red cell haemolysis	Dow, 1986
Rat, CD, 15 M, 15 F	0, 80 400 2,000	7 d/wk, 13 wk	No effects ALP elevated (M) at wk 4, not wk 13. Some renal damage (M) Deaths (4 F). ↓ RBC (F wk 4, M/F wk 13). ↓ Hb/Hct (F wk 4, M/F wk 13). ↓ Hb/Hct (M/F wk 13). ↑ urea/glucose at wk 4, not wk 13. ↑ ALP/SGPT (M, wk 4). ↑ ALP (M, wk 13). ↑ urine volume, cells present. ↑ liver, kidney and thyroid weights, renal damage (M/F). 4/15 M some testicular atrophy	Nipa, 1977
Mouse, ICL-ICR, 5 M	0 500, 1,000 2,000	5 d/wk, 5 wk	No effects No significant effects on the weight and morphology of testes, seminal vesicles and coagulating glands, RBC, Hct, Hb or WBC All died before examination	Nagano *et al*, 1979, 1984
Rabbit, NZW, 3 F	800	1 x	↑ erythrocyte fragility after 1 h and 3 h. Red urine; 2 animals had splenic congestion, erythro-phagocytosis, Hb in renal tubules	Breslin *et al*, 1991

Table 4.20.1: Systemic toxicity of EGPhE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Results	Reference
Oral, gavage (cont'd)	**(mg/kgbw)**			
Rabbit, NZW, 3 F	0	10 d (consecutive)	No effects	Breslin *et al*, 1991
	100		Slight ↓ in bw, RBC, Hct and Hb. ↑ WBC, platelets, reticulocytes. Minor urinary changes. Slight erythroid hyperplasia, splenic extra-medullary haematopoiesis	
	300		1 died at d 10. Anorexia, lethargy. Slight ↓ in bw, RBC, Hct, Hb, WBC and platelets; ↑ reticulocytes. Indication of renal tubule damage. Bone marrow erythroid hyperplasia, splenic necrosis (1 animal). Some necrosis of gastric glandular mucosa	
	600		1 died, 2 killed (moribund) on days 3 and 6. Anorexia, lethargy, red urine. ↓ RBC, Hct, Hb; ↑ WBC, platelets and reticulocytes. Kidney/spleen dark and enlarged, renal tubule damage. Bone marrow erythroid hyperplasia, splenic congestion and erythro-phagocytosis. Some necrosis of gastric glandular mucosa	
	1,000		1 died, 3 killed (moribund) on day 2. Anorexia, lethargy, red urine. ↓ RBC, Hct and Hb; ↑ WBC and platelets. Kidney/spleen dark and enlarged, renal tubule damage. Splenic congestion and erythro-phagocytosis. Some necrosis of gastric glandular mucosa	
Oral, diet	**(mg/kgbw)**			
Rat, Wistar, 10 M, 10 F	0, 50, 100, 200	7 d/wk, 4 wk	No significant effects	Unilever, 1991a
	500 (calculated 550)		↓ bw gain; M ↑ plasma ALP. No other significant effects	

Table 4.20.1: Systemic toxicity of EGPhE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Results	Reference
Oral, diet (cont'd)	**(mg/kgbw)**				
Rat, Wistar, 20 M, 20 F (5 M, 5 F for recovery)	0, 50, 100, 200 500		7 d/wk, 13 wk	No significant effects M ↓ food efficiency, present after 5 wk recovery. M ↓ cholesterol, still present after 5 wk recovery. M ↓ serum protein, not present after 5 wk recovery. F ↓ platelets, still present after 5 wk recovery. M ↓ hepatic parenchymal lipid, not present after 5 wk recovery. M ↑ serum ALP	Unilever, 1991b
	(%)	**(mg/kgbw)**			
Mouse, Swiss CD-1, 20 M, 20 F	0, 0.25	(0, 400	14 wk	No effects observed	Heindel *et al*, 1990; NTP 1984
	1.25	2,000		No effects in M, 1 F died	
	2.5	4,000)		2 F died. Slight ↓ bw M and ↑ liver weight	
Dermal, occluded	**(mg/kgbw)**				
Rabbit, NZW, 10 F	0 1,000		14 d (consecutive)	No effects 3 deaths, 4 moribund and killed after 5 - 8 applications. ↓ RBC, Hct and Hb in descendents. ↑ nucleated RBCs, reticulocytes and leukocytes. Skin dark at application site. Pale livers, dark kidneys/spleen. Haemoglobinuria	Dow, 1985
Rabbit, NZW, 10 M, 10 F	0, 50, 150		6 h/d, 5 d/wk, 13 wk	No effects	Breslin *et al*, 1991
	500			Sporadic erythema/scaling at application site. No other effects	

Table 4.20.2: Genotoxicity of EGPhE

Endpoint / Organism	Strain or type / Target	Concentration	Result	Remark	Reference
In vitro					
Gene mutation					
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	50 - 5,000 µg/plate	**–ve**	+/– S9	Nipa, 1982a
CHO cells	HGPRT locus	2,500 - 3,500 µg/ml 2,000 - 3,500 µg/ml	**–ve** **–ve**	–S9 +S9	Dow, 1987
Chromosome aberration					
CHO cells		125 - 1,000 µg/ml 500 - 3,000 µg/ml	**–ve** **–ve**	–S9 +S9	Unilever, 1985
In vivo					
Chromosome aberration		**(mg/kgbw)**			
Rat, SD, 5 M, 5 F	Bone marrow	1 x 280, 933, 2,800, oral gavage	–ve	Sampling at 6, 24 and 48 h	Dow, 1988
Micronucleus frequency		**(mg/kgbw)**			
Mouse, CD-1, 10 M, 10 F	Bone marrow	0, 300, 600, 1,200 (total dose: 2 equal doses 24 h apart), oral gavage	–ve	Sampling at 24 and 48 h after second dose	Nipa, 1982b

Table 4.20.3: Reproductive and developmental toxicity of EGPhE

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, diet	**%**	**(mg/kgbw)**			
Mouse, Swiss CD-1, 20 F	0, 0.25	0, 375	Continuous breeding	No effects on mothers or on reproductive performance of offspring; 375 mg/kgbw NOAEL	NTP, 1984; Heindel *et al*, 1990; Chapin and Sloane, 1997
	1.25	1,875		No effects on mothers (continuous breeding) ; ↑ neonate and pup mortality	
	2.5	3,700		Foetotoxicity indicated;↓ fertility; severe neonatal toxicity, marked ↑ liver weight in parents	
Dermal	**(mg/kgbw)**				
Rabbit, NZW, 25 F	0, 300		g.d. 6 - 18	No embryotoxic, foetotoxic, teratogenic effects. 300 mg/kgbw NOAEL	Scortichini *et al*, 1987
	600			Maternal toxicity (5 deaths, intravascular haemolysis and sequelae); no embryotoxic, foetotoxic or teratogenic effects	
	1,000			Severe maternal toxicity (9 deaths, intravascular haemolysis and sequelae); 5 survived to gestation before group was terminated. No evidence of adverse effects on conception	
Subcutaneous	**(ml/kgbw)**	**(mg/kgbw)**			
Rat, Wistar, 30 F	0	0	g.d. 6 - 15	No embryotoxic, foetotoxic, teratogenic effects	Unilever, 1984f
	0.1	88		NOAEL (maternal)	
	0.2	175		Slight maternal toxicity; no embryotoxic, foetotoxic or teratogenic effects; reproductive NOAEL	
	0.4	350		Maternal and embryotoxicity; no foetotoxicity or teratogenic effects	

Table 4.20.4: Absorption (uptake), distribution, metabolism and elimination of EGPhE

Route / Species, number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
Oral, gavage				
Rabbit, NZW, 3 F	800	1 x	PhAA at 1,000 µg/ml identified in serum up to 25 h after dosing. EGPhE 25 µg/ml at 1 h after dosing, not detectable at 3 h	Breslin *et al*, 1991
Dermal / oral, gavage				
Rat, Wistar, 3 - 4 M or F	6.2 - 160	1 x , 48 and 96 h collection	Analysis of urinary radioactivity identified 2 major components (PhAA > 75% and EGPhE) and 2 minor components	Howes, 1988
Dermal, occluded				
Rat, Wistar, 4 M, 4 F	18	1 x, 48 h collection	Recovery 76% (M) or 65% (F); 55 - 60% urine, 0.6 - 1% faeces, 1.1 - 1.3% CO_2, 2 - 2.5% carcass and 6.5 - 11% skin/patch	Howes, 1988
4M, 4 F	24	1 x, 48 h collection	Recovery 73% (M) or 67% (F); 55 - 58% urine, 1.7 - 2.2% faeces, 1.5 - 1.6% CO_2, 2.3 - 2.4% carcass and 6.5 - 8% skin/patch	
4 F	24	1 x, 48 h collection	Recovery 93% : 80% urine, 3.1% faeces, 2.2% CO_2, 3.7% carcass and 5% skin/patch	
4 F	24	1 x, 48 h collection	Recovery 98%; 84% urine, 1.5% faeces, 2.2% CO_2, 3.1% carcass and 6.6% skin/patch	
3 F	24	1 x, 48 h collection	Recovery > 99%; 94 - 99% in rinsing, 1.1 - 2.5% skin/patch, 0.5% (1 min) 1.2% (5 min) or 2% (10 min) in urine, < 0.1% faeces and 1 - 1.2% carcass	

4.21 Substance profile: EGBE

4.21.1 Identity

Name:	Ethylene glycol (mono) *n*-butyl ether
IUPAC name:	2-Butoxyethanol
CAS registry No.:	111-76-2
Molecular formula:	$C_6H_{14}O_2$
Structural formula:	$C_4H_9–O–CH_2–CH_2–OH$
Molecular weight:	118.2
Other components:	Diethylene glycol (mono) *n*-butyl ether

4.21.2 Physico-chemical properties

Melting point:	–77°C
Boiling point:	171°C
Vapour pressure:	1.17 hPa at 25°C
Solubility in water:	Soluble
Relative density:	$D_4^{25} = 0.898$

4.21.3 Conversion factors

1 ppm = 4.914 mg/m^3
1 mg/m^3 = 0.204 ppm

4.21.4 Toxicological data

A number of comprehensive toxicological reviews of EGBE have been published including ECETOC Special Report No. 7 (1994); the IRIS Support document on EGBE (US-EPA, 1999); the Concise International Chemical Assessment Document on EGBE (IPCS, 1998); the ATSDR Toxicological Profile for EGBE (ATSDR, 1998); "EGBE: A World of Solutions" (American Chemistry Council, 2000); and Section 6 of Chapter 86 of "Patty’s Toxicology" (Boatman and Knaak, 2001).

From the earliest studies conducted with EGBE (Ghanayem *et al*, 1987a and other studies in Table 4.21.1), the RBC was identified as the sensitive target tissue. In sensitive species, toxicity is manifested as intravascular haemolysis caused by the major metabolite BAA (Section 4.21.4.7), and secondary effects resulting from haemolysis are observed in the kidney, liver, and

spleen as well as other tissues. Not all species are equally sensitive to the haemolytic effects of EGBE. Thus, rabbits, rats and mice are sensitive while guinea pigs and humans are not (Ghanayem and Sullivan, 1993). Haemolytic effects seen in female rats are often more pronounced than in males. Also, older animals are more susceptible than young. However, tolerance to EGBE-induced haemolysis has been reported in rats following multiple exposures (Ghanayem *et al*, 1987a, 1992; Grant *et al*, 1985; Sivarao and Mehendale, 1995).

4.21.4.1 Acute toxicity

Oral

Rat:	LD_{50} 560 - 3,000 mg/kgbw (male); 530 - 2,500 mg/kgbw (female). The LD_{50} was age and sex dependent, with females and older animals more susceptible to toxicity. Signs of toxicity included congested or haemorrhagic lungs, mottled livers, congested kidneys and haemoglobinuria (Smyth *et al*, 1941; Carpenter *et al*, 1956; Weil and Wright, 1967; Truhaut *et al*, 1979).
Mouse:	LD_{50} 1,230 mg/kgbw (male) (Carpenter *et al*, 1956).
Guinea pig:	LD_{50} 1,200 mg/kgbw (Carpenter *et al*, 1956). LD_{50} 1,400 mg/kgbw (Gingell *et al*, 1998).
Rabbit:	LD_{50} 320 - 370 mg/kgbw (male) (Carpenter *et al*, 1956).

The blood of male F344 rats, treated with 0, 150, 250, or 500 mg/kgbw, showed an early (after 1 and 2 h) dose- and time-dependent increase in Hct, PCV and MCV. Hct and PCV thereafter fell below control values. This data indicate that the EGBE-induced haemolysis is preceded by a massive swelling suggesting the erythrocyte membrane as the target (Ghanayem *et al*, 1990b). Single oral administration of EGBE at 5 mmol/kgbw (corresponding to 591 mg/kgbw) to male SD rats resulted in a 26% decrease in the number of circulating RBC and a strong (250-fold) increase in the level of plasma haemoglobin 4 hours after treatment (Morel *et al*, 1996).

Dermal

Rat:	LD_{50} > 2,000 mg/kgbw (24 h), occluded or semi-occluded application. Effects more severe following occlusive treatment and included one death and secondary signs of haemolytic toxicity including

haemorrhagic lungs, dark liver and kidney, and haemorrhage of the small and large intestines (Allen, 1993a,b).

Guinea pig: LD_{50} 1,200 - 4,800 mg/kgbw (1 wk), occluded application (Wahlberg and Boman, 1979). LD_{50} > 2,000 mg/kgbw, occluded application (Gingell *et al*, 1998).

LD_{50} 0.23 ml/kgbw (210 mg/kgbw) or 0.30 ml/kgbw (270 mg/kgbw) on intact and abraded skin respectively (Roudabush *et al*, 1965).

Rabbit: LD_{50} 0.45 - 0.56 ml/kgbw (400 - 500 mg/kgbw) (male) (Carpenter *et al*, 1956); 0.11 ml/kgbw (100 mg/kgbw) (female) (Duprat and Gradiski, 1979). Effects on liver, spleen, kidney and lung. Haemoglobinuria was noted.

LD_{50} > 2,000 mg/kgbw (24 h), semi-occluded application (Allen, 1993c).

LD_{50} (male and female) 841 mg/kgbw (24 h), occluded application (Allen, 1993d).

Inhalation

Rat: LC_{50} 486 ppm (male); 450 ppm (female) (4-h exposure) (2,388 and 2,210 mg/m^3, respectively). Effects on kidneys. Haemoglobinuria (Dodd *et al*, 1983).

Mouse: LC_{50} 700 ppm (3,400 mg/m^3) (7-h exposure) (Werner *et al*, 1943a).

Guinea pig: One of 6 guinea pigs died after 4-hour exposure to saturated vapour (actual level not reported); no haemoglobinuria was observed (Carpenter *et al*, 1956).

LC_{50} > 600 ppm (2,900 mg/m^3) (Gingell *et al*, 1998).

Intraperitoneal

Rat: LD_{50} 550 mg/kgbw (Carpenter *et al*, 1956).

Intravenous

Rat: LD_{50} 300 - 500 mg/kgbw (Carpenter *et al*, 1956).

Mouse: LD_{50} 1,130 mg/kgbw (Carpenter *et al*, 1956).

Rabbit: LD_{50} 250 - 500 mg/kgbw (Carpenter *et al*, 1956).

4.21.4.2 Irritation and sensitisation

Skin irritation

EGBE was slightly irritant to rabbit skin following 4-hour non-occluded exposure. Moderate irritation was reported in percutaneous toxicity studies over 24 hours (Tyler, 1984). Under occlusive patch for 4 hours, EGBE (0.5 ml) was irritant (Rohm and Haas, 1989). EGBE was rated irritant to rabbit skin under the EEC testing protocol and severely irritant under the Draize protocol (Zissu, 1995).

Eye irritation

EGBE was severely irritant to the rabbit eye (Tyler, 1984; Rohm and Haas, 1989; Kennah *et al*, 1989).

The potential of EGBE to cause eye irritation in NZW rabbits was assessed in a GLP study conducted to a standard protocol (OECD 405). The average score for irritation (24 - 72 h, 3 animals) was calculated to be 0.9 for corneal opacity, 0.6 for iris, 2.6 for conjunctiva redness, and 1.8 for chemosis. The irritation was reversible within 21 days post-exposure (BASF, 2000).

Sensitisation

EGBE was not a skin sensitiser in the guinea pig maximisation test (0.5% injection induction, 25% application induction, 10% application challenge) (Unilever, 1989; Zissu, 1995). A human repeated-insult patch test indicated no evidence for sensitisation to EGBE (Greenspan *et al*, 1995).

4.21.4.3 Repeated-dose toxicity (Table 4.21.1)

Subacute toxicity

Male and female rats were whole-body exposed to vapour concentrations ranging from 54 to 432 ppm EGBE for 6 weeks. All female rats at 314 and 432 ppm died, while 3 males survived 30 exposures at 432 ppm (314 ppm not tested in males). Relative liver and kidney weights were increased at 107 and 203 ppm. Congestion of the lungs and of most abdominal viscera as well as cloudy swelling of the liver were observed in these animals. Haemoglobinuria was observed in most of the rats at 314 and 432 ppm, but only in 1 of 15 males and 10 of 15 females at 203 ppm. The NOAEC was stated to be 54 ppm (Carpenter *et al*, 1956).

Male guinea pigs were whole-body exposed to vapour concentrations ranging from 54 to 494 ppm, and female guinea pigs to 376 or 494 ppm for 6 weeks. Mortality was slightly increased and body weights decreased at 376 and 494 ppm, the relative kidney weight increased at 203 ppm and above. The NOAEL was stated to be 107 ppm. Additional groups of male and female guinea pigs exposed to 376 or 494 ppm under otherwise identical conditions did not reveal any significant differences in sex response (Carpenter *et al*, 1956).

Following repeated exposure to EGBE for up to 4 weeks, erythrocyte damage or destruction was the major feature in rats, with bone marrow, spleen and liver involved in erythrocyte replacement. Liver, spleen, bone marrow, and kidney effects were essentially sequelae to the primary effect of erythrocyte haemolysis (Grant *et al*, 1985; Krasavage, 1986). Neither EGBE, nor its metabolite BAA, produced significant testicular toxicity (Krasavage, 1986; NTP, 1993, 2000).

In more recent work, female rats were more sensitive than males to the haemolytic toxicity of EGBE following one to three days of dosing, as evidenced by ocular thrombosis and erythrocyte morphological changes (Nyska *et al*, 1999a; Ghanayem *et al*, 2000, 2001).

In the few studies performed with mice no (NTP, 1993) or only slight (Nagano *et al*, 1979, 1984) effects indicative of haemolysis have been reported.

Subchronic toxicity

Feeding of Sherman Wistar rats with dietary concentrations of 0, 300, 1,250, 5,000 or 20,000 ppm (EGBE intake 0, 18, 76, 310 or 1,540 mg/kgbw/d, respectively). Body weight gain was reduced at 20,000 ppm, and relative liver (5,000 and 20,000 ppm) and kidney (20,000 ppm) weights increased without histopathological correlate. Indications for haemolytic effects have not been reported (Carpenter *et al*, 1956).

Subchronic toxicity in rats was similar to that seen in subacute studies, with females more susceptible to the haemolytic effects of EGBE (Dodd *et al*, 1983; NTP, 1993, 2000). In recently reported work, female rats exposed to 500 ppm EGBE and sacrificed moribund in 13-week inhalation toxicity studies displayed disseminated thrombosis of a number of tissues including vertebrae, heart, lungs, liver and pulp of incisors (Nyska *et al*, 1999b; Long *et al*, 2000). Those effects were not present in males.

Male C3H mice were exposed (whole-body) to 100, 200, and 400 ppm EGBE, for up to 18 weeks. Serial examinations were made from each group after 6 weeks (30 exposures), 12 weeks (60 exposures) and 18 weeks (90 exposures), and in groups treated for 18 weeks followed by a 42-days recovery period. No significant mortality, gross pathology or kidney histopathology was observed. Haemoglobinuria was evident at 200 and 400 ppm after the first and first 2 exposures respectively. Increased erythrocyte osmotic fragility occurred at all concentrations (Carpenter *et al*, 1956).

After subchronic exposure of mice by inhalation to concentrations of 0, 31.2, 62.5, 125, 250 or 500 ppm EGME, haemato-toxicity was evident at concentrations of 62.5 ppm and above, more pronounced in females. Mortality was 40% in males and females at 500 ppm. Inflammation, necrosis, and ulceration of the forestomach, liver necrosis, renal tubule degeneration, atrophy of the spleen, thymus, and lymph nodes, and testicular degeneration were diagnosed in animals died or killed in moribund condition. Histopathology revealed increased haematopoietic cell proliferation and haemosiderin pigmentation of the spleen, Kupffer cell haemosiderin pigmentation of the liver, inflammation and epithelial hyperplasia of the forestomach, and renal tubule haemosiderin pigmentation in animals at 500 ppm surviving to the end of study. Liver weight of 500 ppm males was increased (NTP, 2000).

4.21.4.4 Genotoxicity and cell transformation (Table 4.21.2)

In vitro

EGBE was largely without mutagenic activity in the *Salmonella typhimurium* assay (Ames test) with and without metabolic activation (Zeiger *et al*, 1992; NTP, 2000). Hoflack *et al* (1995) reported EGBE to be positive in the Ames test using *S. typhimurium* strain TA97a at concentrations of 2.2 mg/plate or higher. This result could not be repeated when this strain was tested by Gollapudi *et al* (1996) at levels up to 10 mg/plate. At the HGPRT locus of V79 cells EGBE induced a significant increase in mutants at concentrations ranging between about 20 and 90 mmol (Elias *et al*, 1996). In CHO cells, at 0.38 or 38.1 mmol EGBE/l (Chiewchanwit and Au, 1995) or 0.03 to 1% (Union Carbide, 1989a) there was no mutagenic effect at the HGPRT locus.

The overall evaluation of a series of tests for SCE induction and chromosomal aberration in CHO cells by the NTP was negative with and without metabolic activation (NTP, 2000).

There were no chromosomal aberrations noted in human peripheral lymphocytes at incubation concentrations up to 3000 ppm (Villalobos-Pietrini *et al*, 1989). However, EGBE was judged positive in human lymphocytes with regard to SCE induction (Villalobos-Pietrini *et al*, 1989), but negative in CHO cells (Union Carbide, 1989a).

According to Elias *et al* (1996) EGBE weakly increased the frequency of micronuclei/aneuploidy, chromosome aberrations and SCE in V79 cells at the high concentrations of 4 to 17, 8 to 34, and 8 to 34 mmol/l, respectively. The high exposure concentrations in these assays could have resulted in artificial results. Further, the studies reported by Elias *et al* suffer from a lack of reported detail, unusual dose-response curves, and lack of reproducibility (Elliot and Ashby, 1997).

Kerckaert *et al* (1996) reported EGBE to be capable of cell transformation in SHE cells, but a test with the same cell line was judged negative by Elias *et al* (1996). In addition, Park *et al* (2002b) reported a negative response in this same assay for both EGBE and BAA. (It has to be considered that this assay is not generally accepted as a genotoxicity test). Metabolic cooperation was inhibited by non-cytotoxic concentrations (8.5 - 34 mmol) in V79 cells (Elias *et al*, 1996).

In primary rat hepatocytes a significant induction of unscheduled DNA synthesis was observed at the two lowest of six concentrations investigated, which led the authors conclude that EGBE probably a weak inducer of DNA damage (Union Carbide, 1980).

Hoflack *et al* (1997) postulated, based on studies on human lymphocytes, where EGBE was pre-incubated followed by treatment with mutagenic substances, that EGBE might increase the genotoxicity of DNA-damaging agents by some as yet unspecified mechanism, but that at anticipated occupational exposure concentrations, tissue levels of sufficient magnitude are not expected.

In mouse fibroblasts EGBE did not induce p53 (Duerksen-Hughes *et al*, 1999).

In vivo

EGBE did not increase the frequency of micronucleated PCE in the bone marrow of F344 rats or $B6C3F_1$ mice after i.p. administration (NTP, 2000), nor did it produce DNA adducts (^{32}P-postlabeling) when administered orally to SD rats at 120 mg/kgbw or by osmotic mini-pump to transgenic mice at 1,500 mg/kgbw (Keith *et al*, 1996).

Reviews by Elliot and Ashby (1997) as well as by the US-NTP (2000) have concluded that, in view of the many well-documented negative test results, EGBE is non-mutagenic.

4.21.4.5 Chronic toxicity and carcinogenicity (Table 4.21.1)

Lifetime carcinogenicity studies in rats and mice have been completed by the US NTP (2000).

Rats were exposed by inhalation at 0, 31.2, 62.5, or 125 ppm EGBE for 104 weeks (2 years). Significant haemato-toxicity (consisting of a concentration-dependent regenerative anaemia present at 3, 6 and 12 months of exposure) was reported, more pronounced in females than males. Exposure-related increases in the incidences of hyaline degeneration of the olfactory epithelium and Kupffer-cell pigmentation were observed in the liver of exposed males and females. Benign or malignant phaeochromocytomas of the adrenal glands at in females exposed to 125 ppm produced a rating of "equivocal evidence" for carcinogenicity. The incidence of phaeochromocytomas in female rats (125 ppm) showed a positive trend with dose. A single malignant phaeochromocytoma was observed in a high dose female. However, the incidence of this tumour type (combined benign and malignant) was not significantly greater than chamber controls but did exceed historical control values from other inhalation studies. The slight increase in phaeochromocytomas was considered an "equivocal" finding that could not, with certainty, be attributed to EGBE exposure (NTP, 2000).

Mice were exposed by inhalation to concentrations of 0, 62.5, 125, or 250 ppm EGBE for 104 weeks. Survival of male mice was significantly reduced at 125 and 250 ppm. Body weights of exposed male and female mice were less than those of the controls, females being earlier and more affected. Significant haemato-toxicity consisting of a concentration-dependent regenerative anaemia was present at 3, 6 and 12 months of exposure. Female animals were more affected than males. A dose-related increase in Kupffer-cell pigmentation was observed in the liver of exposed male (125, 250 ppm) and female (all dose levels) animals. Significant non-neoplastic lesions were present in the bone marrow (hyperplasia in males at 125 and 250 ppm), olfactory and respiratory epithelia (females at all levels but without relation to concentration), and spleen (haematopoietic cell proliferation and haemosiderin pigmentation at all levels). In addition, ulcers and epithelial hyperplasia of the forestomach were present with effects in males (at 125 and 250 ppm) and, more severe, in females (all concentrations). A significant increase in the combined incidence of squamous-cell papilloma or carcinoma of the forestomach (single carcinoma at highest dose) in female mice produced a rating of "some evidence" for carcinogenicity. In male mice, the presence of haemangiosarcomas of the liver (significant at 250 ppm) led to a rating of "some evidence" of carcinogenicity. Liver neoplasms (in particular haemangiosarcomas) were observed in male mice but not female mice (NTP, 2000).

Work supported primarily by the American Chemistry Council (ACC) Ethylene Glycol Ethers Panel suggests that the liver neoplasms in male mice reported by NTP (2000) were produced as a consequence of oxidative stress subsequent to RBC haemolysis and haemosiderin (iron) deposition in the liver (Boatman *et al*, 2004). Xue *et al* (1999), Kamendulis *et al* (1999), Siesky *et al* (2001), and Park *et al* (2002a) suggest a strong correlation between iron deposition in hepatocytes or Kupffer cells and the production of oxidative stress in the livers of mice. In mice orally dosed with EGBE up to 600 mg/kgbw/d for up to 90 days, there was a dose-related increase in Perl's staining (iron) in Kupffer cells and hepatocytes and an increase in DNA synthesis, indicating cell proliferation. Oxidative damage, as measured by an increase in 8-hydroxydeoxyguanosine and lipid peroxidation, was accompanied by a decrease in liver vitamin E content. EGBE induces DNA synthesis in the livers of mice having induced hepatic focal lesions (DEN treated) but this synthesis is prevented by vitamin E supplementation (Gottschling *et al*, 2000).

In Syrian hamster embryo (SHE) cells *in vitro*, neither EGBE nor BAA produced cell transformation (Park *et al*, 2002b). However, in the same study, ferrous sulphate induced significant cell transformation and DNA damage, which was reduced or eliminated by antioxidant treatment. Similarly, for cultured hepatocytes from rats or mice or with a mouse alveolar macrophage cell line (CRL-2019), neither EGBE nor BAA up to 25 mmol/l produced oxidative stress (Xue *et al*, 1999; Park *et al*, 2002a), whereas ferrous sulphate treatment produced a positive response. This research further suggests that this effect is seen only in the male mouse liver *in vivo* due to the greater sensitivity of male mouse liver cells to oxidative damage compared to those of either the female mouse or rat. Humans, who are insensitive to the haemolytic effects of EGBE, and have higher levels of hepatic antioxidant capacity compared to rodents, are not expected to show these effects.

The forestomach neoplasms (primarily benign papillomas) reported for female mice in the NTP (2000) bioassay are most likely the result of prolonged exposure-induced irritation with subsequent tumour formation. In work supported by the European Oxygenated Solvents Producers Association and the ACC, it has been concluded that the mouse forestomach is a target organ for EGBE toxicity as a consequence of either direct ingestion of material present on the skin and fur (grooming) or indirectly as a result of muco-ciliary clearance from the nasopharynx. Alternatively, the forestomach may act as sink for EGBE (or BAA) while functioning as a food storage organ, allowing greater residence time in contact with the forestomach epithelium.

In this regard, a dose-related proliferative response was seen in the forestomach following either oral or i.p. dosing (Corley *et al*, 1999). Green *et al* (2002) reported a rapid distribution of ^{14}C-EGBE following i.v. or inhalation exposure, with levels of radioactivity present in the forestomach higher than in the glandular stomach and significant radioactivity deposited on the fur (inhalation). However, i.v. administration resulted in an equal distribution of label to the

forestomach and glandular stomach. A number of tissues are also selectively labelled, including liver, bone, Harderian glands, and buccal cavity following i.v. administration (Green *et al*, 2002). These i.v. results provide evidence for ingestion of material from the buccal cavity, the origin of which may be the salivary or Harderian glands (Boatman *et al*, 2004).

Both EGBE and BAA produce hyperkeratosis in the forestomach of female B6C3F_1 mice after 10 days of oral administration, producing an identical lesion (Green *et al*, 2002). However, BAA was the more potent, with a NOAEL of 50 mg/kgbw/d for BAA versus 150 mg/kgbw/d for EGBE. Poet *et al* (2002, 2003) reported high levels of EGBE in female mouse forestomach following either oral or i.p. dosing. BAA levels paralleled EGBE. The increased levels were maintained throughout a 24-hour sampling period. Also, EGBE in saliva was present at concentrations similar to blood. These authors proposed that food remaining in the stomach may act as a sink for EGBE and BAA, providing continued contact with and delivery of EGBE/BAA to the forestomach epithelium resulting in elevated tissue levels.

Humans have no organ that is either morphologically or functionally comparable to the rodent forestomach. The human tissue most closely resembling the rodent forestomach is the oesophagus, and this does not function as a food storage organ nor is there appreciable residence time to allow for delivery of the chemical to occur. It is accepted that for non-genotoxic carcinogens such as BHA or propionic acid, that carcinogenic risk to humans based on forestomach tumour formation in rodents is minimal (Kroes and Wester, 1986).

4.21.4.6 Reproductive and developmental toxicity (Table 4.21.3)

EGBE was not teratogenic in all those studies with rats, mice or rabbits which have been performed following standard guidelines and/or using a sufficiently high number of animals. Maternally toxic dose levels caused embryotoxicity and foetotoxicity (Sleet *et al*, 1989; Nelson *et al*, 1984b; Tyl *et al*, 1984; Unilever, 1976). Therefore, the very few cleft palates observed in a "teratology probe study" with only 6 female mice/group and using excessively high dose levels (Wier *et al*, 1987) are not considered as an indication of a teratogenic potential of EGBE. EGBE did not cause heart or great vessel defects in rats (Sleet *et al*, 1989). In this latter study, maternal toxicity was observed at 100 mg/kgbw/d or higher (maternal NOAEL: 30 mg/kgbw/d), whereas developmental toxicity, as reduced prenatal viability, was only seen at the highest dose level (200 mg/kgbw/d) with a NOAEL of 100 mg/kgbw/d.

In a continuous breeding study with male and female CD-1 mice EGBE was administered at concentrations of 0, 0.5, 1.0, and 2.0% in the drinking water. At 1.0 and 2.0 % mortality in females was 30 and 65%, respectively, and body weights were decreased. Water consumption was reduced in all treatment groups up to 50%. The number of live pups was reduced at 1.0 and

2.0%. Pup weights were dose-dependently reduced at all levels, the difference to controls being very small (5%) at 0.5% (Heindel *et al*, 1990; Chapin and Sloane, 1997).

Single doses of BAA administered to rats up to 868 mg/kgbw failed to produce testicular toxicity (Foster *et al*, 1987).

4.21.4.7 Kinetics and metabolism (Table 4.21.4)

Urine is the major route of excretion following administration of EGBE. The primary urinary metabolite of EGBE was BAA in all species studied *in vivo* (rat, mouse, guinea pig and man).

BAA was identified in the urine of male albino rats exposed by inhalation to an atmospheric concentration of 2,000 mg/m^3 for 1 hour (Jönsson and Steen, 1978).

Uptake and elimination of inhaled EGBE in the rat was essentially linear over the range 43 to 438 ppm (Sabourin *et al*, 1992a). In humans, the glutamine conjugate of BAA has been reported in urine samples of exposed workers (lacquerers) (Rettenmeier *et al*, 1993). However, no similar amino acid conjugate has been identified in rodents (Table 4.21.4).

Data from 48 exposed workers suggested that an estimated 57% (95% confidence interval 44 - 70%) of the total BAA is excreted in the conjugated form, and that conjugation may be activated above a certain exposure level. Using total BAA significantly reduced the inter-individual variation. Elimination half-lives for free and total BAA were similar (approximately 6 h) and there was no delay in excretion of the conjugated metabolite. Peak excretion of both free and total BAA was between 6 and 12 hours after the end of exposure (Jones and Cocker, 2003).

The influence of temperature, humidity, and clothing on the dermal absorption of EGBE vapours was studied in 4 human volunteers exposed on 9 occasions. Baseline absorption was, on average, 11% of the total absorbed dose. Higher temperature (30°C instead of 20°C) and greater humidity (65% instead of 60%) increased dermal absorption to 14% and 13%, respectively. The wearing of whole-body overalls did not attenuate absorption (mean 10%) (Jones *et al*, 2003).

EGBE was orally administered once to young (4- to 5-week old) or adult (9- to 13-week old) male F344 rats at 500 mg/kgbw. There was a significantly higher portion of the administered dose eliminated by the young rats as CO_2 and in urine as compared to the older rats. The ratio of BAA:EGBE-glucuronide + EGBE-sulphate was significantly higher in older rats, indicating that a decreased degradation/depressed urinary excretion of BAA is responsible for the higher sensitivity of older rats to the haemolytic effects of EGBE (Ghanayem *et al*, 1987a).

The metabolism of ^{14}C-EGBE in male F344 rats has been investigated following single oral administrations at 125 and 500 mg/kgbw. EGBE was rapidly absorbed after oral administration and is rapidly metabolised and eliminated. The major route of EGBE elimination was in the urine (70% at 125 mg/kg and 40% at 500 mg/kg) followed by $^{14}CO_2$ exhalation (18% at 125 mg/kg and 10% at 500 mg/kgbw). Tissue distributions of residual radioactivity revealed the highest levels present in the forestomach followed by liver, kidney, spleen, and glandular stomach. The increased amounts of EGBE dose eliminated in the urine or as $^{14}CO_2$ at the lower dose suggested that saturation of EGBE metabolizing enzymes has occurred at the 500 mg/kgbw dose. A small portion (8%) of a 500 mg/kgbw dose was eliminated in the bile by 8 hours. The major metabolite of EGBE in urine was BAA, which accounts for more than 75% of radioactivity excreted in urine. The second most abundant metabolite in urine was the glucuronide conjugate of EGBE. In bile the opposite was true, with the glucuronide conjugate present as the most abundant component. A small amount of the sulphate conjugate of EGBE was excreted in urine at the low dose but none was detected at the higher dose (Ghanayem *et al*, 1987b).

Pretreatment with the ADH inhibitors pyrazole or cyanamide protected F344 rats against EGBE-induced haemato-toxicity following oral doses of 500 mg EGBE/kgbw; cyanamide also protected against 2-butoxy acetaldehyde (BAL) induced haemato-toxicity. This protection was associated with significant reductions in the conversion of EGBE to BAA and an increase in EGBE glucuronide and sulphate conjugates. Substitution of methylene group hydrogens on EGBE with deuterium delayed, but did not abolish, the haemato-toxic effects of EGBE. These results were consistent with the metabolism of EGBE to BAA being a primary cause of haemato-toxicity following administration of EGBE. Comparison of tissue radioactivity levels 48 hours after oral administration of ^{14}C-EGBE (500 mg/kgbw) showed significantly lower levels of tissue radioactivity in rats where conversion of EGBE to BAA had been blocked (Ghanayem *et al*, 1987c).

The effects of dose, age, inhibition of metabolism and elimination on the toxicokinetics of EGBE and its metabolites have been studied in mature (3 - 4 months) or old (12 - 13 months) male F344 rats; EGBE (31.25, 62.5 or 125 mg/kgbw) was administered by single i.v. injection. Only EGBE and BAA were identified in the plasma. Toxicokinetic parameters for EGBE were measured in EGBE-treated animals alone or in animals pretreated with metabolic inhibitors including 4-methylpyrazole, cyanamide, or probenecid (blocks renal anion transport). Age had no apparent effect on half-life, volume of distribution, or clearance but maximum blood concentrations (C_{max}) for EGBE did increase with age. 4-Methylpyrazole or cyanamide inhibition of EGBE metabolism resulted in increased half-lives and decreased clearance rates for EGBE. Probenecid pretreatment resulted in 2- to 6-fold increases in the elimination half-lives for BAA (Ghanayem *et al*, 1990). These results help to confirm that BAA is responsible for the haemolytic toxicity of EGBE and indicate renal organic acid transport as vital for the detoxification. The authors attributed increased sensitivity of older rats to erythrocyte haemolysis to a number of factors contributing to

increased exposure of erythrocytes to BAA, including compromised renal clearance of BAA, increased conversion of EGBE to BAA and reduced degradation of BAA to CO_2. Erythrocytes from older animals were also more susceptible to BAA-induced haemolysis than erythrocytes from younger animals. Further studies established that rats developed tolerance to EGBE-induced haemolytic anaemia as a result of replacement of old erythrocytes with younger cells (Ghanayem *et al*, 1992; Sivarao and Mehendale, 1995). More recently, Sawant *et al* (1999) have reported that unexpected erythropoietic responses occurred in rats treated successively with EGBE. These authors showed that release of reticulocytes is faster in this case and suggests that a pool of nearly mature reticulocytes remains active in the bone marrow for several days following a haemolytic event.

Non-oxidative metabolism of EGBE via fatty acid conjugation was studied in the liver of F344 rats following a single oral dose of 500 mg/kgbw. Animals were killed 2 hours after treatment, hepatic lipids extracted, and the neutral lipids were separated using solid-phase extraction. It could be demonstrated that EGBE is metabolised non-oxidatively via conjugation with long-chain fatty acids, and the formation of these esters appears to be catalysed by the enzymes involved in fatty acid conjugation of xenobiotic alcohols (Kaphalia *et al*, 1996).

Female $B6C3F_1$ mice were administered (1 x) doses of 50 or 250 mg EGBE/kgbw i.p. or 250 mg/kgbw by oral gavage. EGBE rapidly disappeared from blood and was no longer detectable after 1 hour. At 250 mg/kgbw i.p. or orally, BAA was detectable in the blood up to 12 hours. No BAA was detected in blood at 50 mg/kgbw after 1 hour. At 250 mg/kgbw, forestomach and glandular stomach concentrations were considerably higher than in blood or any other tissue. EGBE concentrations in the forestomach persisted longer than in any other tissue. EGBE half-live and AUC were higher for gavage dosing than i.p. dosing and higher in the forestomach than in any other tissue regardless of route. By 24 hours, 53.8 and 48.4% of the total dose were eliminated in the urine as EGBE, BAA or a conjugate following 250 mg/kg by i.p. and oral gavage, respectively. Peak blood and saliva concentrations of EBGE were detected after 15 and 7.5 min, respectively, regardless of route. Concentrations of EGBE in blood and saliva were nearly identical at all times and were below the detection limit after 1.3 hours. BAA levels did increase over the first 15 to 30 minutes and declined thereafter. The authors concluded that the increased levels of EGBE and BAA in the forestomach can contribute to a prolonged contact irritation, compensatory hyperplasia and tumorigenicity in mice (Poet *et al*, 2003).

The toxicokinetics of EGBE have been reported by Dill *et al* (1998) following inhalation exposures at 31.2, 62.5, or 125 ppm in F344 rats or 62.5, 125, or 250 ppm in $B6C3F_1$ mice for periods up to 18 months. Female rats eliminated BAA more slowly than males but this gender difference was less pronounced for mice. Elimination rates for EGBE and BAA decreased over time in both species.

Percutaneous absorption of neat and in water diluted EGBE was investigated in female guinea pigs. The individual uptake rates varied between 1.3 and 24.8 μmol/cm^2/h, the average was 15 μmol/cm^2/h with neat EGBE. The relative percutaneous uptake rates were approximately equal from the 5, 10, 20, and 100% solutions, while they were approximately twice from the 40 and 80% solutions (Johanson and Fernström, 1986, 1988).

Concomitant *i.p.* administration of ethanol with EGBE in SD rats delayed the elimination of EGBE from the blood (Römer *et al*, 1985). In addition, clear inhibitory effects of concomitant oral administration of three alcohols (ethanol, *n*-propanol, *n*-butanol) on the conversion of EGBE to *n*-butoxyacetic acid (*n*-BAA) were observed in SD rats (Morel *et al*, 1996). Studies on the elimination kinetics of EGBE in perfused rat liver also showed that ethanol inhibited the removal of EGBE (Johanson *et al*, 1986b).

In vitro studies of the metabolic and cellular basis of EGBE-induced haemolytic anaemia in rats were used to assess the risk to man (Ghanayem, 1989; Ghanayem *et al*, 1989). EGBE (10 mmol/l; 118 mg/l) incubated with rat blood caused no haemolysis, whereas 20 mmol/l caused significant haemolysis. BAL and BAA (0.5, 1.0 or 2.0 mmol/l) caused time and concentration dependent swelling of erythrocytes followed by erythrocyte haemolysis. ADH potentiated the effect of BAL on erythrocytes. BAA and BAL (but not EGBE) also caused a time- and concentration-dependent reduction in blood ATP concentration. Partitioning of BAA between plasma and erythrocytes favoured plasma by 6.5:1, but this changed to 3.25:1 after 4 hours. It was not clear whether the ATP fall caused the erythrocyte swelling or whether the swelling led to ATP depletion; the reason for the change in distribution of BAA between plasma and erythrocytes was not defined.

Levels of ADH in female rat livers are greater than those in males (Aasmoe *et al*, 1998). Of the two forms of ADH in rat liver, ADH-2 and ADH-3, the ADH-3 isoenzyme is primarily responsible for glycol ether metabolism.

Johanson *et al* (1986a) evaluated the toxicokinetics of inhaled EGBE in human volunteers exposed to 20 ppm EGBE (100 mg/m^3) during light physical exercise for 2 hours. None of the subjects complained of or showed any signs of adverse effects. The respiratory uptake rate averaged 10 μmol/min (118 mg/l), which corresponded to 57% of the inhaled dose, as judged by blood levels of EGBE. Blood concentrations of EGBE reached a plateau of 7.4 μmol/l (870 μg/l**)** during exposure and fell rapidly at the end of the exposure period with a half life of 40 minutes. Within 24 hours after exposure, 41% of the absorbed amount was eliminated in urine as BAA (the majority appearing within 12 hours) and < 0.03% was excreted as EGBE. The half-life for the decay of BAA in urine from 4 hours after the end of exposure was 5.77 hours (Section 3.1.1).

Johanson and Boman (1991) evaluated systemic exposure to EGBE arising from nose-only and whole-body (no inhalation) exposure in volunteers exposed to 50 ppm EGBE (250 mg/m^3) for

2 hours. Blood EGBE levels were 2 to 3 times higher following whole-body exposure compared to inhalation exposure at 23°C (29% humidity) and 4 to 5 times higher at 33°C (71% humidity). The Task Force concluded that dermal uptake of EGBE may therefore account for around 75% of the total systemic body burden of EGBE (as measured by blood EGBE concentrations) following exposure to 50 ppm EGBE vapour (Section 3.1.1).

Percutaneous absorption was investigated in 5 men keeping 2 or 4 fingers immersed in neat EGBE for 2 hours. Blood samples were analysed for EGBE, urine samples for BAA. The uptake rates ranged from 0.42 to 5.76 µmol/cm^2/h (Johanson *et al*, 1988).

Respiratory uptake was investigated for 10 polar organic solvents with high blood/air partition coefficients, among them EGBE (25 ppm), in 4 healthy male volunteers inhaling the test air for 10 minutes at rest and then room air for 5 minutes. The percentage of solvent in the end-exhaled air and in the mixed-exhaled air increased after the start of the test-air respiration, and reached a quasi-steady-state level within a few minutes. The mean uptake for the last 5 minutes of the test air respiration was 79.7% (Kumagai *et al*, 1999).

Dermal absorption after EGBE vapour exposure was studied with 4 volunteers exposed on 9 occasions under different conditions (whole-body, skin only, varying humidity, temperature and clothing). Baseline dermal absorption was 11% of the total absorbed dose. Higher temperature and greater humidity increased dermal absorption. By combining several factors together in an "industrial scenario" dermal absorption was increased to 39% of the total absorbed dose (Jones *et al*, 2003).

Six male volunteers were dermally exposed to 50% and 90% aqueous solutions or neat EGBE applied to 40 cm² of the volar forearm for 4 hours. Dermal absorption parameters were determined from the 24-hours excretion rate of total BAA (including conjugates) and from EGBE levels in blood. The absorption of EGBE from the aqueous dilutions was markedly higher and accounted (when determined upon BAA in urine) for mean dermal flux rates of 1.34 ± 0.49; 0.92 ± 0.60 and 0.26 ± 0.17 mg/cm²/h with increasing net concentrations of EGBE. Similar relations were observed when EGBE in blood was used for the calculation. The permeability coefficient k_p for 50% and 90% solutions was $1.75 \pm 0.53 \times 10^{-3}$ and $0.88 \pm 0.42 \times 10^{-3}$, respectively (Jakasa *et al* (2003). The data indicate that compared to the neat material, water content as low as 10% may lead to a 4-fold increase in skin permeation (Jakasa *et al*, 2004).

An *in vitro* human skin penetration study on abdominal epidermis, where EGBE was applied undiluted to the outer surface for 8 hours, revealed an absorption rate of 0.198 mg/cm^2/h and a permeability constant of 2.14 cm/h x 10^4 (Dugard *et al*, 1984).

The penetration rate in rat, pig and human skin after 1, 6 or 16 hours was faster for aqueous EGBE than for pure EGBE (3 x faster for rat than for pig or human). The rate was not significantly altered by solvents like isopropanol or linear alkyl sulphonate (LAS) (Bartnik *et al*, 1987).

Percutaneous absorption of EGBE, in aqueous solution or undiluted, through full thickness or dermatomed human breast skin was measured for 24 hours using flow-through diffusion cells. In aqueous solution, steady-state flux was 544 nmol/cm^2/h, time to steady state 0.30 hour and the final level of absorption 1.39 μmol in dermatomed skin. In full-thickness skin time to steady state was increased (1.14 h), while the steady-state flux was reduced to 135 nmol/cm^2/h, as was the final level of absorption (6.7%) (Wilkinson and Williams, 2002).

Physiologically-based pharmacokinetic (PBPK) models

A number of physiologically-based pharmacokinetic (PBPK) models have been developed that describe the absorption, distribution, and elimination of EGBE and BAA in both rats and humans.

Johanson (1986) first modelled EGBE inhalation pharmacokinetics in rats and adapted the model to humans by applying appropriate scaling parameters. Shyr *et al* (1993) incorporated and expanded the model with data from drinking water, dermal, and inhalation exposures in rats. Further refinements by Corley *et al* (1994) produced a model able to simulate blood levels for both EGBE and BAA. Lee *et al* (1998) subsequently have published a model based on that of Corley *et al* (1994) which describes EGBE and BAA pharmacokinetics in rats and mice following long-term (18-month) exposures as reported by Dill *et al* (1998). This latter model accounts for differences in metabolism between male and female rats but underestimates BAA levels in blood. Saturation of elimination may account for the discrepancy. Alternatively, increased plasma protein binding of BAA in female rats is proposed. Varying a number of age-related parameters had little effect on model fit, however, maintaining protein binding capacity constant in rats with age while increasing this same parameter with age in mice led to improved fits.

Johanson (1986) developed a PBPK model for inhalation of EGBE in man which indicated there was unlikely progressive accumulation in workers taking the equivalent of light exercise and exposed to 20 ppm for 8 hours a day. However, the possibility of accumulation by high affinity binding to a specific organ or tissue was not excluded.

Johanson and Fernstrom (1988) related the above inhalation studies in man at 20 ppm EGBE to dermal penetration studies in guinea pigs. Based on the rate of uptake, they calculated that placing both hands into EGBE would be equivalent to an inhalation exposure level of 370 ppm of EGBE during light physical exercise or in excess of 1,000 ppm if at rest (1,800 and 5,000 mg/m^3,

respectively). On the basis of this calculation, they considered that dermal exposure to EGBE should be avoided.

Corley *et al* (1997), using a PBPK model and data obtained from human dermal exposures, estimated that no more than 15 to 27% of EGBE uptake (at rest) could be due to dermal absorption during simulated 8-h exposure to the ACGIH TLV concentration of 25 ppm. Moderate exercise (50 - 100 Watt) reduced the estimated dermal component to 5 to 9% (Section 3.1.1).

4.21.4.8 Mechanistic and in vitro studies on haemolysis

Erythrocytes prepared from human, rat, dog and rabbit blood samples were incubated with different concentrations of EGBE and BAA. Rat erythrocytes lysed at concentrations of 0.05% BAA and above, whereas those of the other species were stable up to 2%. Dog erythrocytes were lysed at 0.05% EGBE and above, whereas the stability of the other species was similar, lysing at 0.25% or higher (Hext, 1984).

In vitro studies examined the metabolic and cellular basis of EGBE-induced haemolytic anaemia in rats and assessed the risk to man (Ghanayem, 1989; Ghanayem *et al*, 1989). Incubation of human blood with BAA (up to 4 mmol/l) *in vitro* caused minimal swelling or haemolysis of erythrocytes and there was no reduction in blood ATP (0.5 - 2.0 mmol/l BAA produced total rat erythrocyte haemolysis). There was a slight increase in haemolysis of human erythrocytes incubated with 8 mmol/l BAA. Female erythrocytes were slightly more sensitive than male erythrocytes. The study was conducted with blood from young volunteers. EGBE reduced the deformability of rat erythrocytes *in vitro* and this was proposed as a possible reason for rat erythrocyte haemolysis *in vivo;* no studies were reported for BAA (Kurantsin-Mills *et al*, 1992). Erythrocyte deformability and haemolysis have been studied with rat and human erythrocytes *in vitro* following exposure to BAA (up to 2.0 mmol/l for up to 4 h). Human erythrocytes were unaffected, whereas rat erythrocytes were haemolysed and showed decreased deformability (Udden, 1994; Udden and Patton, 1994). Human erythrocytes show some minimal decrease in deformability and increased osmotic fragility when incubated at concentrations of 7.5 to 10 mmol/l. These pre-haemolytic effects were seen at concentrations 150-fold greater than concentrations producing similar effects in rat blood (0.05 mmol/l). BAA had no effect on abnormal human erythrocytes (sickle cell, hereditary spherocytosis) or erythrocytes from older subjects (Udden, 1994). Erythrocytes from rats form cup-shaped cells, stomatocytes, and spherocytes when exposed to EGBE *in vivo* or *in vitro* (Udden, 2000). Human blood incubated with BAA up to 2.0 mmol/l showed no similar changes. No haemolysis was seen in blood samples from 97 individuals (adults and children, healthy and ill) when incubated at 10 mmol/l for 4 hours (Udden, 2002). These results suggest that differences in sensitivity to EGBE-induced haemolysis in human populations is minimal.

EGBE penetrated human abdominal skin *in vitro* at a rate of 1.67 µmol/cm^2/h (Dugard *et al*, 1984) and human stratum corneum at 2.5 µmol/cm^2/h (Barber *et al*, 1992), EGBE was absorbed at a rate of 1.56 µmol/cm^2/h (range 0.42 to 5.76 µmol/cm^2/h) during immersion of 2 or 4 fingers of volunteers into undiluted EGBE for 2 hours. Exposure of 4 fingers to liquid EGBE was calculated to be approximately equivalent to an inhalation exposure at 20 ppm based on the rate of uptake of EGBE by both routes (Johanson *et al*, 1988) (Section 3.1.1).

4.21.4.9 Neurotoxicity

Trained female CFE rats were exposed by inhalation (whole-body, 4 h/d, 5 d/wk) for 2 weeks to concentrations of 50, 100, 200 or 400 ppm EGBE. Tests included conditioned avoidance-escape behaviour by a modification of the pole-climb method. Besides a transient haematuria at 200 and 400 ppm no other effect was observed (Goldberg *et al*, 1964).

In addition, EGBE has not produced signs of neurotoxicity in animals exposed either subchronically or in chronic bioassays (Section 4.21.4.4).

4.21.4.10 Immunotoxicity

Subacute toxicity studies conducted with EGBE have not demonstrated degenerative changes in the bone marrow, as has been noted for EGME and EGEE. Studies reporting effects on WBC are inconsistent with more recent robust studies demonstrating a lack of effects following EGBE exposure.

Singh *et al* (2001) reported increased splenic cellularity and spleen weight/body weight ratios in mice exposed dermally to EGBE at 1,500 mg/kgbw/d for 4 days. Topical applications also reduced splenic T cell proliferative and mixed lymphocyte responses but failed to affect a number of other immunological endpoints.

In other studies, EGBE had no significant effect on cellular or humoral measures of immune function. Thus, Exon *et al* (1991) concluded, after comparing the immunotoxicity of EGME with that of EGBE, that the immune system is a sensitive target of the former but not of EGBE. In an experiment designed to compare the potential immunosuppressive activity of various glycol ethers EGBE was administered orally to male F344 rats at dosages ranging from 50 to 400 mg/kgbw on 2 consecutive days. EGBE (in contrast to EGME, EGMEA, and MAA) did not suppress PFC response to TNP-LPS (Smialowicz *et al*, 1992b).

The proliferative activity of guinea pig lymphocytes *in vitro* was not affected by non-cytotoxic doses of BAA (1 mmol/l) or EGBE (2 mmol/l) (Unilever, 1990).

4.21.5 Human effects data

Human poisonings with EGBE have been summarised and reviewed by Udden (1996).

In the first published human volunteer study, 2 men and 2 women were whole-body exposed to vapour concentrations of 98 ppm EGBE for 8 hours, 2 men to 113 ppm for 4 hours, and 2 men and 1 woman to 195 ppm for 8 hours. The 98 ppm concentration was tolerated without any effect (except increased urinary BAA excretion). At 113 and 195 ppm various clinical symptoms were reported (Carpenter *et al*, 1947).

Three cases of human poisonings with EGBE involved probable doses of EGBE of 25 to 60 g. Coma and metabolic acidosis were common features, with hypokalaemia and haemoglobinuria following ingestion of the higher doses. None died as a result of EGBE ingestion, but all required hospitalisation and supportive treatment (Rambourg-Schepens *et al,* 1988; Gisgenbergh *et al,* 1989; Bauer *et al*, 1992; Litovitz *et al*, 1991).

Dean and Krenzelok (1992) reported that of 24 children ingesting EGBE-containing household cleaners, 2 required gastric emptying or lavage followed by hospitalisation, with recovery uneventful. All others were given fluids at home.

Anaemia reported in some cases of human poisonings is most likely not a result of haemolytic toxicity (Udden, 1996).

Massive ingestion of EGBE by a 19-year-old, mentally retarded patient produced hypotension and hypoxia, believed to account for neurological injury that persisted following recovery (Burkhart and Donovan, 1998). An 18-year-old male ingested cleaner containing 22% EGBE on two separate occasions within a 12-day period. Estimated doses received were 1.0 to 1.34 g/kgbw. Minimal hepatic abnormalities following the first episode were not seen following the second ingestion. Acid-base imbalance responded rapidly to haemodialysis and ethanol treatment (Gualtieri *et al*, 1995). No haematological or renal abnormalities were present following either incident.

A 51-year old female who ingested up to 8 ounces (about 230 g) of a cleaner (EGBE and isopropanol) developed prolonged hyperchloremic metabolic acidosis and mental depression. She received ethanol treatment but not haemodialysis. There was no renal dysfunction, oxaluria,

or haemolysis present in this patient during the course of treatment and she was discharged without apparent sequelae (McKinney *et al*, 2000).

No cytogenetic effects were noted in varnish production workers exposed to EGBE, EGEE, and EGEE acetate (Söhnlein *et al* 1993).

Haufroid *et al* (1997) reported slight but significant decreases in Hct levels and increases in MCHb concentration in workers exposed to low levels of EGBE. Urinary BAA excretion was low in these latter studies. Low-level exposure to EGBE in foundry workers has been correlated with increased D-glucaric acid excretion (Collinot *et al*, 1996). This latter effect may be an adaptive rather than toxic response.

Glycol ether exposures have been correlated with a number of congenital malformations including cleft lip. Maternal occupational exposures to glycol ethers were determined through questionnaires, with women divided primarily into two groups: those exposed to EGBE and EGPE and the acetates; and those exposed to methoxypropanol and it's acetate, as well as polyethylene and polypropylene compounds. The authors suggested that this represented evidence of teratogenic effects of EGBE and compounds of the propylene glycol series in humans. They also suggested that EGBE, rather than a causative agent, is acting as a marker for a wider range of occupational exposures (Cordier *et al*, 1997). This study suffers from a lack of biological plausibility, since there is no indication for these effects from animal studies. Selection and recall bias may have contributed to the positive findings.

Carpenter *et al* (1956) reported headaches in human volunteers following inhalation exposure to between 100 and 198 ppm EGBE (490 and 970 mg/m^3) for 8 hours; women appeared to be more severely affected than men.

Seven clerical workers exposed to estimated vapour concentrations of 200 to 300 ppm EGBE for 0.5 to 4 hours experienced intense eye and respiratory irritation, marked dyspnoea, nausea and faintness at the time of exposure, and recurrent eye and respiratory irritation, dry cough and headache. Starting 4 months later, cherry angiomas began to appear on the arms, trunk and thighs of 6 workers over a period of 5 years (Raymond *et al*, 1998).

Table 4.21.1: Systemic toxicity of EGBE

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, feeding	**(ppm)**	**(mg/kgbw)**			
Rat, Sherman Wistar, 5 M, 5 F	0, 300, 1,250	(0, 18, 76	90 d, continuous feeding	No adverse effects; 1,250 NOAEL	Carpenter *et al*, 1956
	5,000	310	↑ relative liver weight		
	20,000	1,540)	↓ bw, ↑ relative liver and kidney weight		
Oral, gavage	**(mg/kgbw)**				
Rat, F344/N, ≥ 5 M at ages 4-5, 9-13, 22-26, or 69 wk	0 32, 63, 125, 250, 500		1 x	No effects Dose- and age-related erythrocyte haemolysis and haemoglobinuria with the oldest rats showing haemoglobinuria at 32 mg/kgbw and all the youngest rats at 250 mg/kgbw	Ghanayem *et al*, 1987a
Rat, F344, 24 M	0		1 x/d, 4 d with 1, 4, 8 or 22 d recovery	No effects	Grant *et al*, 1985
	500			↑ liver/spleen weights to d 8; bone marrow hyperplasia, splenic extra-medullary haematopoiesis (EMH) to d 4; lymphocyte depletion of thymus to d 1. ↓ RBC, Hb and lymphocytes; ↑ MCV, MCHb and reticulocytes. Slow recovery to d 22	
	1,000			↑ liver/spleen weights to d 22, bone marrow hyperplasia; splenic EMH (to d 4); lymphocyte depletion of thymus (to d 1). ↓ RBC, Hb, Hct and lymphocytes; ↑ MCV, MCHb, reticulocytes. Slow recovery to d 22	

Table 4.21.1: Systemic toxicity of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage (cont'd)	**(mg/kgbw)**			
Rat, COBS CD, 10 M	0	1 x/d, 5 d/wk, 6 wk	No effects	Krasavage, 1986
	222		↓ Hb and RBC; ↑ MCHb. Splenic congestion. Minor histopathological changes. ↑ Relative liver weight	
	443		1 death. ↓ Hb and RBC; ↑ MCV, MCHb, and MCHb concentration. Haemoglobinuria. ↑liver, spleen, kidney, heart and brain weights. ↑ serum ALP. Focal haemosiderin deposition in liver and kidney. Splenic congestion	
	885		2 deaths. Changes as for 443 mg/kgbw group plus ↑ serum ALT and ↓ glucose. No testicular effects	
Rat, F344, 6 M (8 - 10 wk)	0, 50, 100	1 x/d, 2 d	No significant effect in antibody response (plaque-forming cell response to trinitrophenyl lipo-polysaccharide)	Smialowicz *et al*, 1992
	200		1 died, 1 moribund	
	400		All died	

Table 4.21.1: Systemic toxicity of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage (cont'd)	**(mg/kgbw)**			
Rat, SD, 6 M, 6 F	0	1 x/d, 21 d	No effects	Exon *et al*, 1991
6 M	180		Dose-related ↓ bw. No significant effect on weight of spleen, kidney, thymus, testis or liver. Slight ↑ NK cell cytotoxic response	
6 M	506		↓ water intake. No effect on IgG antibody production, delayed type hypersensitivity response, cytokine production or splenocyte numbers	
6 F	204		↓ bw. No significant effect on weight of spleen, kidney, thymus or liver. Slight ↑ NK cell cytotoxic response	
6 F	444		↓ water intake. No other effects.	
Rat, F344, 6 M	0	1 x/d, up to 12 d (consecutive)	No effects	Ghanayem *et al*, 1992
	125		Treatment 1 - 3 d: ↑ haemolysis. Following this, RBC rebounded and approached pretreatment levels by d 12. Treatment for 3 d followed by 7 d recovery provided protection with a subsequent challenge dose of 125 or 250 mg/kgbw. Also, rats made anaemic by bleeding followed by 7 d recovery were similarly resistant. Resistance attributed to younger erythrocytes formed	

Table 4.21.1: Systemic toxicity of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage (cont'd)	**(mg/kgbw)**			
Rat, SD, 12 F	0 500, 1,500	1 x/d, up to 21 d	No effects Protective dose (PD): rats receiving toxic but non-lethal dose (500 mg/kgbw) 7 d prior to a toxic LD_{90} (1,500 mg/kgbw) were able to survive. This auto-protective effect was 100% at 7 d but falling to 12% by 21 d. Resistance attributed to newly formed cells which, over 21 d, loose resistance to haemolysis	Sivarao and Mehendale, 1995
Rat, SD, 10 F, 3 F, 5 F	0 500, 1,500	1 x, up to 42 d	No effects In one protocol, rats received a PD (500 mg/kgbw) on days 0, 23, or 35 (or various combinations) followed by LD_{90} (1,500 mg/kgbw) on d 42. Results indicate multiple PD's protected against lethality, although haemolysis (to varying extents) present in all groups. In a second study aimed at determining reticulocytosis, PD's administered on d 0 or 7 followed by 1,500 mg/kgbw on d 7 or 14 indicated that a second PD caused a higher peak of reticulocytes than a single PD. Erythropoetin levels increase steadily after PD but return to normal after 7 d. Increased number of erythrocyte predecessor cells speculated in bone marrow and responsible for rapid increase in reticulocytes after second PD	Sawant *et al*, 1999

Table 4.21.1: Systemic toxicity of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage (cont'd)	**(mg/kgbw)**			
Rat, F344/N, 25 M, 15 F 5 M 5 F	0 13 - 346 77 - 265	14 d	No effects Trend for ↓ water consumption. F ↓ bw in top dose. No chemical related gross lesions. No histopathological change in testes/epididymides (only tissues studied). No deaths	NTP, 1993
Rat, F344/N, 25 M, 15 F 10 M 10 F	0 69 - 452 82 - 470	90 d	No effects Dose related anaemia and erythrocyte haemolysis with liver, spleen, kidney and bone marrow effects associated with disposal/renewal of erythrocytes. Dose-related ↓ bw, ↓ thymus weight at top 2 doses (M) and top dose (F). Uterine atrophy at top 2 doses (363 and 470 mg/kgbw). No deaths	NTP, 1993
Rat, F344/N, 25 M, 15 F 10 M	0 124 - 443	60 d, recovery to 90 d	No effects ↓ bw at d 60 and d 90 in top dose. No other significant effects	NTP, 1993
Rat, F344, 8 F	0 250	3 d, killed after 24 h	No effects Haemorrhages noted in sclera. Histopathological changes in eyes included haemorrhages localised in posterior layers of the retina and leading to photoreceptor degeneration. Thrombi in ciliary processes and limbal blood vessels. Changes indicative of an ischaemic-infarctive process	Nyska *et al*, 1999a

Table 4.21.1: Systemic toxicity of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage (cont'd)	**(mg/kgbw)**			
Rat, F344, 8-10 M, 8-10 F	0		No effects	Ghanayem *et al*, 2000
3-6 M, 3-6 F	250	1 x, blood collected at 4, 8, 24 h	F rats more sensitive *in vivo* to haemolytic effects. Morphological changes in erythrocytes noted earlier and more frequently in F and included stomatocytosis, spherocytosis, and schistocytosis. Aggregation of RBC was more prominent in F. No differences were noted for erythrocytes from M and F when treated with BAA *in vitro*. These gender differences in haemolytic sensitivity to EGBE proposed to account for thrombosis and tissue infarctions noted in F rats following 13 wk inhalation exposures	
Rat, F344, 10-12 M, 10-12 F	0	1 x/d, 1, 2, 3 d; killed 24 or 48 h	No effects	Ghanayem *et al*, 2001
5-8 M, 5-8 F	250		Time-dependent haemolytic anaemia reported as macrocytic, hypochromic, and regenerative in both genders. Morphological changes included stomatocytosis, macrocytosis, and moderate rouleaux formation and spherocytosis. Changes more severe as dosing progressed. Changes suggest faster onset in F rats	
Mouse, ICL-ICR, 5 M	0	5 d/wk, 5 wk	No effects	Nagano *et al*, 1979, 1984
	500, 1,000		↓ RBC	
	2,000		All animals died	

Table 4.21.1: Systemic toxicity of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, gavage (cont'd)	**(mg/kgbw)**				
Guinea pig, 5 M, 5 F	0, 500, 1,000 2,000		1 x	No effects 3/5 M and 5/5 F deaths. Surviving animals gained wt normally. Necrosis and haemorrhage of the gastric mucosa in affected animals. No signs of haemolysis	Gingell *et al*, 1998
	(%)	**(mg/kgbw)**			
Mouse, Swiss CD-1, 20 M, 20 F	0	0	Continuous breeding	No effects	Heindel *et al*, 1990; Chapin and Sloane, 1997
	0.5	700		1 F died. ↑ kidney (M/F) and liver (F) weights in F_1 animals	
	1	1,300		6 F died. ↑ kidney M/F and liver F weights. ↓ bw F	
	2	2,100		13 F died	
Mouse, B6C3F_1, 10 M, 10 F	NS	0	14 d	No effects	NTP, 1993
5 M		93 - 627		No biologically significant effects	
5 F		150 - 1,364		No biologically significant effects	
Mouse, B6C3F_1, 10 M, 10 F	NS	0	90 d	No effects	NTP, 1993
10 M		118 - 694		Slightly ↓ bw gain. No other biologically significant effects	
10 F		185 - 1,306		Slightly ↓ bw gain. No other biologically significant effects	

Table 4.21.1: Systemic toxicity of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Dermal	**(mg/kgbw)**				
Rat, Wistar, 6 F 3 F	0, 200 260 320 375 500		1 x	No haemolysis 2 serum haemolysis, 1 haemoglobinuria 3 serum haemolysis, 3 haemoglobinuria 1 serum haemolysis, 1 haemoglobinuria 3 serum haemolysis, 1 haemoglobinuria	Bartnik *et al*, 1987
Rabbit, NZW, 10 M, 10 F	0, 10, 50, 150		5 d/wk, 90 d	No effects	Mayhew *et al*, 1983
Mouse, BALB/c, 5 F	0 25, 100, 1,000, 1,500		4 d	No effects No effect on thymus cellularity or thymus to bw ratios. ↑ spleen cellularity and spleen weight/bw ratios at 1,500 mg/kgbw/d. Topical EGBE reduced splenic T cell proliferative response to Concanavalin A and mixed lymphocyte response to allogenic antigen. Several other immune endpoints were not affected	Singh *et al*, 2001
Dermal, occluded	**(mg/kgbw/d)**				
Guinea pig, 5 M, 5 F	0 2,000		24 h	No effects No deaths or clinical signs of toxicity	Gingell *et al*, 1998
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, Sherman Wistar, 14 - 15 M, 14 - 15 F	0, 54 107, 203 314, 432	(0, 265 526, 998 1,543, 2,123)	7 h/d, 5 d/wk, 6 wk	No effects ↑ relative liver and kidney weight, haemoglobinuria (203 ppm only) Haemoglobinuria. All F died	Carpenter *et al*, 1947

Table 4.21.1: Systemic toxicity of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Rat, F344, 8 M, 8 F	0, 20	(0, 98	6 h/d; 5 d + 4 d	No effects	Dodd *et al*, 1983
	86	423		↓ bw F, Hb; ↑ MCV	
	245	1,204)		↓ bw , RBC, Hb, and MCHb concentration; ↑ WC, MCV, reticulocytes and nucleated erythrocytes	
Rat, F344, 16 M, 16 F	0, 5, 25	(0, 25, 123	6 h/d, 5 d/wk, 90 d	No effects	Dodd *et al*, 1983
	77	378)		↓ RBC, Hb and MCHb	
Rat, F344, 10 M, 10 F	0	(0	6 h/d, 5 d/wk, 14 wk	No effects	NTP, 2000
	31.2, 62.5	153, 307		Haematological effects in F ≥ 31.2 ppm	
	125	614		Haematological effects in M ≥ 125 ppm	
	250	1,230		M 1/10 died	
	500	2,460)		All M survived but F 5/10 died. F ↓ bw and bw gain. ↓ Hct and RBC. ↑ reticulocytes, MCV, and MCHb. F affected more than M. Anaemia characterised as macrocytic, normochromic, and responsive. Tail lesions in F	

Table 4.21.1: Systemic toxicity of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Rat, F344/N, 50 M, 50 F	0 31.2, 62.5 125	(0 153, 307) 614	6 h/d, 5 d/wk, 104 wk	No effects Olfactory epithelium: hyaline degeneration. Liver Kupffer cell pigmentation F ↓ bw. Olfactory epithelium: hyaline degeneration. Liver Kupffer cell pigmentation. Mild macrocytic, normochromic, regenerative anaemia present at 3, 6 and 12 months with F more affected than M. ↑ bone marrow cellularity and ↓ myeloid / erythrocyte ratios. Benign or malignant phaeochromocytomas in F rated "equivocal" evidence of carcinogenic activity at the highest dose	NTP, 2000
Rat, F344/N, 10 F , 10 M	0, 31, 62.5, 125, 250 500	(0, 152, 307, 614, 1,230 2,460)	6 h/d, 5d/wk, 13 wk	No effects F rats killed moribund: loss of distal tails observed. Histological changes in these animals indicated disseminated thrombosis involving coccygeal vertebrae, cardiac atrium, lungs, liver, pulp of incisor teeth, and submucosa of the anterior nasal cavity. Changes in coccygeal vertebrae included ischemic necrosis and/or degeneration of bone marrow cells, bone lining cells, osteocytes, and chondrocytes. No bone lesions or thrombi in M rats	Nyska *et al,* 1999b

Table 4.21.1: Systemic toxicity of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Rat, F344/N, 10 F	0, 31, 62.5 125, 250 500	(0, 152, 307 614, 1,230 2,460)	6 h/d, 5d/wk, 13 wk	No effects In moribund F rats killed after 1 wk (4 F), dental lesions present consisted of dental pulp thrombosis, pulp infarction, and odontoblast infarction of maxillary incisors. Rats killed after 13 wk lacked lesions. This was attributed to development of tolerance to EGBE-induced haemolysis and incisor regeneration	Long *et al*, 2000
Mouse, $B6C3F_1$, 10 M, 10 F	0, 31.2 62.5 125 250 500	(0, 153 307 614 1,230 2,460)	6 h/d, 5 d/wk, 14 wk	No effects F anaemia in all groups ≥ 62.5 ppm more pronounced in F ↓ bw, M anaemia at ≥ 125 ppm, characterised as normocytic and normochromic ↓ bw, ↓ Hct, RBC, and Hb. ↓ bw, abnormal breathing, red urine stains, lethargy, 4/10 M and 4/10 F died. Epithelial hyperplasia and inflammation of forestomach at sacrifice. Haemosiderin in Kupffer cells of liver and renal tubule haemosiderin pigmentation, ↑ liver weight in M	NTP, 2000
Mouse, C3H, 10 - 16 M	0 100 200, 400	(0, 490, 980, 1,970)	7 h/d, 5 d/wk, 30, 60 or 90 exposures (one 90-d group followed by 42 d recovery)	No effects ↑ erythrocyte osmotic fragility ↑ erythrocyte osmotic fragility, haemoglobinuria	Carpenter *et al*, 1956

Table 4.21.1: Systemic toxicity of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Mouse, B6C3F$_1$, 50 M, 50 F	0	(0	6 h/d, 5 d/wk, 104 wk	No effects	NTP, 2000
	62.5	307		↑ haemosiderin pigmentation in Kupffer cells, present in all F exposed ≥ 62.5 ppm	
	125	614		M ↓ survival at 125 and 250 ppm. ↓ bw (M and F), with reductions earlier and greater for F. Minimal normocytic, normochromic, regenerative anaemia present at 3, 6, and 12 months. F more affected than M. Ulcer and epithelial hyperplasia of forestomach, more pronounced in F. ↑ splenic haematopoietic cell proliferation and haemosiderin pigmentation (M and F). ↑ bone marrow hyperplasia (M). ↑ hyaline degeneration of olfactory and respiratory epithelia (F). ↑ haemosiderin pigmentation in Kupffer cells, present in M at ≥ 125 ppm	
	250	1,230)		↑ F: combined squamous-cell papilloma or carcinoma of forestomach, rated as "some" evidence of carcinogenicity. ↑ M: haemangiosarcomas of liver, rated as "some" evidence of carcinogenicity	
Guinea pig, 10 M	0, 54, 107, 203	(0, 265, 526, 998	7 h/d, 5 d/wk, 6 wk	No effects	Carpenter *et al*, 1956
	376, 494	1,543, 2,123)		↑ mortality. ↓ bw	
Guinea pig, 6 M, 6 F	376, 494	(1,543, 2,123)	7 h/d, 5 d/wk, 6 wk	Same mortality in M and F; no significant difference in sex response	Carpenter *et al*, 1956

Table 4.21.1: Systemic toxicity of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Guinea pig, 5 M, 5 F 5 M 5 F	0 633 691	(0 3,110 3,400)	1 h, whole-body	No effects No deaths or clinical signs of toxicity at maximum achievable vapour concentration	Gingell *et al*, 1998
Subcutaneous	**(ml/kgbw)**	**(mg/kgbw)**			
Guinea pig, 3 F	0, 0.1, 0.5, 1.0	(0, 90, 450, 900)	1 x	No effects	Unilever, 1984e
Intravenous					
Rat, Wistar, 4 M	NS	0, 25 - 62.5 75	1 x	No haemolysis Some haemolysis	Bartnik *et al*, 1987

Table 4.21.2: Genotoxicity of EGBE

Endpoint / Organism	Strain or type / Target	Concentration	Result	Remark	Reference
In vitro					
Gene mutation					
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	Up to 10,000 µg/plate 20 - 111 µl/ml	–ve	+/– S9 (rat, hamster)	Zeiger *et al*, 1992
Salmonella typhimurium	TA1535, TA100, TA1537, TA97, TA98	100 - 10,000 µg/plate	–ve	+/– S9 (rat, hamster)	NTP, 2000
Salmonella typhimurium	TA97a TA98, TA100, TA102	Up to 9,000 µg/plate	+ve in TA97a ≥ 2,200 µg/plate –ve in other strains	+/– S9	Hoflack *et al*, 1995
Salmonella typhimurium	TA97a, TA100	Up to 10,000 µg/plate	–ve	+/– S9	Gallapudi *et al*, 1996
Escherichia coli	WP2uvrA	Up to 10,000 µg/plate	–ve	+/– S9	Gallapudi *et al*, 1996
Bacteriophage T4D, with *E. coli* B	CR63 and K12 (Lambda h)	20 - 111 µl/ml	–ve	Significant phage toxicity	Kvelland, 1988
CHO cells	HGPRT locus	0.03 - 0.5% 0.06 - 1%	–ve	+ S9 – S9	Union Carbide, 1989a
CHO-AS52 cells	HGPRT locus	0.38 - 38.1 mmol/l 45 - 4,500 mg/l	–ve	Strong cytotoxicity at 38.1 mmol/l	Chiewchanwit and Au, 1995
Chinese hamster V79 cells		Up to 8 mmol/l 950 mg/l	–ve	Exceedingly high concentrations, lack of reported detail	Elias *et al*, 1996
Sister chromatid exchange					
CHO cells		0.016 - 0.25%	–ve	+/– S9	Union Carbide, 1989a

Table 4.21.2: Genotoxicity of EGBE (cont'd)

Endpoint / Organism	Strain or type / Target	Concentration		Result	Remark	Reference
Sister chromatid exchange (cont'd)						
Chinese hamster V79 cells		8 - 34 mmol/l 900 - 4,000 mg/l		Weakly +ve	Exceedingly high concentrations, lack of reported detail	Elias *et al*, 1996
CHO cells		3,000 - 5,000 µg/ml		–ve	EGBE induced cell cycle delay, reported as an indication of cytotoxicity	NTP, 2000
Human lymphocytes		Up to 3,000 µg/ml		+ve	↑ from 500 to 3,000 µg/ml	Villalabos-Pietrini *et al,* 1989
Chromosome aberration						
CHO cells		2,500, 3,000, 3,500µg/ml		–ve	EGBE induced cell cycle delay, reported as an indication of cytotoxicity	NTP, 2000
CHO cells		1,510, 2,220, 3,000 µg/ml		±ve	– S9	NTP, 2000
CHO cells		500, 1,670, 5,000 µg/ml		– ve	+ S9	NTP, 2000
		(µmol/l)	**(µg/ml)**			
V79 cells		≥ 5	≥ 600	+ve	Pre-incubated with EGBE. +ve response of cells to methylmethane sulphonate, mitomycin C and bleomycin following preincubation with EGBE.	Elias *et al,* 1996
Chinese hamster V79 cells		8 - 34	900 - 4,000	Weakly +ve	Exceedingly high concentrations, lack of reported detail	Elias *et al*, 1996

Table 4.21.2: Genotoxicity of EGBE (cont'd)

Endpoint / Organism	Strain or type / Target	Concentration		Result	Remark	Reference
Chromosome aberration (cont'd)		**(µmol/l)**	**(µg/ml)**			
Human lymphocytes		≥ 5	≥ 600	+ve	Pre-incubated with EGBE. +ve response of cells to methylmethane-sulphonate, mitomycin C and bleomycin following preincubation with EGBE	Hoflack *et al*, 1997
Human lymphocytes		NS		–ve	Exceedingly high concentrations, lack of reported detail	Elias *et al*, 1996
Human lymphocytes		Up to 3,000 µg/ml		–ve	48 h incubation	Villalabos-Pietrini *et al*, 1989
CHO cells		2,513, 3,750, 5,000 µg/ml		–ve	– S9; harvest time: 10.5 h	NTP, 2000
CHO cells		2,513, 3,750, 5,000 µg/ml		+ve at 5,000 µg/ml only	– S9; harvest time: 20.5 h	NTP, 2000
CHO cells		4,500, 4,700, 5,000 µg/ml		–ve	– S9; harvest time: 20.7 h	NTP, 2000
CHO cells		2,513, 3,750, 5,000 µg/ml		–ve	+ S9; harvest time: 12.5 h	NTP, 2000
Unscheduled DNA Synthesis						
Rat hepatocytes	Primary	1 - 1,000 µg/ml		Unclear	Not reproducible	Union Carbide, 1989a
Cell transformation						
SHE cells		Up to 1,500 µg/ml		+ve at 1,000 and 1,250 µg/ml		Kerckaert *et al*, 1996

Table 4.21.2: Genotoxicity of EGBE (cont'd)

Endpoint / Organism	Strain or type / Target	Concentration		Result	Remark	Reference
Cell transformation (cont'd)		**(µmol/ml)**	**(µg/ml)**			
SHE cells		0.5 - 20 EGBE or BAA	60 - 2,400	–ve, EGBE or BAA alone	In contrast, +ve with ferrous sulphate (2.5 and 5.0 µg/ml), as well as DNA damage. Co-treatment with antioxidants prevented this transformation and decreased DNA damage	Park *et al*, 2002b
Syrian hamster embryo cells (SHE)		8 - 34	950 - 4,000	Weakly +ve	Exceedingly high concentrations, lack of reported detail	Elias *et al*, 1996
p53 induction						
Mouse NCTC 929 cell	Fibroblasts	1 - 100 µg/ml		–ve	No cytotoxicity	Duerksen-Hughes *et al*, 1999
In vivo						
Micronucleus frequency						
Rat, F344/N, 5 M	Bone marrow	Up to 0.4 ml (450 mg/kgbw), 3 x i.p. every 24 h		–ve	Killed 24 h after last injection	NTP, 2000
Mouse, B6C3F_1, 5 M	Bone marrow	Up to 0.4 ml (550 mg/kgbw), 3 x i.p. every 24 h		–ve	Killed 24 h after last injection	NTP, 2000
Mouse, CD-1, 4 M, 4 F	Bone marrow	150 - 1,000 mg/kgbw		–ve	Lack of reported detail	Elias *et al*, 1996

Table 4.21.2: Genotoxicity of EGBE (cont'd)

Endpoint / Organism	Strain or type / Target	Concentration	Result	Remark	Reference
DNA adduct formation					
Rat, SD	Liver, brain, kidney, spleen, and testis	1 x 120 mg/kgbw, administered orally for 2 wk	–ve. No increased DNA methylation		Keith *et al*, 1996
Mouse, transgenic (Onco-mouse Neo01)	Liver, brain, kidney, spleen and testis	1 x 1,500 mg/kgbw, administered by osmotic mini-pump for 2 wk	–ve. No increased DNA methylation	No increased tumour formation after 120 d	Keith *et al*, 1996

Table 4.21.3: Reproductive and developmental toxicity of EGBE

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**				
Rat, F344, F	0, 30, 100, 200		1 x/d, g.d. 9 - 11 or 11 - 13 (to cover specific periods of cardiovascular development)	Top 2 doses were maternally toxic (↓ bw and severe haemato-toxicity). Prenatal viability only reduced at top dose and only during 9 - 11 d dosing. No heart or great vessel defects. NOAEL maternal toxicity, 30 mg/kgbw/d. NOAEL developmental toxicity, 100 mg/kgbw/d	Sleet *et al*, 1989
Mouse, CD-1, 6 F	0, 350, 650 1,000 1,500 2,000		1 x/d, g.d. 8 - 14	No effects ↑ resorptions; cleft palate in 1/5 litters (4/43 foetuses) 3/6 mothers died. ↑ resorptions, cleft palate in 1/3 litters (1/25 foetuses) 6/6 mothers died. No effect on number of implantation sites	Wier *et al*, 1987
Oral, drinking water	**(%)**	**(mg/kgbw)**			
Mouse, CD-1, 20 F	0	(0	*Ad libitum*, 14 wk, continuous breeding	No effects	Heindel *et al*, 1990; Chapin and Sloane, 1997
	0.5	700		1 mother died; no other effects on mothers or on reproductive performance of offspring. Slight ↓ in live pup weight. Offspring fertility normal.	
	1	1,300		Maternal toxicity (6 deaths); fewer litters, pups and ↓ pup weight; ↑ pup mortality; ↓ female fertility	
	2	2,100)		Maternal toxicity (13 deaths); fewer litters pups and ↓ pup weight; ↑ pup mortality; ↓ female fertility	

Table 4.21.3: Reproductive and developmental toxicity of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Dermal	**(ml/kgbw)**	**(mg/kgbw)**			
Rat, SD, 9 F	0, 0.12	(0, 110)	4 x/d, g.d. 7 - 16	No effects	Hardin *et al,* 1984
Inhalation	**(ppm)**	(mg/m^3)			
Rat, SD, 15-16 F	0,150 200	(0, 740 980)	6 h/d, g.d. 7 - 15	No effects Haematuria	Nelson *et al,* 1984b
Rat, F344, 36 F	0, 25, 50 100 200	(0, 123, 246 490 980)	6 h/d, g.d. 6 - 15	No effects Maternal-, embryo- and foetotoxicity Maternal toxicity, embryo-lethality and foetotoxicity	Tyl *et al,* 1984
Rabbit, NZW, 24 F	0, 25, 50, 100 200	(0, 123, 246, 490 980)	6 h/d, g.d. 6 - 18	No effects Maternal- and embryotoxicity	Tyl *et al* ,1984
Subcutaneous	**(ml/kgbw)**	**(mg/kgbw)**			
Rat, CD, 20 F	0 0.05 0.1 0.2	(0 45 90 180)	1 x/d, g.d. 6 - 15	No effects Slight ↑ in rib defects; slightly retarded ossification Initial maternal toxicity (weight loss and haematuria). Slight ↑ in rib defects, slightly retarded ossification Initial maternal toxicity (weight loss and haematuria). Slight ↑ in rib defects, slightly retarded ossification	Unilever, 1976

Table 4.21.4: Absorption (uptake), distribution, metabolism and elimination of EGBE

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime / Sampling time	Result	Reference
Oral, gavage	**(mg/kgbw)**			
Rat, F344, 3 M, 4 - 5 wk 3 M, 9 - 13 wk old	500	1 x, 48 h	> 60% of radiolabel excreted, CO_2 exhalation > 20% < 40% of radiolabel excreted, CO_2 exhalation 11% Young rats: higher amounts of EGBE glucuronide and an unidentified metabolite and lower amounts of BAA	Ghanayem *et al*, 1987a
Rat, F344, 3 M	125	1 x, 48 h	Recovery 92%: 70% urine, 2% faeces, 18% CO_2, < 2% exhaled. Urinary metabolites: BAA (> 75%), glucuronide and sulphate conjugates of EGBE and < 5% of an unidentified metabolite. Radiolabel localised in glandular stomach, ^{14}C > blood level in liver, kidney, skin	Ghanayem *et al*, 1987b
	500		Recovery 55%: 40% urine, 3% faeces, 10% CO_2, < 2% exhaled. Urinary metabolites were BAA (> 75%), EGBE glucuronide and < 1% of unidentified metabolite. Radiolabel localised in glandular stomach, ^{14}C > blood level in liver	
	500	1 x, 8 h bile	8% excreted in bile, primarily as EGBE glucuronide. Some EGBE in bile up to 2 h after dose, with BAA excretion in bile ↑ steadily	
Oral, drinking water	**(mg/kgbw)**			
Rat, F344/N, 4 M	28, 47, 140	*Ad libitum*, 24 h, 72 h	Details of % recovered NS. No significant differences in excretion pattern for 3 doses. Urine excretion 50 - 60% of dose as BAA, 8 - 10% as CO_2 and 10% as MEG	Medinsky *et al*, 1990

Table 4.21.4: Absorption (uptake), distribution, metabolism and elimination of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime / Sampling time	Result	Reference
Dermal, non-occluded	**(mg/kgbw)**			
Rat, F344/N, 4 M	14, 43, 77 (disposition part), 61, 181, 299 (metabolism part)	6 h, 72 h	78 - 90% recovery, 21 - 26% absorbed irrespective of dose. 83% of absorbed dose recovered in urine, primarily as BAA. EGBE glucuronide a lesser metabolite, with small amounts of MEG and CO_2. BAA major plasma metabolite	Sabourin *et al*, 1992b
Rat, Wistar, 6 M, 6 F	200	1 x, 48 h	20 - 23% urine, 19 - 23% application site + glass capsule (only samples taken); percutaneous absorption: 28.6% (M), 25.1% (F)	Bartnik *et al*, 1987
Dermal, non-occluded	**(mg/kgbw)**			
Rat, Wistar, 24 F			Highest radioactivity in blood and plasma after 2 h; major part of absorbed EGBE is BAA	Bartnik *et al*, 1987
Guinea pig, 10 F	1.0 ml (mg/kgbw NS)	1 x, blood up to 2 h	Penetration rate 15 µmol/cm^2/h (1,800 µg/cm^2/h)	Johanson and Fernstrom, 1986
Guinea pig, 2 F	1.0 ml of 100%, 80%, 40%, 20%, 5% EGBE in water (corresponding to 0.38 - 7.6 mol/l)	1 x, blood up to 2 h	Penetration rates of 2.3, 5.2, 6.2, 5.9, 4.5 and 4.4 µmol/cm^2/h for 100%, 80%, 40%, 20%, 10% and 5% EGBE in water, respectively. Penetration rate 7.9 µmol/cm^2/h (930 µg/cm^2/h), including 1 outlier. Blood levels 1.8 - 14.2 µmol/ml (210 - 1,680 µg/ml)	Johanson and Fernstrom, 1988
4 F	1.0 ml of 10%			
Guinea pig, 10 F	1.0 ml of 100% EGBE in water	1 x, blood up to 2 h	Penetration rate 7.9 µmol/cm^2/h (930 µg/cm^2/h). Blood levels 1.8 - 14.2 µmol/ml (210 - 1,680 µg/ml)	Johanson and Fernstrom, 1988
Human, skin	Undiluted (3 or 6 mg/ml, 100 or 200 µl)	24 h	Steady-state flux: 544 nmol/cm^2/h (dermatomed skin), 135 nmol/cm^2/h (full-thickness skin)	Wilkinson and Williams, 2002

Table 4.21.4: Absorption (uptake), distribution, metabolism and elimination of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime / Sampling time	Result	Reference
Dermal, *in vitro*					
Rat (hairless), pig, man, 3 skin samples/species	3.5%, 10%. Isopropanol or sodium dodecyl benzene sulphonate (LAS), 100%		1, 6 or 16 h	Penetration rate: rapid, faster for aqueous EGBE than would be predicted from rate for EGBE, 3x faster for hairless rats than for pig or human, not significantly altered by isopropanol or LAS	Bartnik *et al*, 1987
Rat, skin samples from SD, 12 M	Neat (undiluted)		1 x, 8 h	Penetration rate 506 µg/cm^2/h; permeability constant: 5.30 x 10^{-4} cm/h	Barber *et al*, 1992
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, albino, ? M	(408)	2,000	1 h	BAA identified in the urine	Jönsson and Steen, 1978
Rat, F344/N, 11 - 12 wk, 29 M 9 M 9 M	43 49 438	(210 240 2,150)	6 h (nose-only), 66 h	Essentially linear uptake and elimination. Majority of absorbed dose excreted in urine (64 - 76%), 1 - 2% in faeces, 7 - 9% as exhaled CO_2, 13 - 20% in carcass and 2 - 5% as exhaled EGBE. Major urinary metabolite BAA (57 - 68%), lesser amounts of EGBE glucuronide (5 - 16%), MEG (12 - 25%) and unidentified metabolites	Sabourin *et al*, 1992a
Rat, F344, 4 - 12 M, 4 - 12F	31.2, 62.5, 125	153, 307, 614	6h/d, 5d/wk, up to 18 months (whole-body)	F eliminated BAA more slowly than M. ↓ elimination rate for EGBE and BAA over time	Dill *et al*, 1998

Table 4.21.4: Absorption (uptake), distribution, metabolism and elimination of EGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime / Sampling time	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Mouse, $B6C3F_1$, 6 M, 6 F	62.5, 125, 250	307, 614, 1,230	6h/d, 5d/wk, up to 18 months (whole-body)	F eliminated BAA more slowly than M. ↓ elimination rate for EGBE and BAA over time	Dill *et al*, 1998
Human, 6, gender NS	NS		Full shift, 5 d/wk Sampling post-shift last d and pre-shift d 1 of working wk	Pre-shift urine: little or no butoxyethanol-related material. Post-shift urine: BAA and BAA-N-butoxyacetyl glutamine collectively to up to 7 mmol/l. Ratio BAA-N-butoxyacetyl glutamine to total BAA 0.16 to 0.64 (mean 0.48), i.e. substantial fraction of BAA eliminated as amino acid conjugate	Rettenmeier *et al*, 1993
Intravenous	(mg/kgbw)				
Rat, F344, 3 - 4 M, 3 - 4 or 12 - 13 months old	31.25, 62.5, 125		1 x	EGBE and BAA in plasma. Age had no effect on half-life, volume of distribution, or clearance. Maximum blood concentrations (Cmax) for EGBE ↑ with age. Pyrazole or cyanamide inhibition of EGBE metabolism: ↑ half-lives and ↓ clearance rates for EGBE	Ghanayem *et al,* 1990
Guinea pigs, 10 F	10.8		1 x, blood up to 2 h	Average clearance 128 ml/min/kgbw, mean residence time 4.7 min	Johanson and Fernstrom, 1986
Subcutaneous	(mg/kgbw)				
Rat, Wistar, 3 M	18		1 x, 72 h	Recovery 97%: 80% urine, < 1% faeces, 10% CO_2, 2% expired, 5% carcass. Radiolabel localised in spleen, thymus and liver	Bartnik *et al,* 1987

4.22 Substance profile: EGBEA

4.22.1 Identity

Name:	Ethylene glycol (mono) *n*-butyl ether acetate
IUPAC name:	2-*n*-Butoxyethyl acetate
CAS registry No.:	112-07-2
Molecular formula:	$C_8H_{16}O_3$
Structural formula:	$C_4H_9–O–CH_2–CH_2–O–CO–CH_3$
Molecular weight:	160.2
Other components:	No data

4.22.2 Physico-chemical properties

Melting point:	–64°C
Boiling point:	192°C
Vapour pressure:	0.4 hPa
Solubility in water:	15 g/l

4.22.3 Conversion factors

1 ppm = 6.660 mg/m^3
1 mg/m^3 = 0.150 ppm

4.22.4 Toxicological data

A comprehensive toxicological review on EGBEA has been published as ECETOC Special Report No. 7 (ECETOC, 1994).

4.22.4.1 Acute toxicity

Oral

Rat:	LD_{50} 2,400 mg/kgbw (female); 3,000 mg/kgbw (male). Signs of toxicity were haemoglobinuria/haematuria, with extensive renal damage and blood present in the bladder (Truhaut at *al*, 1979).

LD_{50} 7,000 mg/kgbw (Smyth *et al*, 1962).

Mouse: LD_{50} 3,200 mg/kgbw (Eastman Kodak, 1971 cited by BIBRA, 1987).

Dermal

Rabbit: LD_{50} 1,500 mg/kgbw (Smyth *et al*, 1962). Deaths after 24 to 48 hours. RBC and Hb were decreased, with blood in the kidneys and bladder; haemoglobinuria/haematuria (Truhaut *et al*, 1979).

Inhalation

Rat: No mortalities or signs of toxicity in males and females exposed to saturated vapour (nominally 400 ppm; 2,660 mg/m^3) for 4 hours (Truhaut *et al*, 1979) or 8 hours (Smyth *et al*, 1962).

Rabbit: No mortalities in males and females exposed to saturated vapour (nominally 400 ppm) for 4 hours. Slight, transient haemoglobinuria and/or haematuria (Truhaut *et al*, 1979).

4.22.4.2 Irritation and sensitisation

Skin irritation

EGBEA was not a primary irritant to rabbit skin (Truhaut *et al*, 1979). No significant erythema occurred in rabbits after application of EGBEA for 4 hours under covered patch (Jacobs *et al*, 1989). EGBEA applied dermally was non-irritant in rabbits under the EEC protocol but moderately irritant following the Draize method (Zissu, 1995).

Eye irritation

EGBEA was slightly irritant to the eye of rabbits (Truhaut *et al*, 1979).

Sensitisation

No data are available.

4.22.4.3 Repeated-dose toxicity (Table 4.22.1)

Subacute toxicity

EGBEA caused inhibition of renal activity (as judged by decreased urinary succinate dehydrogenase levels) in Wistar rats treated for 2 weeks with 2,900 mg EGBEA/l in drinking water (the dose equalled about one third of the LD_{50}). The authors noted corresponding decreases in urinary ammonia and glycosaminoglycan excretion (Liesivuori *et al*, 1999).

In a 28-day inhalation study, Wistar rats and NZW rabbits exposed to saturated EGBEA vapours (nominally 400 ppm) showed haematuria and haemoglobinuria from week 2. Histopathology in female rats revealed tubular nephrosis. Rabbits of both sexes showed necrotising tubular nephrosis, atrophic tubular dilatation, and luminar granular deposits (Truhaut *et al*, 1979).

Subchronic toxicity

No data are available.

4.22.4.4 Genotoxicity

No data are available.

In view of the rapid hydrolysis of EGBEA to EGBE (Section 4.22.4.7), the mutagenic potential of EGBEA *in vivo* is likely to be similar to that of EGBE (Section 4.21.4.4), i.e. it is unlikely to possess a biologically relevant genotoxic potential.

4.22.4.5 Chronic toxicity and carcinogenicity (Table 4.22.1)

In a 10-month inhalation study, Wistar rats and NZW rabbits exposed to 100 ppm EGBEA showed no treatment related effects (Truhaut *et al*, 1979).

In view of the rapid conversion of EGBEA to EGBE, the chronic toxicity of EGBEA is likely to be similar to that of EGBE (Section 4.21.4.5), i.e. it may cause non-neoplastic lesions, particularly haemato-toxicity, but is unlikely to possess significant carcinogenic potential.

4.22.4.6 Reproductive and developmental toxicity

No data are available.

In view of the rapid conversion of EGBEA to EGBE, the reproductive and developmental effects of EGBEA are likely to be similar to those of EGBE (Section 4.21.4.6), i.e. it is unlikely to be selectively teratogenic or elicit testicular toxicity.

4.22.4.7 Kinetics and metabolism

EGBEA underwent rapid ($t_{1/2}$ 0.96 min) hydrolysis in rat plasma *in vitro* (37°C) to EGBE (Hoffmann and Jäckh, 1985).

4.22.4.8 Neurotoxicity

No data are available.

In view of the rapid conversion of EGBEA to EGBE, any neurological effects of EGBEA are likely to be similar to those of EGBE (Section 4.1.3.8), i.e. it is unlikely to elicit biologically relevant manifestations of neurotoxicity.

4.22.4.9 Immunotoxicity

No data are available.

In view of the rapid conversion of EGBEA to EGBE, any immunological effects of EGBEA are likely to be similar to those of EGBE (Section 4.21.4.9), i.e. it is unlikely to elicit biologically significant effects.

4.22.5 Human effects data

The skin irritation potential of EGBEA under occlusive patch was assessed by determining cutaneous blood flow in human volunteers. EGBEA (48-h patch) or 10% EGBEA in water (3-h patch) did not substantially alter cutaneous blood flow under the conditions of the test (first assessments after 1 and 12 hours respectively). This was in contrast to marked effects seen with toluene, *n*-hexane and *n*-butanol. Erythema and other skin responses were not recorded (Jacobs *et al*, 1989).

EGBEA stimulated the release of prostaglandin E-2 in cultured human keratinocytes, but only at concentrations compromising cellular integrity (Lawrence *et al*, 1997).

Table 4.22.1: Systemic toxicity of EGBEA

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, drinking water	**(mg/l)**	**(mg/kgbw)**			
Rat, Wistar, 16 - 20 M	2,900	NS	2 wk, urine collected last 24 h	↓ succinate dehydrogenase activity in urine suggesting inhibition of renal activity. ↓ ammonia and glycosaminoglycan excretion	Liesivuori *et al,* 1999
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, Wistar, 10 M and 10 F	400 [a,b]	(2,660)	4 h/d, 5 d/wk, 28 d	Slight haematuria and/or haemoglobinuria from wk 2. No other effects	Truhaut *et al,* 1979
Rat, Wistar, 10 M and 10 F	100 [a]	(670)	4 h/d, 5 d/wk, 10 months	No effects	Truhaut *et al,* 1979
Rabbit, NZW, 2 M and 2 F	400 [a,b]	(2,660)	4 h/d, 5 d/wk, 28 d	Haematuria and/or haemoglobinuria from wk 2. ↓ RBC and Hb after 3 wk, slightly (2) or severely (2). The latter 2 died in wk 4; kidneys were hypertrophic and swollen with blood; bladders were filled with blood	Truhaut *et al,* 1979
Rabbit, NZW, 2 M and 2 F	100 [a]	(670)	4 h/d, 5 d/wk, 10 months	No effects	Truhaut *et al,* 1979

[a] Nominal, actual concentration NS

[b] Saturated vapour

4.23 Substance profile: DEGBE

4.23.1 Identity

Name:	Diethylene glycol (mono) *n*-butyl ether
IUPAC name:	2-(2-Butoxyethoxy)ethanol
CAS registry No.:	112-34-5
Molecular formula:	$C_8H_{18}O_3$
Structural formula:	$C_4H_9–(O–CH_2–CH_2)_2–OH$
Molecular weight:	162.2
Other components:	No data

4.23.2 Physico-chemical properties

Melting point:	–68°C
Boiling point:	230°C
Vapour pressure:	0.057 hPa at 25°C
Solubility in water:	Soluble
Relative density:	$D_4^{20} = 0.967$

4.23.3 Conversion factors

1 ppm = 6.743 mg/m^3
1 mg/m^3 = 0.148 ppm

4.23.4 Toxicological data

4.23.4.1 Acute toxicity

Oral

Rat:	LD_{50} 7,300 mg/kgbw (fasted) or 9,600 mg/kgbw (fed) (Krasavage and Terhaar, 1981a).
Mouse:	LD_{50} 2,400 mg/kgbw (fasted) or 5,500 mg/kgbw (fed) (Krasavage and Terhaar, 1981a).
Guinea pig:	LD_{50} 2,000 mg/kgbw (Smyth *et al*, 1941).

Rabbit: LD_{50} 2,200 mg/kgbw (Boatman and Knaak, 2001).

Dermal

Rabbit: LD_{50} 2,800 mg/kgbw (Krasavage and Terhaar, 1981b).

Inhalation

Rat: LC_{50} not established; no mortalities occurred following 7 hours exposure to saturated vapour (Boatman and Knaak, 2001).

Subcutaneous

Rat: 0.5, 1.0 or 1.5 ml/kgbw (480, 970 or 1,450 mg/kgbw) caused erythrocyte toxicity and related changes in female rats; older rats were more susceptible (Unilever, 1987b).

Guinea pig: No signs of toxicity at 0.1, 0.5 or 1.0 ml/kgbw (97, 480 or 970 mg/kgbw) (Unilever, 1984e).

4.23.4.2 Irritation and sensitisation

Skin irritation

DEGBE was slightly irritant to rabbit skin upon prolonged or repeated exposure (Boatman and Knaak, 2001).

Eye irritation

DEGBE (0.1 ml) was moderately irritant to the rabbit eye. Effects were most severe within the first 24 hours; the eye returned to normal within 14 days (Ballantyne, 1984a).

Sensitisation

DEGBE was a non-sensitiser in a guinea pig maximisation test (25% injection induction, 100% application induction and application challenge) (Unilever, 1984a).

4.23.4.3 Repeated-dose toxicity (Table 4.23.1)

Subacute toxicity

DEGBE was of low toxicity by the oral, dermal or inhalation routes in rats and rabbits (Table 4.23.1). High oral doses (above 1,000 mg/kgbw) in the rat showed toxicity consistent with erythrocyte damage as well as changes in the liver, kidneys, and stomach (Smyth and Carpenter, 1948; Krasavage and Vlaovic, 1982; Procter and Gamble, 1985).

Subchronic toxicity

In a number of studies, DEGBE caused only slight toxicity in rats over periods of 13 weeks and at doses of 1,000 mg/kgbw/d and above (oral or dermal) or 14 ppm (inhalation) (Auletta *et al*, 1993; Beyrouty *et al*, 1993; Hobson *et al*, 1986b; Klimisch *et al*, 1992).

Only minimal effects were reported when DEGBE was administered to F344 rats in drinking water at doses of 0, 50, 250, or 1,000 mg/kgbw/d for 13 weeks. The effects seen included slight decreases in blood parameters at the high dose, however, there were no indications of haemolysis. Furthermore, RBC morphology, RBC indices and reticulocyte counts were unaffected. Bone marrow was also unaffected. The liver was the primary target of toxicity at the 1,000 mg/kgbw level producing slight increases in a number of enzymes, changes in measured serum parameters, minor histopathological effects (females), and minimal nephropathy. The 250 mg/kgbw level was considered the NOAEL for this study (Johnson *et al*, 2002).

F344 rats were exposed to 0, 2, 6 or 18 ppm DEGBE vapour for 5 weeks. No treatment-related effects were seen in male rats of any exposure group nor in female rats exposed to 2 ppm. A slightly increased degree of hepatocyte vacuolisation (apparent fat accumulation) was observed in female rats exposed to 6 or 18 ppm, compatible with slightly increased group mean relative liver weight and paleness of liver in 3 of 10 females at 18 ppm. The slight increase of vacuolisation observed in the livers of the 6 and 18 ppm females was considered by the authors to be of questionable toxicological significance (Gushow *et al*, 1981).

Wistar rats were exposed to a DEGBE vapour concentration of 100 mg/m^3 (close to the highest technically feasible value) or to aerosol at 350 or 1,000 mg/m^3 for 2 weeks. In rats exposed to

DEGBE vapour, the only effect was a slight decrease in the spleen weights among the male animals. In the exposed aerosol groups, spleen weights were decreased in a dose-dependent manner. Furthermore, the lung weights were increased in both sexes. Histological examination revealed perivascular and peribronchial mixed cellular infiltration with an increased incidence of granulocytes. In addition, activated alveolar cells, foam cells and distended goblet cells were observed in all bronchial sections. These effects were considered to be sequels of unspecific irritation by the aerosol. Body weight gain was marginally decreased at the high-dose of 1,000 mg/m^3 (BASF, 1991a).

Exposure of female Wistar rats to 350 mg DEGBE/m^3 for 2 weeks was repeated in a new experiment, this time including a 4-week recovery period, which showed that the effects were reversible (BASF, 1991b).

In a subsequent 90-day inhalation study, Wistar rats were exposed by whole-body inhalation to vapour concentrations of 0, 14.5, 43.5 or 105 mg DEGBE/m^3. Satellite groups were included for a 4-week post-exposure period. No exposure-related effects were observed in the course of this study. Thus, 105 mg/m^3 for 90 exposure days were considered as the NOAEL. The slight vacuolation of the hepatocytes observed after 5 weeks in the Gushow, 1981 study could not be confirmed in the 90-day study employing a similar top concentration (saturated vapour concentration) (BASF, 1992).

4.23.4.4 Genotoxicity (Table 4.23.2)

In vitro

DEGBE was not mutagenic in a range of *in vitro* tests. An isolated weak positive result in a mouse lymphoma test contrasts with the negative results from other studies (Thompson *et al*, 1984; Unilever 1984c,d).

In vivo

DEGBE was not mutagenic when tested for sex-linked recessive lethal mutations in *Drosophila melanogaster* (Thompson *et al*, 1984).

In the mouse micronucleus test, DGBE did not increase the incidence of micronucleated PCE when tested in both sexes up to a maximum tolerated oral dose of 3,300 mg/kgbw (Gollapudi *et al*, 1993).

In all, the available data indicate a general lack of genotoxic potential for DGBE.

4.23.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.23.4.6 Reproductive and developmental toxicity (Table 4.23.3)

Orally (rat and mouse), dermally (rat and rabbit) or subcutaneously (rat) administered DEGBE showed no teratogenic, foetotoxic or embryotoxic effects at doses up to 2,050 mg/kgbw/d (Schuler *et al*, 1984; Nolen *et al*, 1985; Procter and Gamble, 1985; Hardin *et al*, 1987; Unilever 1987a; Ema *et al*, 1988; Auletta *et al*, 1993). Maternal toxicity (reduced weight gain) was seen in some studies (Hardin *et al*, 1987; Unilever, 1987a; Ema *et al*, 1988).

There were no effects on sperm motility, morphology and on testis and epididymal sperm counts, or on testis histopathology in rats dosed up to 1,000 mg/kgbw/d (drinking water) for 13 weeks (Johnson *et al*, 2002) (Section 4.23.4.3).

4.23.4.7 Kinetics and metabolism (Table 4.23.4)

DEGBE was excreted primarily in urine following oral, dermal or parenteral administration to rats (Dugard *et al*, 1984; Unilever 1984b; Boatman *et al*, 1993). The major urinary metabolite was 2-(2-butoxyethoxy)acetic acid (BEAA). In view of the rapid conversion of DEGBEA to DEGBE, studies on the metabolism of DEGBEA are also relevant (Section 4.24.4.7).

In vitro human skin penetration rates from a cleaning product containing 4% DEGBE applied at 100%, 50% and 1.5% dilutions were 0.98, 0.40 and 0.007 μmol/cm^2/h, respectively (Procter and Gamble, 1985).

Absorption across isolated human abdominal epidermis after application of undiluted DEGBE for 8 hours was 0.035 mg/cm^2/h. The permeability constant was 0.357 cm/h x 10^4 (Dugard *et al*, 1984).

Maximal human inhalation and dermal exposure to DEGBE from using a hard surface cleaner containing 4% DEGBE (used at 1.5% dilution) for 15 minutes was 0.06 mg/kgbw (Procter and Gamble, 1985; Gibson *et al*, 1991).

4.23.4.8 Neurotoxicity

DEGBE produced no signs of neurotoxicity in rats in a 90-day dermal application study at doses of 0.2, 0.6 or 2 ml DEGBE/kgbw/d (190, 580 or 1,900 mg/kgbw) and produced no neurotoxic effects using a FOB or with motor activity testing. No treatment-related effects were seen in the central and peripheral nervous system by special neuropathology investigations (Beyrouty *et al*, 1993) (Table 4.23.1).

No neurotoxic effects as evaluated by detailed clinical observations, grip strength measurement, motor activity testing and sensory evaluations were observed in F344 rats dosed up to 1,000 mg/kgbw/d (drinking water) for 13 weeks (Johnson *et al*, 2002).

4.23.4.9 Immunotoxicity

No data are available.

4.23.5 Human effects data

A female office clerk reported symptoms of irritation of the upper airways, erythema of the face, and swollen eyelids. Patch testing showed a positive response to DEGBE and a paint additive containing DEGBE (Berlin *et al*, 1995). Lack of workplace monitoring information does not allow confirmation of DEGBE as the causative agent.

Table 4.23.1: Systemic toxicity of DEGBE

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**			
Rat, Sherman, 5 M, 5 F	51 650, 1,830	1 x/d, 30 d	No effects; NOAEL 51 mg/kgbw. ↓ food intake. Slight (unspecified micro-pathological) changes in liver, kidney, spleen or testis	Smyth and Carpenter, 1948
Rat, CD, 10 M	0 891 1,782 3,564	1 x/d, 7 d/wk, 6 wk	No effects Hyperkeratosis of stomach (all levels). No other significant effects ↓ RBC, Hb and MCHb concentration; ↑ MCV; ↑ liver/spleen weight (relative and absolute). Congested spleen and proteinaceous casts in kidneys. Haemosiderin-like pigmentation in kidneys ↓ RBC, Hb and MCHb concentration; ↑ MCV; ↑ liver/spleen weight (relative and absolute). Congested spleen and proteinaceous casts in kidneys. Haemosiderin-like pigmentation in kidneys and spleen	Krasavage and Vlaovic, 1982
Rat, F344, 16 M	0 65 327 1,630	1 x/d, 7 d/wk, 13 wk	No effects Slight ↑ liver weight, no other effects Slight ↑ liver/spleen weight, no other effects Slight ↑ liver weight; ↓ bw. Very high mortality (dosing accidents)	Hobson *et al*, 1986b
Rat, F344, 16 F	0 51 254 1,270	1 x/d, 7 d/wk, 13 wk	No effects Slight ↓ lymphocytes, no other effects Slight ↓ lymphocytes, no other effects Slight ↓ lymphocytes, ↓ bw. Very high mortality (dosing accidents)	Hobson *et al*, 1986b

Table 4.23.1: Systemic toxicity of DEGBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, drinking water	**(mg/kgbw/d)**				
Rat, F344, 10 M, 10 F	0, 50 250 1,000		*Ad libitum*, 13 wk	No treatment related effects. NOAEL; equivocal ↓ in RBC, Hb and Hct. ↓ bw (4%). ↑ Relative liver weight (7-10%). Liver primary target organ: Slight ↑ in several cytochromes P450 and UDP- glucuronosyl transferase (UGT); slight ↓ in serum total proteins, cholesterol, and aspartate aminotransferase. Minor histopathological changes in the liver (females only). No effects on sperm motility, morphology, sperm counts, or testis histopathology. ↓ RBC, Hb and Hct (minimal). Reticulocyte counts and bone marrow unaffected. Slight ↑ kidney weight (relative and absolute). Minimal nephropathy with no associated adverse effects on urinalysis or clinical parameters. No effects on sperm parameters.	Johnson *et al*, 2002
Dermal, occluded	**(ml/kgbw)**	**(mg/kgbwd)**			
Rat, SD, 12 M, 12 F (2 studies)	0, 0.2. 0.6	(0, 190, 580	6 h/d, 5 d/wk, 13 wk	No systemic or neurotoxic effects	Beyrouty *et al*, 1993
	2	1,900)		Renal tubular epithelium degeneration in 2 M. No other systemic or neurotoxic effects	
Rat, SD, 10 M, 10 F	2 (10, 30, 100% v/v)	0, 200, 600, 2,000	6 h/d, 5 d/wk, 13 wk	No systemic toxicity, (dermal irritation in all treated groups)	Auletta *et al*, 1993
		666, 2,000		Transient haematuria (1 F). No other effects	

Table 4.23.1: Systemic toxicity of DEGBE (cont'd)

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Dermal, non-occluded	**(ml/kgbw)**	**(mg/kgbwd)**			
Rabbit, NZW, 3 M, 3 F	0, 2 (1.5% w/v)	(0, 30)	7 h/d, 5 d/wk, 4 wk	No local or systemic effects	Procter and Gamble, 1982
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, F344, 15 M, 15 F	0, 2	(0, 13	6 h/d, 5 d/wk, 5 wk	No effects	Gushow *et al*, 1981
	6, 18	40, 120)		M no effects. F slight ↑ of hepatocyte vacuolisation, slight ↑ of group mean relative liver weight and paleness of liver in 3/10 F at 18 ppm	
Rat, Wistar, 5 M, 5 F	-	0	6 h/d, 5 d/wk, 2 wk, no recovery	No effects	BASF, 1991a
	(15)	100 (vapour)		M ↓ spleen weight	
	-	350, 1,000 (aerosol)		M ↓ spleen weight, dose-dependant. M, F ↑ lung weight. Lung infiltration and bronchiolisation	
Rat, Wistar, 10 F	(0)	0	6 h/d, 5 d/wk, 2 wk, recovery 4 wk	No effects	BASF, 1991b
	-	350 (aerosol)		At 2 wk (no recovery): granulocyte infiltrations, bronchiolisation. Upon recovery: minimal to slight granulocyte accumulation in lungs	
Rat, Wistar, 10 M, 10 F	0, 2, 6	(0, 13, 40	6 h/d, 5 d/wk, 90 d, recovery 4 wk	No effects	Klimisch *et al*, 1992
	14	90)		NOAEL	

Table 4.23.1: Systemic toxicity of DEGBE (cont'd)

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Subcutaneous	**(ml/kgbw)**	**(mg/kgbw)**			
Rat, Wistar, (6-8 wk old)	0	(0	1 x	No effects	Unilever, 1987b
4 F	0.5	480		Lethargy, minor haematological changes	
8 F	1.0	970		Lethargy, haemoglobinuria (1/8). ↓ RBC, Hb and Hct; ↑ MCV. Minor renal and hepatic changes	
4 F	1.5	1,450)		Lethargy for 24 hours. Haemoglobinuria (3/4) and ↑ urine volume. Markedly ↓ RBC, Hb and Hct; ↑ MCV and reticulocytes. ↑ RBC osmotic fragility. Liver, kidney and spleen changes	
Rat, Wistar, (13-14 wk old)	0	(0	1 x		Unilever, 1987b
4 F	0.5	480		Lethargy, minor haematological changes. ↑ RBC osmotic fragility	
4 F	1.0	970		Lethargy for 24 hours, haemoglobinuria (4/4). ↓ RBC, Hb, Hct (markedly); slight ↓ WBC, reticulocytes. ↑ RBC osmotic fragility. Liver, kidney and spleen changes	
4 F	1.5	1,450)		Similar changes to those at 1.0 ml/kgbw	
Guinea pig, 3 F	0, 0.1, 0.5, 1.0	(0, 97, 480, 970)	1 x (killed at d 5)	No effects	Unilever, 1984e

Table 4.23.2: Genotoxicity of DEGBE

Endpoint / Organism	Strain or type / Target	Concentration	Result	Remark	Reference
In vitro					
Gene mutation					
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	Up to 20 µl/plate	–ve	+/– S9	Thompson *et al*, 1984; Unilever, 1984c,d
CHO cell	HGPRT locus	1,000 - 5,000 µg/ml	–ve	+/– S9	Gollapudi *et al*, 1993
Mouse lymphoma cell	L5178Y TK+/–	0.42 - 10 µl/ml	Weakly +ve	– S9	Thompson *et al*, 1984
Chromosome aberration					
CHO cell		0.1 - 0.79 µl/ml	–ve		Thompson *et al*, 1984
Unscheduled DNA Synthesis					
Rat hepatocyte	Primary	0.26 - 10 µl/ml	–ve	Grain counts	Thompson *et al*, 1984
In vivo					
Sex-linked recessive lethal mutations					
Drosophila melanogaster	Maturing germinal cells	Feeding 11,000 mg/l, 3 d Injection 0.3 µl of 14,000 mg/l	–ve –ve		Thompson *et al*, 1984
Micronucleus frequency					
Mouse, CD-1, 5 M, 5 F	Bone marrow	330, 1,100, 3,300 mg/kgbw, 1 x oral gavage	–ve	Mice killed at 24, 48 and 72 h	Gollapudi *et al*, 1993

Table 4.23.3: Reproductive and developmental toxicity of DEGBE

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral	**(mg/kgbw)**			
Rat, Wistar, 14-16 F	0 25, 115, 633	g.d. 0 - 20	No effects Maternal toxicity (↓ bw gain), no other effects	Ema *et al*, 1988
Rat, CD, 25 M (treated), 25 F (untreated)	0, 250, 500	1 x/d, 60 d (M), prior to mating, until end of mating period	No effects on male fertility	Nolen *et al*, 1985
	1,000		No effects on male fertility. Small ↑ in post-implantation losses; small ↓ in implantation sites and delivered pups (not statistically significant); ↓ in mean bw of pups	
Rat, CD, 25 F	0, 250, 500 1,000	14 d to g.d. 13 14 d to d 21 post partum	No effects No maternal or foetal effects; ↓ pup weight d 14 post partum	Nolen *et al*, 1985
Mouse, CD-1, 50 F	0, 500	g.d. 6 - 13	No effects. Chernoff-Kavlock protocol	Schuler *et al*, 1984; Hardin *et al*, 1987
	2,050		25% maternal mortality. No embryo- or foetotoxicity noted.	
Dermal	**(mg/kgbw)**			
Rat, SD, 25 M, 25 F	0	6 h/d, 5 d/wk, 13 wk prior to mating, F to g.d. 20 (occluded)	No effects	Auletta *et al*, 1993
	200, 666, 2,000		No adverse effects on reproductive performance or fertility in any group.	
Rabbit, NZW, 20 F	0, 100, 300, 1,000	g.d. 7 - 18 (non-occluded)	No effects, except a mild dose-dependent skin irritation	Nolen *et al*, 1985

Table 4.23.3: Reproductive and developmental toxicity of DEGBE (cont'd)

Route / Species, strain, number and sex/group	**Dose or concentration**	**Exposure regime**	**Result**	**Reference**
Subcutaneous	**(mg/kgbw)**			
Rat, Wistar, 20 F	0, 120, 240 480 730	g.d. 6 - 15	No effects Maternal toxicity (↓ bw gain) Maternal toxicity (↓ bw gain); transient haemoglobinuria. Foetotoxicity	Unilever, 1987a

Table 4.23.4: Absorption (uptake), distribution, metabolism and elimination of DEGBE

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**			
Rat, Wistar, NS	7 mg/kg	1 x, 96 h collection	Recovery 90-93%: 78 - 86% urine (66 - 75% within 6 h); 0.5 - 1.3% faeces, 6.7 - 11% CO_2, 1% or less carcass	Unilever, 1984b
Dermal, occluded	**(mg/kgbw)**			
Rat, Wistar, NS	730	1 x, clipped or unclipped	Excretion pattern similar for clipped and unclipped animals. Recovery 67-73%: 37% urine, 1.7% faeces, 4 - 6% CO_2, 3% carcass, 20 - 26% skin/patch. Major urinary metabolite was not DEGBE. Penetration rate 0.55 μmol/cm^2/h	Unilever, 1984b
Rat, SD, 4 M, 4 F	200, 2,000	1 x, 24 h	Recovery: 83 - 89%. Major urinary metabolite 2-(2-butoxyethoxy)acetic acid; 5.2 - 8.2% of urinary radioactivity was labile to β-glucuronidase, releasing DEGBE. Traces of BAA were not quantified. Penetration rate 4.5 μmol/cm^2/h (M) or 9 μmol/cm^2/h (F)	Boatman *et al*, 1993
Dermal, *in vitro*				
Human abdominal whole skin	Undiluted	1 x, 8 h	Penetration rate 0.035 mg/cm^2/h, measured in diffusion cell	Dugard *et al*, 1984
Subcutaneous	**(mg/kgbw)**			
Rat, Wistar, NS	7	1 x, 96 h collection	Recovery 90-93%: 78 - 86% urine (66 - 75% within 6 h); 0.5 - 1.3% faeces, 6.7-11% CO_2, 1% or less carcass	Unilever, 1984b
Intraperitoneal	**(mg/kgbw)**			
Rat, Wistar, NS	7	1 x, 96 h collection	Recovery 90-93%: 78 - 86% urine (66 - 75% within 6 h); 0.5 - 1.3% faeces, 6.7 - 11% CO_2, 1% or less carcass	Unilever, 1984b

4.24 Substance profile: DEGBEA

4.24.1 Identity

Name:	Diethylene glycol (mono) *n*-butyl ether acetate
IUPAC name:	2-(2-butoxyethoxy)ethyl acetate
CAS registry No.	124-17-4
Molecular formula:	$C_{10}H_{20}O_4$
Structural formula:	$C_4H_9–(O–CH_2–CH_2)_2–O–CO–CH_3$
Molecular weight:	204.3
Other components:	No data

4.24.2 Physico-chemical properties

Melting point:	Not known
Boiling point:	247°C
Vapour pressure:	< 0.013 hPa
Solubility in water:	65 g/l
Relative density:	$D_4^{20} = 0.985$

4.24.3 Conversion factors

1 ppm = 8.493 mg/m^3
1 mg/m^3 = 0.118 ppm

4.24.4 Toxicological data

4.24.4.1 Acute toxicity

Oral

Rat:	LD_{50} 6,500 - 11,900 mg/kgbw (Smyth *et al*, 1941; Draize *et al*, 1948).
Rabbit:	LD_{50} 2,300 - 2,700 mg/kgbw (Draize *et al*, 1944, 1948).
Guinea pig:	LD_{50} 2300 - 2,600 mg/kgbw (Smyth *et al*, 1941; Draize *et al*, 1948).
Mouse:	LD_{50} 6,500 mg/kgbw (Draize *et al*, 1948).

No signs of toxicity were described in these tests.

Dermal

Rabbit: LD_{50} 5,400 mg/kgbw (male) (Draize *et al*, 1948).

LD_{50} 14.8 ml/kgbw (14,500 mg/kgbw) (female); signs of toxicity were renal damage (Union Carbide, 1984).

Inhalation

Rat: 4-h LC_{50} 8,693 ppm (73,800 mg/m^3). Signs of toxicity were red foci and congestion in the lungs (DuPont, 1984). One of 6 rats died following a single 8-hour exposure to saturated vapour (actual level not reported) (Union Carbide, 1984).

4.24.4.2 Irritation and sensitisation

Skin irritation

DEGBEA was slightly irritant to rabbit skin in a Draize test (Draize *et al*, 1944, 1948).

Eye irritation

DEGBEA caused mild to moderate irritation to the rabbit eye in a Draize test (Carpenter and Smyth, 1946).

Sensitisation

No data are available.

4.24.4.3 Repeated-dose toxicity

In view of the rapid hydrolysis of DEGBEA to DEGBE (Section 4.24.4.7), the effects following repeated exposure are likely to be similar to that of DEGBE (Section 4.23.4.3).

Subacute toxicity

No data are available.

Subchronic toxicity

A 13-week dermal non-occluded application study in rabbits, with dosage levels of 0.5, 1.0, 2.0, and 4.0 ml/kgbw applied daily to the clipped intact skin, showed a dose-related increase in renal tubular damage and haematuria. Treatment caused slight dermatitis (Draize *et al*, 1944, 1948).

4.24.4.4 Genotoxicity

No data are available.

In view of the rapid conversion of DEGBEA to DEGBE, the mutagenic potential of DEGBEA *in vivo* is likely to be similar to that of DEGBE (Section 4.23.4.4), i.e. it is unlikely to elicit genotoxicity.

4.24.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.24.4.6 Reproductive and developmental toxicity

No data are available.

In view of the rapid conversion of DEGBEA to DEGBE, the reproductive toxicity and teratogenic potential of DEGBEA is likely to be similar to that of DEGBE (Section 4.23.4.6), i.e. it is unlikely to elicit developmental or reproductive toxicity.

4.24.4.7 Kinetics and metabolism (Table 4.24.1)

DEGBEA is readily absorbed by intact as well as abraded skin (Draize *et al*, 1948).

DEGBEA underwent rapid hydrolysis in rat blood to DEGBE with a half-life of less than 3 minutes. The urine was the major route of excretion after oral and dermal administration to rats. The major metabolite was BEAA (Deisinger and Guest, 1989).

Following dermal applications of 200 or 2,000 mg/kgbw ^{14}C-DEGBEA for 24 hours, 80 to 88% of the applied radioactivity was recovered in urine. The dermal absorption rate was estimated to be 1.58 (males) and 1.46 (females) mg/cm^2/h (Boatman *et al*, 1993).

4.24.4.8 Neurotoxicity

No data are available.

In view of the rapid conversion of DEGBEA to DEGBE, the lack of neurological effects of DEGBE should apply to DEGBEA (Section 4.23.4.8).

4.24.4.9 Immunotoxicity

No data are available.

4.24.5 Human effects data

Dawson *et al* (1989) reported a single case of allergic contact dermatitis following occupational exposure over a 20-year period.

Repeated applications of an insect repellent containing 50% DEGBEA (and 15% DEGEE, 28% ethanol, 7% corn oil and a trace of lavender oil) over several months produced kidney failure in a 3-year old child (Hoehn, 1945; Draize *et al*, 1948).

Table 4.24.1: Absorption (uptake), distribution, metabolism and elimination of DEGBEA in SD rats

Route / Number and sex/group	Dose		Exposure regime	Result	Reference
In vitro	**(mmol/l)**	**(mg/l)**			
Blood	5	(1,000)	1 x, samples taken 0 - 14 min	94% hydrolysis of DEGBEA to DEGBE after 10 min; half-life 3 min.	Deisinger and Guest, 1989
Oral, gavage	**(mg/kgbw)**				
5 M	200		1 x, 72 h	97% recovery; 83% urine, 2% faeces, 5% CO_2, 4% carcass. 59% urinary radiolabel was BEAA, 15% aryl sulphatase labile conjugates, 12% diethylene glycol, 12% 2-(2-, (3- or 4-hydroxybutoxy)-ethoxy) ethanol, 2% acid labile. No DEGBEA or DEGBE in urine, no significant metabolism to EGBE or BAA	Deisinger and Guest, 1989
	2,000		1 x, 72 h	101% recovery; 84% urine, 3% faeces, 5% CO_2, 4% carcass. 53% urinary radiolabel was BEAA, 7% aryl sulphatase labile conjugates, 32% diethylene glycol + 2-(2-, (3- or 4-hydroxybutoxy)-ethoxy) ethanol, 8% acid labile. No DEGBEA or DEGBE in urine, no significant metabolism to EGBE or BAA	
Dermal, occluded	**(mg/kgbw)**				
4 M, 4 F	200, 2,000		1 x, 24 h	Recovery 80 - 88%. Major urinary metabolite BEAA. Dermal absorption rate 1.58 mg/cm^2/h (M) or 1.46 mg/cm^2/h (F)	Boatman *et al*, 1993

4.25 Substance profile: TEGBE

4.25.1 Identity

Name:	Triethylene glycol (mono) *n*-butyl ether
IUPAC name:	2-(2-(2-Butoxyethoxy)ethoxy)ethanol
CAS registry No.	143-22-6
Molecular formula:	$C_{10}H_{22}O_4$
Structural formula:	$C_4H_9–(O–CH_2–CH_2)_3–OH$
Molecular weight:	206.3
Other components:	No data

4.25.2 Physico-chemical properties

Melting point:	–35°C
Boiling point:	255 - 295°C
Vapour pressure:	< 0.01 hPa
Solubility in water:	Soluble
Relative density:	$D_4^{20} = 0.9890$

4.25.3 Conversion factors

1 ppm = 8.576 mg/m^3
1 mg/m^3 = 0.117 ppm

4.25.4 Toxicological data

4.25.4.1 Acute toxicity

Oral

Rat:	LD_{50} 6,730 mg/kgbw and 5,300 mg/kgbw (Smyth *et al*, 1962; US-EPA, 1982h). Signs of toxicity were loss of righting reflex and flaccid muscle tone.

Dermal

Rabbit: LD_{50} 3,540 mg/kgbw (1 x undiluted TEGBE) (Smyth *et al*, 1962).

LC_{50} > 2,000 mg/kgbw (1 x undiluted TEGBE) (US-EPA, 1982i).

Inhalation

Rat: All animals (10 males) survived 1-hour exposure to saturated vapour; no signs of toxicity were observed (US-EPA, 1982c).

4.25.4.2 Irritation and sensitisation

Skin irritation

TEGBE was not irritant when applied to rabbit skin (Smyth *et al*, 1962; US-EPA, 1982j).

Eye irritation

TEGBE was an eye irritant when administered to rabbits (Smyth *et al*, 1962; US-EPA, 1982k).

Sensitisation

No data are available.

4.25.4.3 Repeated-dose toxicity

Subacute toxicity

In a dermal study in NZW rabbits (5 M, 5 F) a limit dose of 1,000 mg TEGBE/kgbw/d was applied (6 h/d; 5d/w) under occlusion for 21 days. A control group was sham-treated. TEGBE did not produce any systemic toxicity in male or female animals. Observations at the application site indicated that TEGBE produced slight erythema and oedema starting on day 6 or 7 of treatment. Erythema continued throughout the study whereas oedema was not observed after day 18 (Leber *et al*, 1990).

Subchronic toxicity

No data are available.

4.25.4.4 Genotoxicity

No data are available.

Genotoxicity studies with the structurally similar TEGME showed no mutagenic activity (Section 4.6.4.4).

4.25.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.25.4.6 Reproductive and developmental toxicity

TEGBE was screened in Wistar rats for potential developmental toxicity following a modified Chernoff-Kavlock protocol. Groups of 10 mated female rats were administered daily doses of 0, 250 and 1,000 mg TEGBE/kgbw by gavage on day 6 to 15 of gestation. No significant changes in clinical conditions or body weight were seen in maternal rats. All rats in the control and treated groups were pregnant and delivered live foetuses. The number of live pups on day 1 and 5 post partum were also comparable in these groups indicating no chemically induced developmental effects (Leber *et al*, 1990).

4.25.4.7 Kinetics and metabolism

Human abdominal whole skin (254 cm^2) was mounted in a glass diffusion apparatus and the diffusion of TEGBE from the donor into the receiver chamber was monitored for 12 hours period using GC analysis. The diffusion rate of TEGBE was determined to be 22 $\mu g/cm^2/h$, indicating a low skin permeability of the compound (Leber *et al*, 1990).

4.25.4.8 Neurotoxicity

No data are available.

4.25.4.9 Immunotoxicity

No data are available.

4.25.5 Human effects data

No data are available.

4.26 Substance profile: EGHE

4.26.1 Identity

Name:	Ethylene glycol (mono) *n*-hexyl ether
IUPAC name:	2-(Hexyloxy)ethanol
CAS registry No.	112-25-4
Molecular formula:	$C_8H_{18}O_2$
Structural formula:	$C_6H_{13}–O–CH_2–CH_2–OH$
Molecular weight:	146.2
Other components:	No data

4.26.2 Physico-chemical properties

Melting point:	–45°C
Boiling point:	208°C
Vapour pressure:	< 0.1 hPa
Solubility in water:	9 g/l
Relative density:	$D_4^{20} = 0.891$

4.26.3 Conversion factors

1 ppm = 6.078 mg/m^3
1 mg/m^3 = 0.165 ppm

4.26.4 Toxicological data

4.26.4.1 Acute toxicity

Oral

Rat:	LD_{50} 1.48 g/kgbw (Smyth *et al*, 1954); LD_{50} 1.67 ml/kgbw (males) and 0.83 ml/kgbw (females) (1,490 and 740 mg/kgbw, respectively). Signs of mucosal irritation were present at doses of 1 ml/kgbw (890 mg/kgbw) and above, indicating potential lung damage by aspiration (Ballantyne and Myers, 1987).

Dermal

Rat: LD_{50} 0.89 ml/kgbw (790 mg/kgbw) (Smyth *et al*, 1954); LD_{50} 0.81 ml/kgbw in males and 0.93 ml/kgbw in females (720 and 830 mg/kgbw, respectively). Signs of toxicity included salivation, sluggishness, unsteady gait, and comatose appearance. The onset of signs occurred within 20 to 30 minutes of application of EGHE (Ballantyne and Myers, 1987).

Inhalation

Rat: Survived 8-hour exposure to saturated vapour (concentration not stated) (Smyth *et al*, 1954). Male and female rats exposed for 6 hours to saturated vapour (approximate concentration 85 ppm) exhibited neither signs of toxicity nor irritancy. In addition, no signs of toxicity were observed during the 14-day post-exposure observation period, nor were any pathological features seen at necropsy following the observation period (Ballantyne and Myers, 1987).

In a 4-hour study with mean chamber concentrations of 83 ppm no mortality, no clinical signs, no effects on body weight gain, and no gross lesions at necropsy were observed (Leber *et al*, 1987).

Intravenous

Rat LD_{50} 53.6 mg/kgbw (males) and 70.0 mg/kgbw (females) (Ballantyne *et al*, 2003).

Rabbit LD_{50} 40.0 mg/kgbw (males) and 30.3 mg/kgbw (females) (Ballantyne *et al*, 2003).

4.26.4.2 Irritation

Skin irritation

EGHE was non-irritant to rabbit skin following short-term contact (Smyth *et al*, 1954). EGHE (0.5 ml) applied to the shaven dorsal skin of 6 rabbits and covered with an occlusive dressing for 4 hours produced erythema and oedema, which disappeared within 2 to 7 days. In addition,

necrosis was observed in 3 rabbits between 1 and 7 days post-application, which persisted through 7 days (Ballantyne and Myers, 1987).

Eye irritation

Instillation of a 5% solution of EGHE into the rabbit eye caused severe injury, while a 1% solution caused minor injury (Smyth *et al*, 1954). Instillation of undiluted EGHE (0.005 ml) into the rabbit eye caused severe conjunctivitis with iritis and diffuse corneal injury, which healed in 3 to 7 days (Ballantyne and Myers, 1987).

Sensitisation

No data are available.

4.26.4.3 Repeated-dose toxicity (Table 4.26.1)

Subacute toxicity

Undiluted EGHE was applied topically to the skin of NZW rabbits at dose levels of 0, 44, 222 or 444 mg/kgbw over an 11-day period. A clear dose-response relationship was observed for local skin irritation (erythema and oedema). Systemic toxicity (decreased food consumption and loss of body weight) was apparent in both sexes from the high-dose (444 mg/kgbw) group. At the same dose level, decreases in erythrocyte counts and Hb and Hct values and increased MCV were reported in both sexes. A similar trend in haematology parameters was also noted in males at all dose levels and in females at 222, or 444 mg/kgbw/d (Union Carbide, 1989b).

F344 rats exposed (9 x) to 0, 20, 40 or 80 ppm EGHE over 11 days exhibited depressed body weight gain and increased liver weight at the highest exposure concentration of 84 ppm. No histopathological changes or alterations in haematological parameters, morphology of the testes or liver were observed (Klonne *et al*, 1987).

Subchronic toxicity

F344 rats were exposed by inhalation to 0, 20, 40 or 70 ppm EGHE for 13 weeks. Sensory irritation, decreased body weight gain and increased liver weight were observed in animals of the highest dose group. Increased liver weight was observed in both sexes of the 40-ppm group with a slight decrease in body weight gain observed in the females of this group, but these changes

were not considered biologically significant. There were no haematological abnormalities or testicular atrophy in any exposure group. Thus, 41 ppm was considered to be the NOAEL (Klonne *et al*, 1987).

Male and female NZW rabbits received daily epicutaneous doses equivalent to 0, 44, 222, and 444 mg EGHE/kgbw on 9 days within a period of 11 days. Applications were for 6 hours with undiluted EGHE under occlusive conditions. Local irritation was observed in all EGHE treated groups, body weights were reduced at 222 and 444 mg/kgbw. At 444 mg/kgbw, there less food consumption and haematological parameters (erythrocyte count, haemoglobin concentration and haematocrit) were reduced; 2 females died at 444 mg/kgbw (Ballantyne *et al*, 2003).

4.26.4.4 Genotoxicity (Table 4.26.2)

In vitro

EGHE was not mutagenic with or without metabolic activation when tested for histidine reversion in a *Salmonella typhimurium* assay (Ames test), at concentrations ranging from 0.3 to 15 mg/plate and at the HGPRT locus of CHO cells at concentrations up to 1.0 mg/ml (Union Carbide, 1985a).

EGHE did not cause increases in chromosome aberrations in CHO cells, with or without addition of a metabolic activation system. EGHE was not considered to be a clastogenic agent in this assay (Union Carbide, 1985b).

EGHE did not produce a dose-related or reproducible response in CHO cells when tested for mutagenicity or increased SCE rate (Union Carbide, 1985c).

4.26.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.26.4.6 Reproductive and developmental toxicity (Table 4.26.3)

Timed-pregnant F344 rats were exposed to EGHE vapour at concentrations of 0, 20, 40 or 80 ppm (near saturation) on day 6 to 15 of gestation, then killed on day 21 of gestation. Maternal toxicity, as shown by transient weight gain reduction, was observed at 40 ppm during exposure only. There were no treatment-related effects with respect to haematology, necropsy, gestational parameters, malformations, or variations. It was concluded that exposure to EGHE vapour

produced maternal toxicity, but no evidence of developmental toxicity or teratogenicity. A NOAEL for maternal toxicity was established at 20 ppm (Tyl *et al*, 1989).

Timed-pregnant NZW rabbits were exposed to EGHE vapour at concentrations of 0, 20, 40 or 80 ppm (near saturation) on day 6 to 18 of gestation, then killed on day 29 of gestation. Maternal toxicity, as shown by transient weight gain reduction, was observed at 80 ppm during exposure only. There were no treatment-related effects with respect to haematology, necropsy, gestational parameters, malformations, or variations. It was concluded that exposure to EGHE vapour produced maternal toxicity, but no evidence of developmental toxicity or teratogenicity. A NOAEL for maternal toxicity was 40 ppm (Tyl *et al*, 1989).

4.26.4.7 Kinetics and metabolism

Aasmoe *et al* (1998) reported that a single isoenzyme of ADH (ADH-3) in rat liver was responsible for oxidising glycol ethers. In the case of EGHE, a V_{max} value of 1.66 nmol NADH/min/mg protein and a K_m value of 0.15 mM were reported. In the series of glycol ethers tested, V_{max} and K_m values decreased with increasing chain lengths suggesting that for equivalent glycol ether concentrations, metabolism of EGHE will be less rapid than for other shorter chain homologues and will be saturated at lower substrate concentrations.

Male and female F344 rats or NZW rabbits were administered a single dermal dose of 25 or 10 mg EGHE/kgbw, respectively, under occlusive conditions for 48 hours. In rats, percutaneous absorption was rapid with more than 95% of the radiochemical dose recovered, bioavailability was greater than 75%, urinary excretion was 21 to 33%, $^{14}CO_2$ and volatiles accounted for 15 to 18%. In rabbits 75% of the dose was recovered, mostly in urine (58 - 60%). In both species extensive metabolism took place. Male F344 rats or NZW rabbits were administered a single i.v. injection of 2.5 and 25 mg/kgbw or 1 and 10 mg/kgbw, respectively. Elimination (as metabolites) in rats was mainly in urine (68 - 74%), without free EGHE being detected. The free EGHE concentration in plasma declined rapidly and was no longer detectable by 8 hours (rats) or 1 hour (rabbits) (Ballantyne *et al*, 2003).

4.26.4.8 Neurotoxicity

No data are available.

4.26.4.9 Immunotoxicity

No data are available.

4.26.5 Human effects data

No data are available.

Table 4.26.1: Systemic toxicity of EGHE

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Dermal	**(mg/kgbw)**				
Rabbit, NZW, 5 M, 5 F	0		9 x 6 h/d, 5 d/wk, 11 d	No effects	Union Carbide, 1989b
	44			Slight erythema M; slight oedema F. Slight alteration in haematology (M)	
	222			Slight erythema M, F; slight oedema M, oedema F. Alteration in haematology (M, F)	
	444			Slight erythema M, moderate F; moderate oedema M, moderate F. ↓ bw and food consumption (M, F). Moderate alteration in haematology (M, F)	
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, F344, 10 M, 10 F + 10 M, 10 F in controls and high dose for 2-week recovery	0, 20, 40 80	(0, 120, 240 490)	9 x 6 h/d, 5 d/wk, 11 d	No effects ↓ bw gain. ↑ liver weight without histopathological alterations	Klonne *et al*, 1987
Rat, F344, 10 M, 10 F + 10 M, 10 F at all levels for 4-week recovery	0, 20 40 70	(0, 120 240 430)	6 h/d, 5 d/wk, 13 wk	No effects NOAEL. ↑ liver weight. Slight ↓ in bw gain (F) Sensory irritation, ↓ bw gain and ↑ liver weight	Klonne *et al*, 1987

Table 4.26.2: Genotoxicity of EGHE in vitro

Endpoint / Organism	Strain or type / Target	Concentration	Result	Remark	Reference
Gene mutation					
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	0.3 - 15 mg/plate	**–ve**	+/– S9	Union Carbide, 1985a
CHO cell	HGPRT locus	Up to 1.0 mg/ml Up to 1.3 mg/ml	**–ve**	– S9 + S9	Union Carbide, 1985c
Chromosome aberration					
CHO cell		0.1 - 0.8 mg/ml 0.08 - 0.4 mg/ml	**–ve**	– S9 + S9	Union Carbide, 1985b
Sister chromatid exchange					
CHO cell		Up to 0.8 mg/ml Up to 1.0 mg/ml	**–ve**	– S9 + S9	Union Carbide, 1985c

Table 4.26.3: Reproductive and developmental toxicity of EGHE

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, F344, 25 F	0, 20 40, 80	(0, 120 240, 490)	6 h/d, g.d. 6 -15	No effects Maternal toxicity (↓ bw), no embryo- or foetotoxicity	Tyl *et al*, 1989
Rabbit, NZW, 22 F	0, 2, 40 80	(0, 120, 240 490)	6 h/d, g.d. 6 - 18	No effects Maternal toxicity (↓ bw), no embryo- or foetotoxicity	Tyl *et al*, 1989

4.27 Substance profile: DEGHE

4.27.1 Identity

Name:	Diethylene glycol (mono) hexyl ether
IUPAC name:	2-(2-(2-Hexyloxy)ethoxy)ethanol
CAS registry No.	112-59-4
Molecular formula:	$C_{10}H_{22}O_3$
Structural formula:	$C_6H_{13}–(O–CH_2–CH_2)_2–OH$
Molecular weight:	190.3
Other components:	No data

4.27.2 Physico-chemical properties

Melting point:	–35°C
Boiling point:	258.3°C
Vapour pressure:	< 0.001 hPa
Solubility in water:	No data
Relative density:	$D_4^{20} = 0.937$

4.27.3 Conversion factors

1 ppm = 7.911 mg/m^3
1 mg/m^3 = 0.126 ppm

4.27.4 Toxicological data

4.27.4.1 Acute toxicity

Oral

Rat:	LD_{50} 4.92 ml/kgbw (males) and 3.73 ml/kgbw (females) (4,610 and 3,500 mg/kgbw, respectively) (Ballantyne and Myers, 1987).
	LD_{50} 3,016 mg/kgbw (males), 1,823 mg/kgbw (females); post-treatment period 3 days (Ballantyne and Vergnes, 2001).

Dermal

Rabbit: LD_{50} 1.5 ml/kgbw (1,410 mg/kgbw) (Union Carbide, 1950), LD_{50} 2.14 ml/kgbw (males) and 2.37 ml/kgbw (females) (2,010 and 2,220 mg/kgbw, respectively) following occlusive dressing for 24 hours (Ballantyne and Myers, 1987).

Inhalation

Rat: Survived saturated vapour concentrations (not stated) for 8 hours, without significant effects (Union Carbide, 1950) or for 6 hours (Ballantyne and Myers, 1987). No signs of toxicity or irritancy were noted during exposure, or during the subsequent 14-day post-exposure observation period. In addition, no gross pathological features were observed at necropsy.

Intraperitoneal

Mouse: LD_{50} 894 mg/kgbw (males), 715 mg/kgbw (females); post-treatment period 3 days (Ballantyne and Vergnes, 2001).

4.27.4.2 Irritation

Skin irritation

DEGHE caused mild erythema and oedema of about 24 hours duration when 0.5 ml of undiluted material was applied under an occlusive dressing to the clipped skin of rabbits for 4 hours. DEGHE was not considered to be corrosive under the conditions of this test. Application of 4 ml/kgbw (3,750 mg/kgbw) under an occlusive dressing for 24 hours led to more severe irritation with persistent erythema, oedema, and necrosis (Union Carbide, 1950).

Eye irritation

DEGHE was severely irritant to the rabbit eye (Ballantyne and Myers, 1987). Instillation of 0.005 or 0.1 ml caused severe conjunctivitis and corneal injury in all 6 rabbits, requiring 7 to 21 days for complete corneal healing (Ballantyne and Myers, 1987).

Sensitisation

No data are available.

4.27.4.3 Repeated-dose toxicity (Table 4.27.1)

Subacute toxicity

NZW rabbits were exposed (9 x) to DEGHE by occluded dermal exposure at dose levels of 0, 100, 300 or 1,000 mg/kgbw over an 11-day period. A dose-response relationship was noted for local skin irritation. Severe irritation was noted in the 1,000-mg/kgbw/d group and mild irritation in the 100-mg/kgbw/d group. There were no treatment-related clinical signs of systemic toxicity (Ballantyne and Vergnes, 2001).

Subchronic toxicity

No data are available.

4.27.4.4 Genotoxicity (Table 4.27.2)

In vitro

DEGHE was not mutagenic in *Salmonella typhimurium* (Ames test), with or without addition of a metabolic activation system (Ballantyne and Vergnes, 2001). In CHO cells, DEGHE produced slight increases in gene mutations both with and without metabolic activation. However, this effect was not reproducible in duplicate cultures. A linear regression analysis of the data in the presence of S9 indicated a significant, although weak, positive response (Union Carbide, 1987c).

DEGHE did not reproducibly increase the incidence of SCEs in CHO cells, with or without the addition of a metabolic activation system (Ballantyne and Vergnes, 2001).

In vivo

Swiss-Webster mice were dosed with DEGHE by oral gavage. No positive or dose-related increases in the frequency of micronucleated PCE of the bone marrow were produced. DEGHE was considered to be inactive as a clastogenic agent (Ballantyne and Vergnes, 2001).

No reproducible statistically significant or dose-related increases in the incidence of chromosomal aberrations were seen in the bone marrow of male and female SD rats injected with DEGHE (Ballantyne and Vergnes, 2001).

4.27.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.27.4.6 Reproductive and developmental toxicity

No data are available.

4.27.4.7 Kinetics and metabolism

No data are available.

4.27.4.8 Neurotoxicity

No data are available.

4.27.4.9 Immunotoxicity

No data are available.

4.27.5 Human effects data

No data are available.

Table 4.27.1: Systemic toxicity of DEGHE in NZW rabbits

Route / Number and sex/group	**Dose**	**Exposure regime**	**Result**	**Reference**
Dermal	**(mg/kgbw)**			
5 M, 5 F	0, 100, 300	9 x 6 h/d, 11 d	Dose-response for skin irritation; no systemic toxicity, all dose levels	Ballantyne and Vergnes, 2001
	1,000		Severe irritation	

Table 4.27.2: Genotoxicity of DEGHE

Endpoint / Species	Strain or type / Target	Concentration	Result	Remark	Reference
In vitro					
Gene mutation					
Salmonella typhimurium	TA98, TA100, TA1535, TA1537, TA1538	0.03 - 3 mg/plate 0.1 - 10 mg/plate	**–ve**	– S9 + S9	Ballantyne and Vergnes, 2001
CHO cells	HGPRT locus	Up to 2.0 mg/ml Up to 2.5 mg/ml	**+/–ve**	– S9 + S9; weakly +ve	Ballantyne and Vergnes, 2001
Sister chromatid exchange					
CHO cell		Up to 2.0 mg/ml	**–ve**	+/– S9	Ballantyne and Vergnes, 2001
In vivo					
Micronucleus frequency		**(mg/kgbw)**			
Mouse	Swiss-Webster Bone marrow	M: 370, 750, 1,500 2,400 F: 450, 900, 1,500 1 x oral gavage	**–ve**	Bone marrow sampled 24 and 48 h after treatment	Ballantyne and Vergnes, 2001
Chromosome aberration		**(mg/kgbw)**			
Rat, 5 M, 5 F	SD Bone marrow	M: 375, 750, 1,500 F: 450, 900, 1,500 1 x injection, perorally	**–ve**	Bone marrow sampled 12, 24, 48 h after treatment	Ballantyne and Vergnes, 2001
Rat, 5 M, 5 F	SD Bone marrow	375, 600, 750, 1,000, 1,500 1 x injection, perorally	**–ve**	Bone marrow sampled 12, 24, 48 h after treatment	Ballantyne and Vergnes, 2001

4.28 Substance profile: 2PG1ME

4.28.1 Identity

Name:	2-Propylene glycol 1-methyl ether
IUPAC name:	1-Methoxy-2-propanol
CAS registry No.	107-98-2
Molecular formula:	$C_4H_{10}O_2$
Structural formula:	$CH_3–\underset{\displaystyle OH}{\underset{\vert}{CH}}–CH_2–O–CH_3$
Molecular weight:	90.1
Other components:	2-Methoxy-1-propanol (< 5%)

4.28.2 Physico-chemical properties

Melting point:	–96°C
Boiling point:	120°C
Vapour pressure:	12.0 hPa
Solubility in water:	Completely soluble
Relative density:	$D_4^{20} = 0.919$

4.28.3 Conversion factors

1 ppm = 3.745 mg/m^3
1 mg/m^3 = 0.267 ppm

4.28.4 Toxicological data

4.28.4.1 Acute toxicity

Oral

Rat:	LD_{50} 6.6 ml/kgbw (6,100 mg/kgbw). Signs of toxicity were dyspnoea, somnolence, uncoordinated gait and ataxia (Rowe *et al*, 1954).
	LD_{50} 5,200 mg/kgbw (Smyth *et al*, 1962).
	LD_{50} 7,510 mg/kgbw (Smyth *et al*, 1941).

Mouse: LD_{50} 10,800 mg/kgbw (Stenger *et al*, 1972).

Rabbit: LD_{50} 5,300 mg/kgbw (Stenger *et al*, 1972).

Dog: LD_{50} 9,200 mg/kgbw. Signs of toxicity included nausea, vomiting, diarrhoea and respiratory arrest (Shideman and Procita, 1951).

CNS depression preceded mortality in all studies.

Dermal

Rabbit: LD_{50} between 11,000 and 13,800 mg/kgbw (24 h, occluded). Signs of toxicity were loss of body weight at all doses (5.0 - 15.0 ml/kgbw; 4,600 - 13,800 mg/kgbw), signs of general narcosis varying in intensity from slight weakness and drowsiness to deep anaesthesia (Rowe *et al*, 1954). LD_{50} 13,000 to 14,000 mg/kgbw (Smyth *et al*, 1962).

Inhalation

Rat: 4-h LC_{50} 15,000 ppm (56,000 mg/m^3) (Rowe *et al*, 1954). Rats survived single, 6-hour exposures to 6,038 and 7,559 ppm 2PG1ME (22,610 and 28,310 mg/m^3) but experienced general narcosis (Cieszlak and Crissman, 1991).

Guinea pig: 10-h LC_{50} 15,000 ppm (56,000 mg/m^3) (Rowe *et al*, 1954).

Intravenous, intraperitoneal and subcutaneous

Rat: LD_{50} 3,900 mg/kgbw (i.v. or i.p.) or 7,200 mg/kgbw (s.c.) (Stenger *et al*, 1972).

Mouse: LD_{50} 4,900 mg/kgbw (i.v.) (Stenger *et al*, 1972).

Rabbit: LD_{50} 1,100 mg/kgbw (i.v.) or 4,600 mg/kgbw (s.c.) (Stenger *et al*, 1972).

Dog: LD_{50} 1,800 to 2,300 mg/kgbw (i.v.) (Stenger *et al*, 1972).

4.28.4.2 Irritation and sensitisation

Skin irritation

2PG1ME was slightly irritant to rabbit skin even upon prolonged or repeated contact. No skin corrosion occurred (Rowe *et al*, 1954; Smyth *et al*, 1962).

Eye irritation

Undiluted 2PG1ME was slightly irritant to the rabbit eye. It may cause slight transient opacity of the cornea (Rowe et al, 1954; Smyth et al, 1962).

Sensitisation

Undiluted 2PG1ME was not sensitising in guinea pigs using the modified Maguire test (Carreon *et al*, 1984).

4.28.4.3 Repeated-dose toxicity (Table 4.28.1)

Subacute toxicity

Oral

Rats received repeated oral doses of approximately 0, 92, 270, 920 or 2,760 mg 2PG1ME/kgbw/d by gavage for 5 weeks. The animals showed no adverse effects as judged by the appearance, growth, organ weights and histopathological examination of the organs. Under the same conditions, 2,760 mg/kgbw produced only minor effects in the liver and kidneys (Rowe et al, 1954).

Dermal

When NZW rabbits received daily dermal applications of 1,000 mg 2PG1ME/kgbw/d for 3 weeks, slight local skin irritation occurred. No signs of systemic toxicity and no haematological, gross or histopathological effects were observed (Calhoun *et al*, 1984).

Inhalation

Wistar rats were exposed by inhalation to 0, 200 or 600 ppm 2PG1ME for 10 days. No alteration in testicular weight or histopathology and no changes in haematology were observed (Doe *et al*, 1983).

When F344 rats were exposed to 2PG1ME at 3,000 ppm for 9 days, the animals were sedated and had a slight increase in liver weight. No haematological or testicular effects were observed. No effects occurred at 300 or 1,000 ppm (Miller *et al*, 1981).

In $B6C3F_1$ mice exposed to 2PG1ME for 9 days, no effects occurred at 300 or 1,000 ppm. In the females, liver weight was slightly increased at 3,000 ppm (Miller *et al*, 1981).

CFE rats were exposed to concentrations of 0, 2,500, 5,000 and 10,000 ppm 2PG1ME for 2 weeks. There was a transient non-specific depression of behaviour for the first several exposures to 5,000 and 10,000 ppm. However, there was a rapid development of tolerance. Decreased growth rate was seen at 10,000 ppm (Goldberg *et al*, 1964).

Subchronic toxicity

Oral

2PG1ME was administered by gavage to Wistar rats at dose levels of 0, 460, 920, 1,800 or 3,700 mg/kgbw/d for 13 weeks. The animals experienced mild to severe dose-related CNS depression and minor kidney injury at dose levels greater than 920 mg/kgbw. The sedation caused decreased growth as a result of decrease food intake. Dose levels greater than 920 mg/kgbw caused enlarged livers accompanied by cell necrosis and increased mortality (Stenger *et al*, 1972).

Dogs were administered 2PG1ME by gavage at dose levels of 0, 460, 920, 1,800 or 2,800 mg/kgbw/d for 14 weeks. Mild to severe dose-related CNS depression and minor kidney injury was seen at dose levels greater than 920 mg/kgbw. Dose levels greater than 920 mg/kgbw caused enlarged livers accompanied by cell necrosis and increased mortality. Male dogs developed numerous spermiophages in the epididymides, the significance of which is not clearly understood (Stenger *et al*, 1972).

Dermal

Repeated occluded dermal application of 6,400 or 9,200 mg 2PG1ME/kgbw for 13 weeks produced narcosis and death in rabbits. Terminal body weights were significantly decreased at these dose levels. Narcosis-related deaths were attributed to pneumonia and emphysema. At 9,200 mg/kgbw, kidney weights were significantly increased. Three rabbits that died exhibited moderate to marked renal tubular necrosis; other rabbits that died had slight granular degeneration of the renal tubules. Doses of 1,800 mg/kgbw/d were well tolerated (Rowe *et al*, 1954).

Inhalation

There were no observable treatment-related effects in F344 rats exposed to 0, 300 or 1,000 ppm 2PG1ME for 13 weeks. At 3,000 ppm, apart from sedation and lower body weight gain, transient CNS depression and slightly increased liver weights were observed (Landry *et al*, 1983).

When F344 rats mice were exposed to 3,000 ppm 2PG1ME for 1, 2 or 13 weeks, the initial hepatic cell proliferation in week 1 returned to normal in week 2. The cell proliferation in the male rat kidneys was 4-fold higher than in the control group over the remaining dosing period, consistent with observed $\alpha_{2\mu}$-globulin accumulation (Cieszlak *et al*, 1996a).

Likewise, when $B6C3F_1$ mice were exposed to 3,000 ppm 2PG1ME for 1, 2 or 13 weeks, the initial hepatic cell proliferation in week 1 returned to normal in week 2 (Cieszlak *et al*, 1996b).

4.28.4.4 Genotoxicity (Table 4.28.2)

In vitro

2PG1ME was not mutagenic in *Salmonella typhimurium* (Ames test), with and without metabolic activation (Kirkland *et al*, 1983).

No clastogenic activity (increase of chromosome aberration rate) was seen in CHO cells (Kirkland and Verschuuren, 1983).

2PG1ME was negative in the rat hepatocyte unscheduled DNA synthesis assay (Mendrala and Schumann, 1983a).

The results of a series of genotoxicity tests in V79 cells suggested 2PG1ME to be a weak inducer of sister chromatid exchanges, a weak enhancer of methylmethane-sulphonate-induced

chromosome aberrations and inhibitor of metabolic cooperation. These effects were observed at excessively high concentrations, which would potentially produce artificial responses due to osmolarity changes in the medium (Elias *et al*, 1996). It does not appear that medium osmolarity was measured in this study.

Elias *et al* (1996) reported 2PG1ME to be without clastogenic activity using the chromosomal aberration test in V79 cells and human lymphocytes, in the *in vitro* and the *in vivo* mouse bone marrow micronucleus assay. 2PG1ME did not induce aneugenic effects in V79 cells *in vitro* (aneuploidy and mitotic division aberrations).

In a morphological transformation assay with SHE cells, 2PG1ME was negative (Elias *et al*, 1996).

4.28.4.5 Chronic toxicity and carcinogenicity

When subjected to 7-hour exposures of 2PG1ME vapour for 4 to 7 months, guinea pigs tolerated 3,000 ppm, rats 1,500 ppm, and monkeys and rabbits 800 ppm without adverse affects. At 6,000 ppm, rats were deeply narcotised (CNS depression) at the end of each exposure in the first few weeks, which led to high mortality. Thereafter, the survivors became tolerant. Guinea pigs and rabbits, tested at 6,000 ppm, exhibited similar narcotic effects. Slight lung (rat, rabbit) and liver (rat, guinea pig) changes were observed at microscopic examination (Rowe *et al*, 1954).

F344 rats were exposed to 0, 300, 1,000 or 3,000 ppm 2PG1ME vapour for 2 years. The following primary treatment-related effects were seen in animals exposed to the highest concentration of 3,000 ppm: initial sedation and its subsequent resolution correlating with induction of hepatic mixed function oxidase activity and S-phase DNA synthesis; elevated mortality in male rats; elevated deposition of $\alpha_{2\mu}$-globulin and associated nephropathy and S-phase DNA synthesis in male rat kidneys and increased occurrence and/or severity of eosinophilic foci of altered hepatocytes in male rats. No toxicologically relevant statistically significant increases in neoplasia occurred. A numerical increase in the incidence of kidney adenomas occurred in male rats at 1,000 ppm; however, the association with $\alpha_{2\mu}$-globulin nephropathy, a male rat specific effect, indicated a lack of relevance for human risk assessment (Spencer *et al*, 2002).

$B6C3F_1$ mice were exposed to 0, 300, 1,000 or 3,000 ppm 2PG1ME vapour for 2 years. Primary treatment-related effects seen at 3,000 ppm included: initial sedation and its subsequent resolution correlating with induction of hepatic mixed function oxidase activity and S-phase DNA synthesis, and elevated mortality in male mice. Statistically significant increases occurring in neoplasia were not toxicologically relevant (Spencer *et al*, 2002).

4.28.4.6 Reproductive and developmental toxicity (Table 4.28.3)

Oral

No teratogenic effects in CFE rats, CFLP mice or Yellow Silver rabbits were seen in oral studies with 2PG1ME at dose levels of up to 740, 1,850 or 924 mg/kgbw, respectively. In rats, the highest dose of 740 mg/kgbw caused developmental toxicity, i.e. delayed ossification of the skull bones of the foetuses; at 370 mg/kgbw no effects were observed (Stenger *et al*, 1972).

In a continuous breeding study in CD-1 mice, 2PG1ME was administered up to 2% (3,328 mg/kg/d) in the drinking water for 2 generations. No effects on fertility or reproduction were observed at 2%, although at this dose level 2PG1ME was moderately toxic as shown by statistically decreased pup body weights from birth to postnatal day 14 in the second generation animals (Gulati *et al*, 1986).

Inhalation

No teratogenic effects were observed in pregnant F344 rats exposed by inhalation to 2PG1ME at concentrations of 0, 500, 1,500 or 3,000 ppm on day 6 to 15 of gestation. At the highest concentration, on the first 2 days of exposure, the dams suffered from mild lethargy. The foetuses revealed delayed ossification at 3,000 ppm (Hanley *et al*, 1984c).

No teratogenic effects were observed in pregnant NZW rabbits exposed by inhalation to 0, 500, 1,500 or 3,000 ppm 2PG1ME on day 6 to 18 of gestation. At 3,000 ppm, on the first 2 days of exposure, the dams suffered from mild lethargy (Hanley *et al*, 1984c).

Pregnant Wistar rats were exposed to 0, 200 or 600 ppm 2PG1ME on day 16 to 17 of gestation. No effects were noted on maternal body weight gain or the number, weight or viability of pups (Doe *et al*, 1983).

Male and female SD rats were exposed by inhalation to 0, 300 1,000 or 3,000 ppm 2PG1ME vapours for 10 days prior to mating and 7 d/wk during mating, gestation and lactation for 2 generations. Marked parental toxicity was seen at 3,000 ppm, as evidenced by changes in various organ and body weights (relative/absolute) of the first and second generation males and females, in particular of the testes and ovaries. In females, toxicity was accompanied by decreased fertility, lengthened oestrous cycle, decreased ovarian weight and histopathological ovarian atrophy. Embryotoxicity and foetotoxicity occurred only at maternally toxic doses. No reproductive/neonatal effects were seen at levels which caused less marked maternal toxicity (Carney *et al*, 1999).

Subcutaneous

No teratogenic effects in CFE rats, CFLP mice or Yellow Silver rabbits were seen following s.c. injection of 2PG1ME at dose levels of up to 740, 370 or 92 mg/kgbw/d, respectively. In rats, the highest dose of 740 mg/kgbw caused delayed ossification of the skull bones; at 370 mg/kgbw no effects were observed (Stenger *et al*, 1972).

4.28.4.7 Kinetics and metabolism (Table 4.28.4)

In vitro

An *in vitro* human skin penetration study, in which 2PG1ME was applied undiluted to the outer surface of abdominal epidermis for 8 hours, revealed an absorption rate of 1.170 mg/cm^2/h and a permeability constant of 12.5 cm/h x 10^4 (Dugard *et al*, 1984).

Skin permeation was calculated using the Franz cell method with human skin. 2PG1ME was tested in pure form and with 70% acetone. In pure form the lag time was 33 minutes, flux at steady state was 0.472 mg/cm^2/h, and permeation 0.512 x 10^{-3} cm/h. In mixture with acetone, the respective values were 26 minutes, 0.605 mg/cm^2/h and 0.729 x 10^{-3} cm/h (Larese *et al*, 1999).

Percutaneous absorption of 2PG1ME, in aqueous solution or undiluted, through full thickness or dermatomed human breast skin was measured for 24 hours using flow-through diffusion cells. In aqueous solution, steady-state flux was 48 nmol/cm^2/h (4.3 µg/cm^2/h), time to steady state 0.68 hours, and the final level of absorption 0.15 µmol (13.5 µg) in dermatomed skin (Wilkinson and Williams, 2002).

In vivo

After oral gavage of 2PG1ME to F344 rats, 10 to 20% of either high or low dose was eliminated within 2 days as the glucuronate or sulphate conjugates in the urine. At the same time, 50 to 60% was eliminated in the expired air as carbon dioxide, following metabolism to propylene glycol. Amongst the metabolites of 2PG1ME, no 2-MPA was identified (Miller *et al*, 1983b).

In a comparative study, F344 rats and B6C3F$_1$ mice were dosed with 2PG1ME, either orally or i.v., at 90 or 450 mg/kgbw. The extent of $^{14}CO_2$ exhalation and urine excretion were identical by either route in both species. The rate of exhalation of $^{14}CO_2$ was faster in mice than in rats. In addition, mice metabolised and eliminated 2PG1ME faster than rats (Ferrala *et al*, 1994).

Oral absorption and metabolism were studied in male $B6C3F_1$ mice following single gavage administration of 90 mg/kgbw. The maximum concentrations of 2PG1ME and propylene glycol (PG) in plasma were attained at 20 and 30 minutes following dosing, respectively (Ferrala *et al*, 1994).

During single exposure by inhalation of F344 rats to 300, 750, 1,500 or 3,000 ppm 2PG1ME for 6 hours, the blood concentration increased throughout the 6-hour exposure period and did not reach an apparent steady state at any of the concentrations tested, indicating that absorption was limited by respiration. At the end of exposure, blood concentrations were not proportional to the exposure concentrations, and the clearance was best described as a zero order process. In a subsequent study, rats were exposed (6 h/d) to 300 or 3,000 ppm 2PG1ME for 1 or 10 days by whole-body inhalation. At 3,000 ppm, rats given repeated exposures had lower 2PG1ME blood levels than rats given a single exposure. After the 10^{th} exposure to 3,000 ppm, the end-of-exposure blood concentrations were 50% lower versus the first day. This indicated clearly an increased elimination capacity in these rats (Morgott and Nolan, 1987).

Blood kinetics of 2PG1ME in F344 rats after i.v. injection of 10 or 100 mg/kgbw were characterised by rapid hydrolysis; half-lives were 1.55 and 3.77 minutes for the low and high doses, respectively (Domoradzki *et al*, 2001).

External and internal exposure to 2PG1ME was examined in 22 workers (20 men and 2 women) during production, leak testing and mounting of brake hoses. Average external exposure concentrations (measured by means of personal air monitoring) of 2PG1ME were 82.2 mg/m^3 (21.95 ppm), 68.6 mg/m^3 (18.32 ppm) and 11.3 mg/m^3 (3.02 ppm) in the brake-hose production, leak test and mounting areas, respectively. Internal exposure of 2PG1ME was measured in both urine and blood; the samples were taken post-shift. The highest internal levels were measured in the brake-hose production section (average 4.6 mg/l in urine and 13.5 mg/l in blood) and in the leak test area (4.2 mg/l urine and 11.0 mg/l blood). 2PG1ME levels in blood and urine samples of workers of the mounting area were below detection limits. Elimination kinetics of 2PG1ME in 3 highly exposed persons were characterised by mean excretion half-lives of approximately 4.4 hours (Hubner *et al*, 1992).

Human volunteers (4 males, 2 females) were exposed to 100 ppm (375 mg/m^3) 2PG1ME vapour for 8 hours, including a 30-min break after 4 hours. Blood, breath and urine samples were collected from all volunteers before, during, and up to 24 hours after exposure for the determination of 2PG1ME. Within the hour a steady state for 2PG1ME in alveolar air was reached while 2PG1ME was rapidly cleared from the lungs. Blood levels of 2PG1ME rose steadily throughout the exposure and post-exposure blood levels of up to 103 μmol/l were attained. The mean elimination half-life was 93 minutes (range 81 - 111 min). Urinary levels of 2PG1ME ranged from 78 to 110 μmol/l (average 92 μmol/l) at the end of exposure. Results were

more consistent when expressed in μmol/l than when corrected for urine volume or creatinine. The urinary half-life averaged 120 minutes (range 50 - 151 min) and elimination was virtually complete after 16 hours (Jones *et al*, 1997).

Human volunteers (2 males and 2 females) were exposed at rest to 100 ppm 2PG1ME (375 mg/m^3) vapour during 4 hours on four separate occasions, twice with and twice without fresh air-fed half masks to reflect skin-only and whole-body exposure, respectively. During exposure the volunteers wore only T-shirts and shorts to optimise the dermal surface available for vapour contact. Blood, breath and urine samples for the determination of 2PG1ME were collected from all volunteers. Single blood samples were collected immediately after exposure, breath samples prior to and immediately after exposure and then at 10- to 15-minute intervals up to 3 hours after exposure. Urine samples were taken prior to exposure and at 2 hourly intervals after exposure up to 12 hours after exposure, and at 22 hours after exposure. Dermal uptake from 2PG1ME vapour was calculated to be 9.6 ± 6.5% (range 4.1 - 18.2) based on breath samples, 8.0 ± 5.7% (range 2.5 - 14.0) based on blood samples, and 4.2 ± 1.7 % (range 2.2 - 6.1) based on urine sample (Brooke *et al*, 1998).

Three male workers occupationally exposed to air concentrations of 20 to 40 ppm 2PG1ME during cleaning of vats in an ink factory had urinary concentrations of up to 7.78 mg/l free and up to 17.06 mg/l total 2PG1ME. The levels of conjugated (with sulphate or glucuronide) 2PG1ME ranged between 30 and 65% (Devanthéry *et al*, 2000).

Respiratory uptake was investigated for 10 polar organic solvents with high blood/air partition coefficients, among them 2PG1ME (25, 50 or 100 ppm; 94, 187 or 375 mg/m^3), in 4 healthy male volunteers inhaling the test air for 10 minutes at rest and then room air for 5 minutes. The percentage of solvent in the end-exhaled air and in the mixed-exhaled air increased after the start of the test-air respiration, and reached a quasi-steady-state level within a few minutes. The mean uptake for the last 5 minutes of the test air respiration was 81.3% (Kumagai *et al*, 1999).

Human volunteers (6 males) were exposed to 2PG1ME vapour, with and without respiratory protection, for 6 hours. During whole-body exposure to 15, 50 or 95 ppm (56, 187 or 356 mg/m^3), levels of free 2PG1ME in urine reached 1.3, 4.4 and 7.9 mg/l, respectively. Maximum total (free and conjugated) 2PG1ME levels were 2.5, 6.2 and 10.3 mg/l in urine, 0.4, 1.4 and 2.9 ppm (1.5, 5.2 and 11 mg/m^3) in exhaled air and 2.0, 4.9 and 11.8 mg/l in blood. PGME half-lives were calculated to be about 3.5 hours in urine and 10 minutes in exhaled air. Following dermal-only exposure, 2PG1ME was not detected in breath, blood or urine (Devanthéry *et al*, 2002). The levels of 2-MPA concentrations (a metabolite of 2PG1ME) in urine at the end of these exposures ranged from 1.19 to 3.29 mg/l for inhalation and dermal exposure and from below the detection limit to 2.10 mg/l for exposure of one hand (Devanthéry *et al*, 2003).

4.28.4.8 Neurotoxicity

Trained female CFE rats were exposed by inhalation (whole-body, 4 h/d, 5 d/wk) for 10 exposures to concentrations of 2,500, 5,000 or 10,000 ppm (9,400, 18,700 or 37,500 mg/m^3). Tests included conditioned avoidance-escape behaviour by a modification of the pole-climb method. Besides a transient non-specific depression of behaviour at 5,000 and 10,000 ppm and a decrease in growth rate at 10,000 ppm no other effect was observed (Goldberg *et al*, 1964).

4.28.4.9 Immunotoxicity

The available studies provide no indication for an immunologic effect. No specific further studies are available.

4.28.5 Human effects data

2PG1ME had a perceptible odour concentration of ≥ 100 ppm (370 mg/m^3). At 1,000 ppm (3,700 mg/m^3), the odour became intolerable. Anaesthetic effects were reported at still higher exposure levels (Stewart *et al*, 1970).

Human volunteers exposed to 2PG1ME for 1 to 7 hours developed tolerance within 25 minutes. At 100 ppm (370 mg/m^3), the odour of 2PG1ME could be detected and mild eye irritation was noted in 3 of 6 individuals. At 250 ppm (940 mg/m^3), the majority of 23 subjects complained of eye, nose or throat irritation; several subjects developed headaches, and one nausea. None of the exposures resulted in changed vision, coordination, neurological parameters or brake-reaction. Clinical tests, including complete urinalysis before and after exposure, showed no effects (Stewart *et al*, 1970).

Table 4.28.1: Systemic toxicity of 2PG1ME

Route / Species, strain, number and sex/group		Dose or concentration		Exposure regime	Result	Reference
Oral, gavage		**(ml/kgbw/d)**	**(mg/kgbw/d)**			
Rat, NS,	5 -10 M	0, 0.1, 0.3, 1.0 3.0	(0, 92, 270, 920 2,760)	1 x/d, 5 d/wk, 35 d (5 wk)	No effects ↑ liver/kidney weight. Minor effects liver/kidney	Rowe *et al*, 1954
Rat, CFE,	10 M, 10 F	0, 0.5 1, 2 4	(0, 460 920, 1,800 3,700)	1 x/d, 7 d/wk, 13 wk	No effects Mild to severe dose-related ↓ CNS, ↓ growth ↑ liver cell necrosis, ↑ mortality, ↓ food intake	Stenger *et al*, 1972
Dog, Beagle,	3 M, 3 F	0, 0.5 1 2, 3	(0, 460 920 1,800, 2,800)	1 x/d, 5 d/wk, 14 wk	No effects Mild to severe dose-related ↓ CNS ↑ mortality. M: spermiophages in epididymis	Stenger *et al*, 1972
Dermal, occluded		**(ml/kgbw/d)**	**(mg/kgbw)**			
Rabbit, NZW,	5 M, 5 F	NS	0 1,000	1 x/d, 5 d/wk, 3 wk	No effects Slight local skin irritation	Calhoun *et al*, 1984
Rabbit, NS,	≥ 5 M	0, 2 7 10	(0, 1,800 6,400 9,200)	1 x/d, 5 d/wk, 13 wk	No effects Narcosis and death, ↓ terminal bw Narcosis and death, ↑ kidney weight; renal tubular necrosis (3)	Rowe *et al*, 1954
Inhalation		**(ppm)**	**(mg/m^3)**			
Rat, NS,	10 M, 10 F	1,500 3,000 6,000	(5,600 11,200 22,400)	4 x 7 h/d, 5 d	NOAEL ↑ liver weight ↓ growth. ↑ liver/kidney weight, lung/liver changes	Rowe *et al*, 1954
Rat, Wistar,	10 M	0, 200, 600	(0, 750, 2,250)	6 h/d, 10 d	No effects	Doe *et al*, 1983

Table 4.28.1: Systemic toxicity of 2PG1ME (cont'd)

Route / Species, strain, number and sex/group		Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)		**(ppm)**	**(mg/m^3)**			
Rat, F344,	5 M, 5 F 10 M, 10 F	0, 300, 1,000 3,000	(0, 1,120, 3,700 11,200)	6 h/d, 9 d	No effects ↓ CNS, ↑ liver weight	Miller *et al*, 1981
Rat, CFE,	8 - 10 F	0, 2,500 5,000 10,000	(0, 9,400 18,700 37,000)	4 h/d, 5 d/wk, 2 wk	No effects ↓ behaviour, ↑ tolerance ↓ behaviour, ↑ tolerance, ↓ growth rate	Goldberg *et al*, 1964
Rat, F344,	10 M, 10 F	0, 300, 1,000 3,000	(0, 1,120, 3,700 11,200)	6 h/d, 5 d/wk, 13 wk	No effects ↓ CNS; ↑ liver weight; hepatocellular hypertrophy in F	Landry *et al*, 1983
Rat, F344,	5 M, 5 F	3,000	(11,200)	6 h/d, 5 d/wk, 1, 2, 13 wk	↑ hepatic cell proliferation wk 1, M ↑ kidney cell proliferation	Cieszlak *et al*, 1996a
Rat, NS,	20 M, 20 F	0, 1,500	(0, 5,600	141 x 7 h/d, 5 d/wk, 198 d (28.3 wk)	No effects	Rowe *et al*, 1954
		3,000	11,200		Narcosis at end of each exposure, rapid recovery, tolerated after 1 wk. ↑ mortality, ↑ liver weight (M, F)	
	10 M, 10 F	6,000	22,400)	81 x 7 h/d, 5 d/wk, 114 d (16.3 wk)	Narcosis at end of each exposure, tolerated for last 2 months. Slight ↓ growth, ↑ liver/kidney weight (M slight, F moderate)	Rowe *et al*, 1954
Rat, F344,	50 M, 50 F	0, 300, 1,000 3,000	(0, 1,120, 3,700 11,200)	6 h/d, 5 d/wk, 2 y	No effects Initial sedation with resolution, M ↑ mortality; M: ↑ $\alpha_{2\mu}$-globulin and S-phase DNA, ↑ eosinophilic foci of altered hepatocytes	Spencer *et al*, 2002

Table 4.28.1: Systemic toxicity of 2PG1ME (cont'd)

Route / Species, strain, number and sex/group		Dose or concentration (ppm)	(mg/m^3)	Exposure regime	Result	Reference
Inhalation (cont'd)						
Mouse, B6C3F$_1$,	5 M, 5 F 10 M, 10 F	0, 300, 1,000 3,000	(0, 1,120, 3,700 11,200)	6 h/d, 5 d/wk 9 d	No effects F ↑ liver weight	Miller *et al*, 1981
Mouse, B6C3F$_1$,	5 M, 5 F	3,000	(11,200)	6 h/d, 5 d/wk, 1, 2, 13 wk	↑ hepatic cell proliferation wk 1	Cieszlak *et al*, 1996b
Mouse, B6C3F$_1$,	50 M, 50 F	0, 300, 1,000 3,000	(0, 1,120, 3,700 11,200)	6 h/d, 5 d/wk, 2 y	No effects Initial sedation with resolution, M ↑ mortality	Spencer *et al*, 2002
Guinea pig,	8 M, 8 F 5 M , 5 F	0, 1,500, 3,000 6,000	(0, 5,600, 11,200 22,400)	7 h/d, 5 d/wk, 184 d (26.2 wk) 7 h/d, 5 d/wk, 113 d (16.1 wk)	No effects Narcosis at end of each exposure. ↓↓ growth. Slight ↑ lung/kidney weight, slight lung changes	Rowe *et al*, 1954
Rabbit, NS,	1 M, 1 F 1M, 2 F 1 M, 1 F 1 F	0, 800 1,500 3,000 6,000	(0, 3,000 5,600 11,200 22,400)	7 h/d, 5 d/wk, 184 d (26.2 wk) 7 h/d, 5 d/wk, 186 d (26.6 wk) 7 h/d, 5 d/wk, 184 d (26.2 wk)	No effects No effects NOAEL. Slight ↑ liver weight, slight changes of lung/liver (F) Slight ↑ liver weight, slight changes of liver (F) and lung (M) Narcosis at end of each exposure. ↓ growth. Slight ↑ lung/kidney weight, slight lung changes	Rowe *et al*, 1954
Rabbit, NZW,	7 M, 7 F	0, 300, 1,000 3,000	(0, 1,120, 3,700 11,200)	6 h/d , 5 d/wk, 13 wk	No effects Narcosis	Landry *et al*, 1983

Table 4.28.1: Systemic toxicity of 2PG1ME (cont'd)

Route / Species, strain, number and sex/group		Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)		**(ppm)**	**(mg/m^3)**			
Monkey, NS,	1 F	0	(0	7 h/d, 5 d/wk, 205 d (29.3 wk)	No effects	Rowe *et al*, 1954
	1 M, 1 F	800	3,000	7 h/d, 5 d/wk, 186 d (26.6 wk)	No effects	
	1 F	1,500	5,600	7 h/d, 5 d/wk, 205 d (29.3 wk)	NOAEL. ↑ liver weight, slight lung and liver changes	
	2 F	3,000	11,200)		↑ liver weight, slight lung and liver changes	

Table 4.28.2: Genotoxicity of 2PG1ME

Endpoint / Species	Strain or type / Target	Concentration		Result	Remark	Reference
In vitro						
Gene mutation						
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	2 - 6,250 µg/plate		**–ve**	+/– **S9**	Kirkland *et al*, 1983
Chromosome aberration		**(mmol/l)**	**(mg/l)**			
CHO cells			1.25, 2.5, 5.0, 10	**–ve**		Kirkland and Verschuuren, 1983
Human lymphocyte		NS	NS	**–ve**	- S9	Elias *et al*, 1996
V79 cells		NS	NS	**–ve**	– S9	Elias *et al*, 1996
V79 cells		50	(4,500	**–ve**	+ Methylmethane-sulphonate (0.2 mmol/l)	Elias *et al*, 1996
		100, 200	9,000, 18,000)	**Weakly +ve**		
Unscheduled DNA synthesis		**(mmol/l)**	**(mg/l)**			
Rat hepatocyte	Primary	0.0316 to 100, in half-log intervals	(2.85 9,000)	**–ve**		Mendrela and Schumann, 1983a
Sister chromatid exchange		**(mmol/l)**	**(mg/l)**			
V79 cells		50, 75, 100	(4,500, 6,800, 9,000)	**Weakly +ve**		Elias *et al*, 1996
Micronucleus formation		**(mmol/l)**	**(mg/l)**			
V79 cell		NS	NS	**–ve**	– S9	Elias *et al*, 1996

Table 4.28.2: Genotoxicity of 2PG1ME (cont'd)

Endpoint / Species	Strain or type / Target	Concentration		Result	Remark	Reference
Aneuploidy		**(mmol/l)**	**(mg/l)**			
V79 cell		NS	NS	**–ve**	– S9	Elias *et al*, 1996
Inhibition of metabolic co-operation		**(mmol/l)**	**(mg/l)**			
V79 cells		13.9 27.8, 55.6	(1,250 2,505, 5,010)	**–ve** **+ve**		Elias *et al*, 1996
In vivo						
Micronucleus frequency		**(mg/kgbw)**				
Mouse, 4/sex/group	CD-1 Bone marrow	0, 2,500, 4,000, 5,000, 6,000, i.p.		**–ve**	Bone marrow collected at 24, 48 and 72 h	Elias *et al*, 1996

Table 4.28.3: Reproductive and developmental toxicity of 2PG1ME

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**				
Rat, CFE, 20 F	0, 46, 92, 185, 370 740		1 x/d, g.d. 1 - 18	No effects Delayed ossification	Stenger *et al*, 1972
Mouse, CFLP, 20 F	0, 460, 924, 1,850		1 x/d, g.d. 1 - 18	No effects	Stenger *et al*, 1972
Rabbit, Silver Yellow, 15 F	0, 230, 460, 924		1 x/d, g.d. 6 - 18	No effects	Stenger *et al*, 1972
Oral, drinking water	**% in water**	**(mg/kgbw)**			
Mouse, CD-1, 20 M, 20 F	0, 0.5	0, 949	*Ad libitum*, continuous for 2 generations	No effects	Gulati *et al*, 1986; Chapin and Sloane, 1997
	1.0	1,885			
	2.0	3,328		↓ pup weights	
F_1	2.0	3,328		↓ pup growth to weaning and bw of M adults, ↓ right epididymal and prostate gland weights	
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, F344, 29 - 32 F	0, 500, 1,500 3,000	(0, 1,900, 5,600 11,200)	6 h/d, g.d. 6 - 15	No effects ↓ ossfication; Mild lethargy in dams on d 1 - 2 of exposure	Hanley *et al*, 1984c
Rat, Wistar, 20 F	0, 200, 600	(0, 750, 2,250)	6 h/d, g.d. 6 - 17	No effects	Doe *et al*, 1983
Rabbit, NZW, 29 - 32 F	0, 500, 1,500 3,000	(0, 1,870, 5,600 11,200)	6 h/d, g.d. 6 - 18	No effects Mild lethargy in dams on d 1 - 2 of exposure	Hanley *et al*, 1984c

Table 4.28.3: Reproductive and developmental toxicity of 2PG1ME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Rat, SD-CD, 30 M, 30 F	0, 300, 1,000 3,000	(0, 1,120, 3,750 11,200)	6 h/d, 5d/wk, 10 d prior to mating; 6 h/d, 7d/wk during mating, gestation and lactation	No effects Parental toxicity: changes in relative/absolute organ/bw, including testes and ovaries. ↓ M fertility, lengthened F oestrous cycle, F ↓ ovarian weight, ovarian atrophy; embryotoxicity and foetotoxicity	Carney *et al*, 1999
Subcutaneous	**(mg/kgbw)**				
Rat, CFE, 20 f	0, 46, 92, 185, 370 740		1 x/d, g.d. 1 - 21	No effects Delayed ossification	Stenger *et al*, 1972
Mouse, CFLP, 20 F	0, 46, 92, 185, 370		1 x/d, g.d. 1 - 18	No effects	Stenger *et al*, 1972
Rabbit, Silver Yellow, 15 F	0, 46, 92		1 x/d, g.d. 6 - 18	No effects	Stenger *et al*, 1972

Table 4.28.4: Absorption (uptake), distribution, metabolism and elimination of 2PG1ME

Route / Species, strain, number and sex/group	Dose		Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**				
Rat, F344, 3 M	90 780		1 x, 48 h collection	50 - 60% exhaled as CO_2 10 - 20% excreted in urine as: unchanged 2PG1ME, propylene glycol and sulphate and glucuronate conjugates of 2PG1ME	Miller *et al*, 1983b
Mouse, $B6C3F_1$, 4 M	90		1 x, blood collection up to 5 h	2PG1ME was rapidly absorbed and metabolized to PG; maximum concentrations of 2PG1ME and PG in plasma at 29 and 30 min after dosing, respectively.	Ferrala *et al*, 1994
Oral or intravenous					
Rat, F344, NS	90 450		1 x	43 - 77% exhaled as CO_2 7 - 16% excreted in urine Exhalation, metabolism and excretion slower than in mice	Ferrala *et al*, 1994
Mouse, $B6C3F_1$, 4 M	90 450		1 x	43 - 77% exhaled as CO_2 7 - 16% excreted in urine Exhalation, metabolism and excretion faster than in rats	Ferrala *et al*, 1994
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, F344, 2 M, 2 F	300 750 1,500 3,000	(1,120 2,800 5,600 11,200)	6 h, nose-only	↑ blood concentration without reaching a plateau: Absorption limited by respiration, not saturated. Clearance best described with pseudo zero order kinetics. End of exposure blood concentrations not proportional to exposure concentration	Morgott and Nolan, 1987

Table 4.28.4: Absorption (uptake), distribution, metabolism and elimination of 2PG1ME (cont'd)

Route / Species, strain, number and sex/group	Dose		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Rat, F344, 6 M, 6 F	300 3,000	(1,120 11,200)	6 h/d, whole-body, 1 or 10 d	At 3,000 ppm, ↑ elimination capacity: 2PG1ME blood levels lower than during single exposure above. Elimination practically completed within 24 h	Morgott and Nolan, 1987
Intravenous	**(mg/kgbw)**				
Rat, F344, 4 M	10 100		1 x, 12 h collection	Half-life 1.55 min Half-life 3.37 min	Domoradzki *et al*, 2001

4.29 Substance profile: 2PG1MEA

4.29.1 Identity

Name:	2-Propylene glycol 1-methyl ether acetate
IUPAC name:	1-Methoxy-2-acetoxypropane
CAS registry No.	108-65-6 (major isomer)
Molecular formula:	$C_6H_{12}O_3$
Structural formula:	$CH_3–CH(O–CO–CH_3)–CH_2–O–CH_3$
Molecular weight:	132.2
Other components:	2-Methoxy-1-acetoxypropane (< 0.5%)

4.29.2 Physico-chemical properties

Melting point:	< –66°C
Boiling point:	146°C
Vapour pressure:	3.7 hPa
Solubility in water:	160 g/l
Relative density:	$D_4^{20} = 0.960$

4.29.3 Conversion factors

1 ppm = 5.496 mg/m^3
1 mg/m^3 = 0.182 ppm

4.29.4 Toxicological data

4.29.4.1 Acute toxicity

Oral

Rat: LD_{50} 8,532 mg/kgbw (Carreon *et al*, 1980).

Dermal

Rabbit: LD_{50} > 5,000 mg/kgbw (Henck *et al*, 1980).

The main signs of toxicity after acute high oral or dermal dosage were lethargy, anorexia, shallow breathing and excess salivation.

Inhalation

Rat: 6-h LC_{50} > 4,345 ppm (23,880 mg/m^3). No signs of toxicity were seen during exposure or upon gross pathological examination (Henck *et al*, 1980).

4.29.4.2 Irritation and sensitisation

Skin irritation

Undiluted 2PG1MEA was not irritant to rabbit skin (Henck *et al*, 1980).

Eye irritation

Undiluted 2PG1MEA caused conjunctival redness in rabbits, slight conjuctival swelling, slight iritis and corneal opacity. The eye returned to normal within 7 days (Henck *et al*, 1980).

Sensitisation

2PG1MEA (10% aqueous solution) was not sensitising in guinea pigs using a modified Maguire test (Carreon *et al*, 1980).

4.29.4.3 Repeated-dose toxicity (Table 4.29.1)

Subacute toxicity

F344 rats were exposed (9 x) to 2PG1MEA by inhalation at concentrations 0, 300, 1,000 or 3,000 ppm over a period of 11 days. Metaplasia was present in the nasal olfactory epithelium of both sexes at 3,000 ppm. In male rats, there was a slight increase in relative liver weight without histopathological changes at 3,000 ppm and kidney effects with reticulated appearance at 1,000 and 3,000 ppm. In the cytoplasm of the proximal tubules, an increased degree of normally occurring eosinophilic granularity was observed in all male rats at 3,000 ppm and in 1 out of 5 males at 1,000 ppm (Miller *et al*, 1984).

$B6C3F_1$ mice were exposed (9 x) to 2PG1MEA by inhalation at concentrations 0, 300, 1,000 or 3,000 ppm over a period of 11 days. Metaplasia was present in the nasal olfactory epithelium at all dose levels (Miller *et al*, 1984).

Following exposure of SD rats by oral (gavage) administration for 44 days, 2PG1MEA was slightly toxic at a dose of 1,000 mg/kgbw/d. Body weight gain was depressed and food consumption reduced, accompanied by decreases in blood glucose and inorganic phosphorus and a slight increase in relative adrenal weight. No effects were noted at the lower dose levels of 300 and 100 mg/kgbw/d. The study was combined with a reproductive/developmental toxicity study (Section 4.29.4.5) (Ito *et al*, 1997 cited by Ministry of Health and Welfare Japan, 1998).

Subchronic toxicity

No data are available.

4.29.4.4 Genotoxicity (Table 4.29.2)

In vitro

2PG1MEA did not cause gene mutations (histidine reversion) in *Salmonella typhimurium* (Ames test), with and without metabolic activation, up to 50,000 µg/plate (Mendrala and Schumann, 1983b; Shibuya *et al*, 1997 cited by Ministry of Health and Welfare Japan, 1998).

2PG1MEA did not induce chromosomal aberrations in Chinese hamster lung (CHL/IU) cells (Tanaka *et al*, 1997 cited by Ministry of Health and Welfare Japan, 1998).

2PG1MEA did not induce unscheduled DNA synthesis in rat hepatocytes (Mendrala and Schumann, 1983c).

4.29.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.29.4.6 Reproductive and developmental toxicity (Table 4.29.3)

Pregnant SD rats were exposed by inhalation to 2PG1MEA concentrations of 0, 500, 2,000 or 4,000 ppm during day 6 to 15 of gestation. Dyspnoea was observed at 4,000 ppm and in one

animal of the 2,000 ppm group. Food consumption and body weight were reduced at 2,000 and 4,000 ppm. In the 500 ppm group, no signs of toxicity were observed. No teratogenic or other developmental effects were seen in foetuses at any of the dose levels (Asaki and Houpt, 1990).

2PG1MEA was administered to SD rats by oral gavage at dose levels of 0, 100, 300 or 1,000 mg/kgbw/d for 44 days (males) or from 14 days before mating to day 3 of lactation (females). Slight toxicity was observed at 1,000 mg/kgbw/d in both sexes (Section 4.29.4.3). No teratogenic or developmental effects occurred at any dose level (Ito *et al*, 1997 cited by Ministry of Health and Welfare Japan, 1998).

4.29.4.7 Kinetics and metabolism (Table 4.29.4)

In vitro

Skin permeation was calculated using the Franz cell method with human skin. 2-PG1MEA was tested in pure form and with 70 % acetone. In pure form the lag time was 30 min, flux at steady state was 0.059 mg/cm^2/h, and permeation 0.061 cm/h x 10^{-3}. In mixture with acetone the respective values were 30 min, 0.067 mg/cm^2/h and 0.079 cm/h x 10^{-3} (Larese *et al*, 1999).

In vivo

Acetate esters of aliphatic alcohols are rapidly hydrolysed by enzymes in the respiratory epithelium, lungs, liver and blood of rats, rabbits and hamsters (Stott and McKenna, 1984, 1985; Dahl *et al*, 1987).

Miller *et al* (1984) reported that metabolism of 2PG1ME and 2PG1MEA in F344 rats followed the same pattern (Section 4.28.4.7). Within 48 hours after oral gavage or inhalation of 2PG1MEA, 64% or 53% of the radioactivity, respectively, was exhaled as CO_2. In the urine, the same metabolites as for 2PG1ME were recovered, amounting to 24% after oral gavage or 26% after inhalation.

The blood kinetics of equimolar doses of 2PG1MEA and 2PG2ME in rats were identical showing rapid initial hydrolysis of the acetate ester to the parent alcohol; half-lives were 1.55 and 3.77 minutes for the low and high doses, respectively (Domoradzki *et al*, 2001) (Section 4.28.4.7). Hydrolysis of 2PG1MEA was slower *in vitro*, the half-lives being approximately 15 minutes in rat blood and human blood, and 30 minutes in rat and human liver homogenates (Domoradzki *et al*, 2001, 2003).

Laitinen *et al* (1997) has published a method for biomonitoring exposure to technical-grade 2PG1MEA based on analysis of urinary 2-MPA (limit of detection 0.1 mg/l). The method was employed to compare post-shift 2-MPA levels in silkscreen workers with breathing-zone measurements of 2PG1MEA. Mean 2-MPA levels were expressed as 1.27 mmol/mol creatinine for exposures of 0 to 33 cm^3/m^3 (0 - 33 ppm, 0 - 180 mg/m^3).

4.29.4.8 Neurotoxicity

No data are available.

4.29.4.9 Immunotoxicity

No specific data are available.

The available studies provide no indication for an immunologic effect.

4.29.5 Human effects data

No data are available.

Table 4.29.1: Systemic toxicity of 2PG1MEA

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral	**(mg/kgbw)**				
Rat, SD, 10 M, 10 F	0, 100, 300		M: 44 d; F: 14 d before mating to d 3 of lactation	No effects	Ito *et al*, 1997 cited by Ministry of Health and Welfare Japan, 1998
	1,000			↓ bw gain M, F; ↓ food consumption, ↓ blood glucose, ↓ inorganic phosphorus and ↓ relative adrenal weight (M)	
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, F344, 5 M, 5 F	0, 300	(0, 1,65	9 x 6 h/d, 11 d	No effects	Miller *et al*, 1984
	1,000	5,500		M ↑ Granularity of kidney tubular cells	
	3,000	16,500)		M ↑ Relative liver weight, granularity of kidney tubular cells, M degenerative changes in olfactory mucosa	
Mouse, $B6C3F_1$, 5 M, 5 F	0	(0,	9 x 6 h/d, 11d	No effects	Miller *et al*, 1984
	300	1,650		F (1) olfactory mucosa replaced by respiratory mucosa	
	1,000	16,500)		M and F olfactory mucosa replaced by respiratory mucosa	

Table 4.29.2: Genotoxicity of 2PG1MEA in vitro

Endpoint / Organism	Strain or type/ Target cells	Concentration	Result	Remark	Reference
Gene mutation		**(µg/plate)**			
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	100 - 50,000	**–ve**	+/– S9	Mendrala and Schumann, 1983b
Salmonella typhimurium	TA100, TA1535, TA98, TA1537	0, 313 - 5,000	**–ve**	+/– S9, pre-incubation	Shibuya *et al*, 1997 cited by Ministry of Health and Welfare Japan, 1998
Chromosome aberration		**(mg/ml)**			
CHL/IU cells		0, 0.33, 0.65, 1.3	**–ve**	+/– S9	Tanaka *et al*, 1997 cited by Ministry of Health and Welfare Japan, 1998
Unscheduled DNA Synthesis		**(mol/l)**			
Rat hepatocyte	Primary	3.16×10^{-5} - 0.1	**–ve**		Mendrala and Schumann, 1983c

Table 4.29.3: Reproductive and developmental toxicity of 2PG1MEA in SD rats

Route / Number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Oral	**(mg/kgbw)**				
10 M, 10 F	0, 100, 300, 1,000		M: 44 d; F: 14 d before mating to d 3 of lactation	No teratogenic or developmental effects	Ito *et al*, 1997 cited by Ministry of Health and Welfare Japan, 1998
Inhalation	**(ppm)**	**(mg/m^3)**			
SD, 20 F	0, 500 2,000 4,000	(0, 2,750 11,000 22,000)	6 h/d, g.d.6 - 15	No effects Dyspnoea in 1 animal. ↓ food consumption; ↓ bw Dyspnoea. ↓ food consumption; ↓ bw	Asaki and Houpt, 1990

Table 4.29.4: Absorption (uptake), distribution, metabolism and elimination of 2PG1MEA

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral	**(mg/kgbw)**				
Rat, F344 3 M	1,150		1 x, 48 h collection	64% exhaled as CO_2; 24% excreted in urine as 2PG1ME, propylene glycol and sulphate, and glucuronate conjugates of 2PG1ME	Miller *et al*, 1984
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, F344, 6 M	3,000	(16,500)	6 h, 48 h collection	53% exhaled as CO_2; 26% excreted in urine as 2PG1ME, propylene glycol and sulphate, and glucuronate conjugates of 2PG1ME	Miller *et al*, 1984
Intravenous	**(mg/kgbw)**				
Rat, F344, 4 M	14.7 147		1 x, 12 h collection	Rapid hydrolysis to 2PG1ME, half-life 1.55 min Rapid hydrolysis to 2PG1ME, half-life 3.37 min	Domoradzki *et al*, 2001, 2003
Rat, F344, 4 M	10 100		1 x, 12 h collection	Half-life 1.55 min Half-life 3.37 min	Domoradzki *et al*, 2001, 2003

4.30 Substance profile: 1PG2ME

4.30.1 Identity

Name:	1-Propylene glycol 2-methyl ether
IUPAC name:	2-Methoxy-1-propanol
CAS registry No.	1589-47-5
Molecular formula:	$C_4H_{10}O_2$
Structural formula:	CH_3-CH(OCH_3)-CH_2-OH
Molecular weight:	90.1
Other components:	No data

4.30.2 Physico-chemical properties

Melting point:	< –50°C
Boiling point:	130 - 133°C
Vapour pressure:	5.5 hPa
Solubility in water:	Completely soluble
Relative density:	D_4^{20} = 0.938

4.30.3 Conversion factors

1 ppm = 3.745 mg/m^3
1 mg/m^3 = 0.267 ppm

4.30.4 Toxicological data

4.30.4.1 Acute toxicity

Oral

Rat: LD_{50} > 5,000 mg/kgbw (BASF, 1979a).

Dermal

No data available.

Inhalation

Rat: 4-h LC_{50} > 6 mg/l (1,600 ppm) (BASF, 1979b).

4.30.4.2 Irritation and sensitisation

Skin irritation

Undiluted 1PG2ME applied (24-h, occluded) to intact and scarified rabbit skin was not irritant (BASF, 1979a).

Eye irritation

Undiluted 1PG2ME was not irritant to rabbit eyes (BASF, 1979a).

Sensitisation

No data are available.

4.30.4.3 Repeated-dose toxicity (Table 4.30.1)

Subacute toxicity

Wistar rats were administered 1,800 mg 1PG2ME/kgbw/d by oral gavage for 10 days. A marginal decrease of RBC and total Hb concentration were the only effects observed. No testicular damage and no leukopenia were observed (BASF, 1982a).

A 28-day inhalation study employing 0, 110, 560 or 2,800 ppm 1PG2ME, revealed reduced body weight gain, respiratory irritation and thymic atrophy at the top concentration. No testicular or bone marrow toxicity was noted. The NOAEC was 560 ppm (BASF, 1984c).

Subchronic toxicity

No data are available.

4.30.4.4 Genotoxicity (Table 4.30.2)

1PG2ME did not cause mutagenic effects in *Salmonella typhimurium* (Ames test), with or without metabolic activation, up to 5,000 µg/plate (BASF, 1988a).

4.30.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.30.4.6 Reproductive and developmental toxicity (Table 4.30.3)

Pregnant Wistar rats exposed by inhalation to 0, 1,000; 2,000 or 2,700 ppm 1PG2ME from day 6 to 15 of gestation developed foetuses with thoracic vertebral incisions and split vertebrae at the highest level and to a slight extent (only incisions) at 1,000 and 2,000 ppm. The dams showed slight sedation and irritation at higher dose levels (BASF, 1984a; Merkle *et al*, 1987).

Himalayan rabbits were exposed by inhalation to 0, 145, 225, 350 or 545 ppm 1PG2ME from day 6 to 18 of gestation. Maternally toxic effects were recorded only at the highest level (decreased weight gain and uterine weights at the end of the post-exposure period; placental weights were increased). Increased foetal resorptions were observed and foetal weights were decreased. Two animals aborted. Malformations occurred in a dose-related manner at 350 and 545 ppm, and to a marginal extent a 225 ppm. The anomalies mostly consisted in the sternebrae; fused ribs and swollen rib cartilage were also observed; 2 foetuses at 350 ppm had cleft palate; at 545 ppm several other types of anomalies were observed including truncus ateriosus communis, missing gall bladder, and aplasia of phalanges). At 225 ppm the numbers of variations in ribs and sternebrae were slightly increased. The NOAEL was 145 ppm (BASF, 1988b; Hellwig *et al*, 1994).

Developmental toxicity studies with the isomer 2PG1ME in rats, containing 1.32% of 1PG2ME as an impurity, did not show effects at the 3,000 ppm level, which was equivalent to 40 ppm of 1PG2ME (Hanley *et al*, 1984b,c).

In a developmental toxicity study, groups of inseminated rabbits were dosed by gavage with 0, 10, 26 or 78 mg/kgbw/d of MPA, the major metabolite of 1PG2ME, on day 7 to 19 of gestation, followed by foetal evaluation on day 28 of gestation. Results with MPA were compared with those of rabbits similarly dosed with 0, 2.5, 7.5 or 15 mg/kgbw/d of MAA, teratogenic metabolic of EGME. The developmental toxicity NOAELs were approximately 10-fold higher for MPA (26 mg/kgbw/d) than for MAA (2.5 mg/kgbw/d) with MPA exhibiting less severity of effects and non-selective foetotoxicity compared to the effects produced by MAA (Carney and Johnson, 2000).

The comparative developmental effects were studied for 1PG2ME (0.5 and 2.0 mM) in rabbit whole-embyro culture and 2-MPA (1.5 mM) and MAA (5 mM) in rat and rabbit whole-embyro cultures. There were no adverse effects of MPA or its parent compound, 1PG2ME, in contrast to severe dysmorphogenesis in 100% of the embryos cultured in 5 mM MAA (Carney and Tornesi, 2001; Tornesi and Carney, 2001). MPA peak blood levels previously associated with a developmental NOAEL and LOAEL in rabbits after oral administration were 0.5 mM and 1.3 mM respectively, suggesting a relatively high threshold based on internal dose (Pottenger *et al*, 1999) (Section 4.30.4.7).

4.30.4.7 Kinetics and metabolism (Table 4.30.4)

F344 rats dosed with 1.0 or 8.7 mmol/kg of ^{14}C-labelled material (at C-atom 2) eliminated 70 to 80% of the radioactivity in the urine and 10 to 20% as $^{14}CO_2$. 2-MPA accounted for 79 to 93% of the urinary radioactivity (Miller *et al*, 1986).

NZW rabbits showed rapid and complete conversion of 1PG2ME (administered by oral gavage) to 2-MPA, with a half-life of 33 to 44 hours for MPA. Peak MPA blood levels associated with a developmental NOAEL and LOAEL were 0.5 mM and 1.3 mM respectively (Pottenger *et al*, 1999).

4.30.4.8 Neurotoxicity

No data are available.

4.30.4.9 Immunotoxicity

The available studies provide no indication for immunologic effects. No specific further studies are available.

4.30.5 Human effects data

No data are available.

Table 4.30.1: Systemic toxicity of 1PG2ME in Wistar rats by oral gavage

Number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
5 M	0	1 x/d, 10 d	No effects	BASF, 1982a
	1,800		↓ erythrocytes and Hb. No testicular effects, no leukopenia. EGEE equimolar level as positive control	

Table 4.30.2: Genotoxicity of 1PG2ME in vitro

Endpoint / Species	Strain	Concentration	Result	Remark	Reference
Gene mutation					
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	Up to 5,000 µg/plate	**–ve**	+/– S9	BASF, 1988a

Table 4.30.3: Reproductive and developmental toxicity of 1PG2ME by inhalation

Route / Species, strain, number and sex/group	Concentration (ppm)	Concentration (mg/m^3)	Exposure regime	Result	Reference
Rat, Wistar, 5 F	0 1,000, 2,000, 3,000	(0 3,750, 7,500, 11,200)	6 h/d, g.d. 6 - 15	No effects ↑ thoracic vertebral anomalies (dumb-bell-shaped notches of central cartilage)	BASF, 1984a; Merkle *et al*, 1987
Rabbit, Himalayan, 10 - 11 F	0 145 225, 350, 545	(0, 543 843, 1,310, 2,040)	6 h/d, g.d. 6 - 18	No effects 1 sternebral malformation ↑ malformations	BASF, 1988b; Hellwig *et al*, 1994

Table 4.30.4: Absorption (uptake), distribution, metabolism and elimination of 1PG2ME by oral gavage

Route / Species, strain, number and sex/group	Dose (mmol/kgbw)	Dose (mg/kgbw)	Exposure regime	Result	Reference
Rat, F344, 3 M	1.0, 8.7	(90, 784)	1 x	10 - 20% as CO_2,, 70 - 80% in urine: 2-MPA accounted for 79 - 93% in urine	Miller *et al*, 1986
Rabbit, NZW, 3 F	0.75, 3.0 0.25, 0.75 MPA	67.5, 270 26, 78 MPA	1 x, 168 h collection	Rapid conversion of 1PG2ME to MPA Half-life of MPA 33 - 44 h, peak MPA blood levels 0.5 and 1.3 mM for low and high dose respectively	Pottenger *et al*, 1999

4.31 Substance profile: 1PG2MEA

4.31.1 Identity

Name:	1-Propylene glycol 2-methyl ether acetate
IUPAC name:	2-Methoxy-1-acetoxypropane
CAS registry No.	70657-70-4
Molecular formula:	$C_6H_{12}O_3$
Structural formula:	CH_3-CH(OCH_3)-CH_2-O-CO-CH_3
Molecular weight:	132.2
Other components:	No data

4.31.2 Physico-chemical properties

Melting point:	< –20°C
Boiling point:	150 - 151°C
Vapour pressure:	2.9 hPa
Solubility in water:	Completely soluble

4.31.3 Conversion factors

1 ppm = 5.496 mg/m^3
1 mg/m^3 = 0.182 ppm

4.31.4 Toxicological data

4.31.4.1 Acute toxicity

Oral

Rat:	LD_{50} > 5,000 mg/kgbw. Sign of toxicity was CNS depression (BASF, 1984b).

Dermal

Rabbit:	LD_{50} > 2,000 mg/kgbw. No signs of toxicity (Merkle *et al*, 1987).

Inhalation

Rabbit: 6-h LC_{50} > 400 ppm (2,200 mg/m^3). No signs of toxicity (BASF, 1984c).

Dog: 6-h LC_{50} > 400 ppm (2,200 mg/m^3). Signs of toxicity were initially increased salivation and eyelid movement (BASF, 1984c).

4.31.4.2 Irritation and sensitisation

Skin irritation

Undiluted 1PG2MEA showed no irritation on rabbit skin (Braun, 1997a).

Eye irritation

1PG2MEA vapours at 400 ppm (2,200 mg/m^3) caused slight irritation to rabbit eyes (Braun, 1997b).

Sensitisation

No data are available.

4.31.4.3 Repeated-dose toxicity (Table 4.31.1)

Subacute toxicity

Wistar rats received 10 daily gavage administrations of 2,600 mg 1PG2MEA/kgbw for 2 weeks. No effects were seen in macroscopic investigations or in routine clinical chemistry (BASF, 1982a).

Wistar rats were exposed by inhalation to 0, 110, 560 or 2,800 ppm 1PG2MEA for 4 weeks. The concentrations 110 and 560 ppm were without adverse effects. Respiratory irritation, reduced weight gain and thymic atrophy were observed at 2,800 ppm. No testicular or bone marrow toxicity was observed (BASF, 1984d; Ma-Hock *et al*, 2005).

Subchronic toxicity

No data are available.

4.31.4.4 Genotoxicity

No data are available.

4.31.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.31.4.6 Reproductive and developmental toxicity (Table 4.31.2)

Pregnant Himalayan rabbits were dermally exposed (6 h/d) to 0, 1,000 or 2,000 mg 1PG2MEA/kgbw/d from day 6 to 18 of gestation. The test material was applied undiluted under semi-occlusive conditions. No adverse effects were observed (Merkle *et al*, 1987).

Pregnant Wistar rats were exposed by inhalation to concentrations of 0, 110, 550 or 2,700 ppm 1PG2MEA from day 6 to 15 of gestation. Maternal effects (irritation, sedation, decreased body weights between days 15 and 20) were observed at 2,700 ppm and, slightly, at 550 ppm. At 2,700 ppm, there was an increased rate of foetal resorptions, a slight decrease of foetal weights, and 12 out of 189 foetuses showed thoracic vertebral incisions, but there were no effects upon the foetuses at 550 ppm (Merkle *et al*, 1987).

Pregnant Himalayan rabbits were exposed by inhalation to 0, 36, 145 or 550 ppm 1PG2MEA from day 6 to 18 of gestation. No maternal effects were observed. At 550 ppm, all foetuses investigated showed malformations of sternum, paws, major blood vessels and heart. No treatment-related effects occurred at 36 and 145 ppm (Merkle *et al*, 1987).

4.31.4.7 Kinetics and metabolism

The *in vitro* half-life for hydrolysis of 1PG2MEA in rat plasma (cleavage of the ester bond) at 37°C was approximately 10 minutes (Hoffmann and Gelbke, 1984; Hoffmann and Jäckh, 1985). The resulting 1PG2ME is oxidised to 2-MPA and excreted via the urine (Miller *et al*, 1986).

4.31.4.8 Neurotoxicity

No data are available.

4.31.4.9 Immunotoxicity

No data are available.

4.31.5 Human effects data

No data are available.

Table 4.31.1: Systemic toxicity of 1PG2MEA in Wistar rats

Route / Number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**				
5 M	2,600		1 x/d, 10 d	No effects	BASF, 1982a
Inhalation	**(ppm)**	**(mg/m^3)**			
5 M, 5 F	0, 110 560 2,800	(0, 605 3,080 15,400)	4 h/d, 5 d/wk, 4 wk	No effects Slight respiratory irritation ↑ irritation. ↓ bw gain. Slight ↑ liver and thymus atrophy	BASF, 1984d; Ma-Hock *et al*, 2005

Table 4.31.2: Reproductive and developmental toxicity of 1PG2MEA

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Dermal, semi-occluded	**(mg/kgbw)**				
Rabbit, Himalayan, 10 F	0, 1,000, 2,000		g.d. 6 - 18	No effects	Merkle *et al*, 1987
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, Wistar, 25 F	0, 110 550 2,700	(0, 600 3,000 14,800)	g.d. 6 - 15	No effects No foetal effects (maternal CNS depression; ↓ maternal weight) Abnormalities in 12/189 foetuses (split vertebrae thoracicae); dead implantations	Merkle *et al*, 1987
Rabbit, Himalayan, 15 F	0, 36, 145 550	(0, 200, 800 3,000)	g.d. 6 - 18	No effects All foetuses (63) severely malformed in the absence of clear maternal toxicity	Merkle *et al*, 1987

4.32 Substance profile: DPGME

4.32.1 Identity

Name:	Dipropylene glycol (mono) methyl ether
IUPAC name:	None
CAS registry No.	34590-94-8
Molecular formula:	$C_7H_{16}O_3$
Structural formula:	$CH_3–(O–CH_2–\underset{\displaystyle CH_3}{\underset{\vert}{C}}H)_2–OH$
Molecular weight:	148.2
Other components:	Mixture of 4 isomers

4.32.2 Physico-chemical properties

Melting point:	–83°C
Vapour pressure:	1 hPa
Solubility in water:	Completely soluble
Relative density:	$D_4^{20} = 0.951$

4.32.3 Conversion factors

1 ppm = 6.161 mg/m^3
1 mg/m^3 = 0. 162 ppm

4.32.4 Toxicological data

4.32.4.1 Acute toxicity

Oral

Rat:	LD_{50} 5.5 ml/kgbw (male); 5.45 ml/kgbw (female) following administration of undiluted material (5,230 and 5,180 mg/kgbw, respectively). Sign of toxicity was depression of the CNS (Rowe *et al*, 1954).
Dog:	LD_{50} 7,500 mg/kgbw. Mortality within 48 hours. Sign of toxicity was respiratory paralysis (Shideman and Procita, 1951).

Dermal

Rabbit: LD_{50} 10,000 to 14,000 mg/kgbw (Browning, 1965). Transient narcosis but no deaths were observed at 20 ml/kgbw (19,000 mg/kgbw) (24-h occluded application) (Draize *et al*, 1944; Rowe *et al*, 1954).

Inhalation

Rat: Exposure to 500 ppm (vapour and aerosol atmosphere) (3,100 mg/m^3) for 7 hours produced mild narcosis (Rowe *et al*, 1954).

4.32.4.2 Irritation and sensitisation

Skin irritation

DPGME was slightly irritant (slight scaliness) to rabbit skin following repeated application for 90 days (Draize *et al*, 1944). No primary skin irritation was seen in a subchronic study (Rowe *et al*, 1954) (Section 4.32.4.3).

Eye irritation

Undiluted DPGME (0.1 ml) was irritant to the rabbit eye, in particular the conjunctivae and margins of the eyelid. These effects declined in severity over 24 hours and had completely resolved by 14 days. Measurement of corneal thickness and intraocular pressure showed minor changes indicating minimal effects on the corneal epithelium (Ballantyne, 1984b).

Sensitisation

No data are available.

4.32.4.3 Repeated-dose toxicity (Table 4.32.1)

Subacute toxicity

Oral administration of DPGME to rats (strain not stated) at doses of 0, 40, 200 or 1,000 mg/kgbw/d for 4 weeks resulted in salivation immediately after dosing and increased liver

weight accompanied by histopathological changes (centrilobular hypertrophy) at 1,000 mg/kgbw/d. Liver effects in males did not resolve in the recovery group. The NOAEL was 200 ppm (Kobayashi, 2000).

Open or occluded daily dermal application of doses of 100 or 1,000 mg DPGME/kgbw/d to the shaved skin of male Porton-Wistar rats for 4 weeks produced no significant changes in clinical chemistry, haematology or pathology (Fairhurst *et al*, 1989).

Small increases in relative liver weight were observed in F344 rats and $B6C3F_1$ mice exposed by inhalation to atmospheres of 50, 140 or 300 ppm for 9 days. These changes occurred in the absence of any histopathological changes and were considered adaptive (Landry *et al*, 1981).

DPGME administered to SD rats as daily i.p. doses of 1,000 mg/kgbw for 2 weeks produced no effects in urinary excretion of N-acetyl-β-glucosaminidase, $\beta_{2\mu}$-globulin and albumin and was therefore judged to have no effect on renal function (Bernard *et al*, 1989).

Subchronic toxicity

Daily dermal occluded application (5 d/wk) of DPGME to the shaved skin of rabbits for 90 days at dose levels of 1.0 to 10 ml/kgbw (950 - 9,500 mg/kgbw/d) resulted in narcosis and death at 10 ml/kgbw/d (9,500 mg/kgbw/d) (Rowe *et al*, 1954).

When F344 rats and NZW rabbits were exposed by inhalation to atmospheres of 0, 15, 50 or 200 ppm for 13 weeks, no effects were seen in either species at any concentration (Landry and Yano, 1984).

4.32.4.4 Genotoxicity (Table 4.32.2)

In vitro

DPGME has been evaluated for mutagenic activity (histidine reversion) in *Salmonella typhimurium* (Ames test) and *Escherichia coli* (Kirkland and Varley, 1983; Sakata, 2000), and for clastogenic activity (increase of chromosome aberration rate) in CHO cells (Kirkland, 1983) and CHL/IU cells (Indo, 2000). In all of these studies, negative results were obtained, both in the presence and absence of metabolic activation.

No unscheduled DNA synthesis was observed in rat hepatocytes (Mendrala, 1983).

4.32.4.5 Chronic toxicity and carcinogenicity (Table 4.32.1)

Rats, rabbits, guinea pigs and monkeys (strains not stated) were exposed by inhalation for various periods of 6.5 to 7.5 months to essentially saturated atmospheres, reported to contain 300 ppm DPGME as measured by spectroscopic analysis. The only observed effects were in rats and consisted of slight narcosis in the first weeks of exposure and a slight increase in liver weight at the end of the study. There were microscopic changes of the liver (granulation of cytoplasm and small and large vacuoles) in female guinea pigs, male and female monkeys, but not in rabbits (Rowe *et al*, 1954).

4.32.4.6 Reproductive and developmental toxicity (Table 4.32.3)

Studies of the developmental toxicity of DPGME in F344 rats and NZW rabbits provided no evidence of any selective toxicity to the foetus (Breslin *et al*, 1990a,b).

4.32.4.7 Kinetics and metabolism

Following single oral administration of ^{14}C-labelled DPGME (1,289 mg/kgbw) to male F344 rats, 60% of the radiolabel was excreted in the urine, most within the first 24 hours, 27% in the expired air and < 3% in the faeces. DPGME, PGME, propylene glycol, dipropylene glycol and the sulphates and glucuronides of DPGME were identified in the urine (Miller, 1985b).

4.32.4.8 Neurotoxicity

No specific data are available.

Acute, subacute and subchronic studies with DPGME in experimental animals have shown that exposure to high concentrations or extensive and prolonged skin contact may produce depression of the CNS leading to narcosis. Death following massive exposures has been ascribed to respiratory paralysis. The CNS effects produced by sub-lethal concentrations appear to be fully reversible and conventional histopathological examination of the CNS and peripheral nerves have not indicated any structural changes (Section 4.32.4.1 and 4.32.4.3).

4.32.4.9 Immunotoxicity

No specific data are available.

Repeated exposure of animals to DPGME has not provided evidence of any effects on the immune system (Section 4.32.4.1 and 4.32.4.3).

4.32.5 Human effects data

Patch tests conducted with DPGME in 50 human subjects produced no evidence of either primary skin irritation or sensitisation (Rowe *et al*, 1954). No evidence of skin sensitisation was reported in another human patch test (Draize *et al*, 1944).

Airborne concentrations of between 300 to 400 ppm (1,850 - 2,500 mg/m^3) have been described as very disagreeable to humans (Rowe *et al*, 1954).

Table 4.32.1: Systemic toxicity of DPGME

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral	**(mg/kgbw)**				
Rat, NS, 5 M, 5 F + 5/sex for recovery	0, 40, 200 1,000		1 x/d, 28 d	No effects Salivation. ↑ liver weight with centrilobular hypertrophy	Kobayashi, 2000
Dermal, occluded	**(ml/kgbw)**	**(mg/kgbw)**			
Rabbit, NS, 5 M	0, 1.0, 3.0, 5.0 10.0	(0, 950, 2,900, 4,800 9,500)	1 x/d, 5 d/wk, 90 d	No effects Narcosis, death	Rowe *et al*, 1954
Rat, Porton-Wistar, 8 M	0, 100, 1,000		1 x/d, 5 d/wk, 4 wk	No effects	Fairhurst *et al*, 1989
Dermal, open application	**(mg/kgbw)**				
Rat, Porton-Wistar, 8 M	0, 100, 1,000		1 x/d, 5 d/wk, 4 wk	No effects	Fairhurst *et al*, 1989
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, F344, 5 M, 5 F	0 50, 140, 330	(0 310, 860, 2,030)	6 h/d, 9 d	No effects ↑ Liver weight (M)	Landry *et al*, 1981
Mouse, B6C3F_1, 5 M, 5 F	0 50, 140, 330	(0 310, 860, 2,030)	6 h/d, 9 d	No effects ↑ Liver weight (M)	Landry *et al*, 1981
Rat, F344, 10 M, 10 F	0, 15, 50, 200	(0, 92, 310, 1,250)	6 h/d, 5 d/wk, 13 wk	No effects	Landry and Yano, 1984
Rabbit, NZW, 7 M, 7 F	0, 15, 50, 200	(0, 92, 310, 1,250)	6 h/d, 5 d/wk, 13 wk	No effects	Landry and Yano, 1984

Table 4.32.1: Systemic toxicity of DPGME (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Rat, NS, 20 M, 20 F	0 300	(0 1,850)	7 h/d, 5 d/wk, 200 d (28.6 wk)	No effects ↓ CNS (narcosis), resistant after few wk. ↑ liver weight (M, F); no other significant effects	Rowe *et al,* 1954
Rabbit, NS, 2 M, 2 F	0, 300	(0 1,850)	7 h/d, 5 d/wk, 221 d (31.6 wk)	No effects No significant effects.	Rowe *et al,* 1954
Guinea pig, NS, 8 M, 8 F	0 300	(0 1,850)	7 h/d, 5 d/wk, 186 d (26.6 wk)	No effects No significant effects. Slight granulation of liver cytoplasm and vacuolation (F)	Rowe *et al,* 1954
Monkey, NS, 1 M, 1 F	0 300	(0 1,850)	7 h/d, 5 d/wk, 221 d (31.6 wk)	No effects No significant effects. Slight granulation of liver cytoplasm and vacuolation (M, F)	Rowe *et al,* 1954
Intraperitoneal	**(mg/kgbw)**				
Rat, SD, 5 F	0, 1,000		1 x/d, 5 d/wk, 2 wk	No effects	Bernard *et al,* 1989

Table 4.32.2: Genotoxicity of DPGME in vitro

Endpoint / Species	Strain or type / Target	Concentration	Result	Remark	Reference
Gene mutation		**(µg/plate)**			
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	2 - 6,250	**–ve**	+/– S9	Kirkland and Varley, 1983
Salmonella typhimurium	TA100, TA1537, TA98	Up to 5,000	**–ve**	+/– S9	Sakata, 2000
Escherichia coli	WP2uvrA	Up to 5,000	**–ve**	+/– S9	Sakata, 2000
Chromosome aberration		**(mg/ml)**			
CHO cell		1.25, 2.5, 5.0, 10	**–ve**	+/– S9	Kirkland, 1983
CHL/IU cell		0.37, 0.74, 1.48	**–ve**	+/– S9	Indo, 2000
Unscheduled DNA synthesis		**(mg/ml)**			
Primary hepatocyte		Up to 14.8	**–ve**		Mendrala, 1983d

Table 4.32.3: Reproductive and developmental toxicity of DPGME

Route / Species, strain, number and sex/group	**Dose or concentration**		**Exposure regime**	**Result**	**Reference**
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, F344, 8 F	0, 50, 150, 300	(0, 310, 920, 1,850)	7 h/d, g.d. 6 - 15	Not maternally toxic, embryo/foetotoxic or teratogenic	Breslin, 1990a
Rabbit, NZW, 4 F	0, 50, 150, 300	(0, 310, 920, 1,850)	7 h/d, g.d. 7 - 19	Not maternally toxic, embryo/foetotoxic or teratogenic	Breslin, 1990a

4.33 Substance profile: TPGME

4.33.1 Identity

Name:	Tripropylene glycol (mono) methyl ether
IUPAC name:	1-(2-(2-Methoxy-1-methylethoxy)-1-methylethoxy)-2-propanol
CAS registry No.	25498-49-1 (all isomers)
Molecular formula:	$C_{10}H_{22}O_4$
Structural formula:	$CH_3–(O–CH_2–\underset{\displaystyle CH_3}{\underset{\vert}{CH}})_3–OH$
Molecular weight:	206.3
Other components:	Contains 8 isomers

4.33.2 Physico-chemical properties

Melting point:	–78°C
Boiling point:	243°C
Vapour pressure:	0.2 hPa
Solubility in water:	Miscible in all proportions
Relative density:	$D_4^{20} = 0.965$

4.33.3 Conversion factors

1 ppm = 8.576 mg/m^3
1 mg/m^3 = 0.117 ppm

4.33.4 Toxicological data

4.33.4.1 Acute toxicity

Oral

Rat:	LD_{50} 3.3 ml/kgbw (3,200 mg/kgbw) following single oral gavage at 9 dose levels. No clinical signs were noted (Rowe *et al*, 1954).

Dermal

Rabbit: 24-h LD_{50} > 20 ml/kgbw (19,300 mg/kgbw). One death occurred at 15 ml/kgbw (14,500 mg/kgbw). There was an appreciable loss in body weight. Recovery was prolonged at 10 ml/kgbw (9,700 mg/kgbw) and above. Definite narcosis began at all dose levels (10 to 20 ml/kgbw; 9,700 - 19,300 mg/kgbw) within few hours of exposure but was usually not apparent at the end of the 24-hour exposure period (Rowe *et al*, 1954).

Inhalation

Rat: Survived 7-hours exposure to an atmosphere saturated with the vapours of TPGME at 25°C (maximum 13.2 ppm; 113 mg/m^3) without adverse effects (Rowe *et al*, 1954).

4.33.4.2 Irritation and sensitisation

Skin irritation

No specific (acute test) data are available.

Ninety-day continuous skin contact caused only very minor irritation in the form of scaliness (Rowe *et al*, 1954) (Section 4.33.4.3).

Eye irritation

One drop of TPGME, undiluted, was placed in the eyes of rabbits on each of 5 consecutive days. A mild transitory irritation of the conjunctival membranes appeared after each dose. There was no cumulative effect. Fluorescein staining revealed no corneal injury (Rowe *et al*, 1954).

Sensitisation

No data are available.

4.33.4.3 Repeated-dose toxicity (Table 4.33.1)

Subacute toxicity

F344 rats were exposed (whole-body) to TPGME aerosol concentrations of 0, 18, 42 or 118 ppm for 9 days. The aerosol particle size (mass median aerodynamic diameter) ranged from 3.4 to 4.1 μm. There were no exposure-related effects on body weight or the weight of brain, heart, kidneys, thymus and testes, and no changes in haematology, clinical chemistry parameters or urinalysis. An increase in liver weight in both sexes was the only exposure-related effect. There were no treatment-related gross or histopathologic changes in the liver, or any other organ or tissue (Miller *et al*, 1985c).

$B6C3F_1$ mice were exposed (whole-body) to TPGME aerosol concentrations of 0, 18, 42 or 118 ppm for 9 days. The aerosol particle size (mass median aerodynamic diameter) ranged from 3.4 to 4.1 μm. There were no exposure-related effects on body weight or the weight of brain, heart, kidneys, thymus and testes, and no changes in haematology, clinical chemistry parameters or urinalysis. An increase in liver weight in both sexes was the only exposure-related effect; the liver weights of male mice were significantly increased at all three exposure concentrations, whereas there was no effect on the liver weights of female mice of the low (18 ppm) exposure group. There were no treatment-related gross or histopathologic changes in the liver, or any other organ or tissue, except for minimal changes in tinctorial staining properties of hepatocytes in the high (118 ppm) exposure group. This was considered to be indicative of an adaptive rather than a toxic response of the liver (Miller *et al*, 1985c).

Subchronic toxicity

TPGME was applied under occlusive wrap to rabbit skin at dose levels up to 9,700 mg/kgbw/d for 90 days. Control animals received distilled water only. None of the rabbits died at doses up to 4,830 mg/kgbw, whereas 1 out of 8 animals survived at 9,700 mg/kgbw following narcosis, showing increased kidney weights. Low doses of 970 to 4,830 mg/kgbw were without effect on weight gain, except for a depression during the last 2 weeks at 4,830 mg/kgbw. Kidney injury was apparent from 970 mg/kgbw. There were no other treatment-related abnormalities upon gross and histological examination. Blood parameters were also normal, even at the highest dose tested. The skin was subject to occasional scaling and erythema, without significant difference between the treated and control animals (Rowe *et al*, 1954).

4.33.4.4 Genotoxicity (Table 4.33.2)

In vitro

TPGME was not mutagenic when tested in *Salmonella typhimurium* strains (Ames test), using a pre-incubation assay in the absence and presence of a metabolic activation system, up to 100,000 µg/plate (Mendrala and Schumann, 1982a).

TPGME did not induce unscheduled DNA synthesis in cultured primary rat hepatocytes at concentrations from 0.02 to 20 mg/ml (half-log intervals) (Mendrala and Schumann, 1982b).

4.33.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.33.4.6 Reproductive and developmental toxicity (Table 4.33.3)

In a range-finding study in female SD rats exposed to TPGME aerosol up to 8,200 mg/m^3 from day 6 to 15 of gestation. No effects were seen in terms of maternal toxicity and embryotoxicity (Breckenridge *et al*, 1985a).

Mated female SD rats were exposed (whole-body) to an TPGME aerosol at concentrations of 0, 100, 300 or 1,000 mg/m^3 from day 6 to 15 of gestation. No deaths occurred during the study. There was an increased incidence of red staining of the muzzle at 1,000 mg/m^3, indicating maternal toxicity. Body weight and weights of selected organs of the TPGME-treated rats were similar to those of the controls. Gross pathology of the TPGME-treated rats was normal, while uterine parameters (number of corpora lutea, live foetuses, dead foetuses, resorptions, implantations, pre- and post-implantation losses, foetal weight and sex ratio) were not affected by treatment. In all groups the pregnancy rate was at least 80%. The overall incidences of foetal findings, major malformations and minor visceral and skeletal anomalies in the treated groups were not significantly different from control values. Significantly decreased incidences of thoracic vertebral skeletal variants at the 100 and 1,000 mg/m^3 levels were considered to be unrelated to treatment. In conclusion, dose levels of up to 1,000 mg TPGME/m^3 did not cause embryo-lethality, foetotoxicity or teratogenicity (Breckenridge *et al*, 1985b).

4.33.4.7 Kinetics and metabolism (Table 4.33.4)

Male F344 rats were given a single oral dose of 206 or 825 mg of ^{14}C-radiolabeled TPGME/kgbw. After dosing, expired air, excreta and tissues were analysed for ^{14}C activity and metabolites in urine were isolated and identified. Approximately 69% (206 mg/kgbw) or 75% (825 mg/kgbw) of the ^{14}C was excreted in urine as tripropylene glycol (TPG) while only 16% (both doses) was eliminated as $^{14}CO_2$ within 48 hours after dosing. Faeces contained 5% (both doses) of the administered radioactivity. At the end of the 48-hour monitoring period, greater than 95% of the radioactive dose was recovered. TPGME and propylene glycol as well as an oxidation product of dipropylene glycol [2-(1-hydroxy-2-propoxy) propanoic acid], dipropylene glycol monomethyl ether (DPGME), TPGME and the sulphate conjugate of TPGME were identified in urine; less than 5% of the high dose was recovered as unchanged TPGME. These results indicate that TPGME is metabolised extensively and is comparable to DPGME in disposition and types of metabolites (Calhoun *et al*, 1986a; Miller 1987).

4.33.4.8 Neurotoxicity

No data are available.

4.33.4.9 Immunotoxicity

No data are available.

4.33.5 Human effects data

No data are available.

Table 4.33.1: Systemic toxicity of TPGME

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Dermal, occluded	**(ml/kgbw)**	**(mg/kgbw)**			
Rabbit, NS, 5 - 8 M	0	(0	1 x/d, 5 d/wk, 90 d (12.8 wk)	No effects	Rowe *et al*, 1954
	1	970		Kidney injury (tubular necrosis)	
	3	2, 900		Kidney injury (slight granular degeneration, hydropic changes, tubular necrosis)	
	5	4,830		Kidney injury (slight granular degeneration, hydropic changes)	
	10	9,650)		Kidney injury; narcosis and death of 7/8 animals	
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, F344, 5 M, 5 F	(0 18, 42, 118)	0 150, 360, 1,010	6 h/d, 9 d	No effects ↑ liver weight at all dose levels. No gross or histopathologic changes in the liver. No changes in testes or haematology nor any other systemic effects	Miller *et al*, 1985c
Mouse, $B6C3F_1$, 5 M, 5 F	(0 18, 42, 118)	0 150, 360, 1,010	6 h/d, 9 d	No effects ↑ liver weight at all dose levels. No gross or histopathologic changes in the liver. No changes in testes or haematology nor any other systemic effects	Miller *et al*, 1985c

Table 4.33.2: Genotoxicity of TPGME in vitro

Endpoint / Species	Strain or type	Concentration		Result	Remark	Reference
Gene mutation		**µg/plate**				
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	10 - 100,000		**–ve**	+/– S9, pre-incubation	Mendrala and Schumann, 1982a
Unscheduled DNA synthesis		**(mol/l)**	**(mg/ml)**			
Rat hepatocyte	Primary	0.0001 - 0.1	(0.02 - 20)	**–ve**		Mendrala and Schumann, 1982b

Table 4.33.3: Reproductive and developmental toxicity of TPGME in SD rats

Route / Number and sex/group	Concentration		Exposure regime	Result	Reference
Inhalation, aerosol	**(mg/l)**	**(mg/m^3)**			
7 F	0, 0.3, 0.9 2.7, 8.2	0, 300, 900 2,700, 8,200	6 h/d, g.d. 6 - 15	No effects Maternal toxicity. No embryotoxicity	Breckenridge *et al*, 1985a
25 F	0, 0.1, 0.3 1.0	0, 100, 300 1,000	6 h/d, g.d. 6 - 15	No effects Maternal toxicity. No embryotoxicity; no foetotoxicity; no teratogenicity	Breckenridge *et al*, 1985b

Table 4.33.4: Absorption (uptake), distribution, metabolism and elimination of TPGME following oral gavage in F344 rats

Number and sex/group	Dose		Exposure regime	Result	Reference
	(mmol/kgbw)	(mg/kgbw)			
3 M	1	206	1 x	Within 48 h, 16% exhaled as CO_2, 69% excreted in urine as TPG, DPGME and subsequent degradation products, but no 2-MPA	Calhoun *et al*, 1986a,b; Miller, 1987
3 M	4	825	1 x	Within 48 h, 16% exhaled as CO_2; 75% excreted in urine as TPG, DPGME and degradation products. Only 5% of unchanged TPGME	Calhoun *et al*, 1986a,b; Miller, 1987

4.34 Substance profile: 2PG1EE

4.34.1 Identity

Name:	2-Propylene glycol (mono) 1-ethyl ether
IUPAC name:	1-Ethoxy-2-propanol
CAS registry No.	1569-02-4 (α-isomer)
Molecular formula:	$C_5H_{12}O_2$
Structural formula:	$CH_3–\underset{\displaystyle OH}{\underset{\vert}{C}}H–CH_2–O–C_2H_5$
Molecular weight:	104.2
Other components:	2-Ethoxy-1-propanol (< 10%)

4.34.2 Physico-chemical properties

Melting point:	–90°C
Boiling point:	132°C
Vapour pressure:	10 hPa
Solubility in water:	Completely soluble
Relative density:	$D_4^{20} = 0.897$

4.34.3 Conversion factors

1 ppm = 4.332 mg/m^3
1 mg/m^3 = 0.231 ppm

4.34.4 Toxicological data

4.34.4.1 Acute toxicity

Oral

Rat:	LD_{50} > 5,000 mg/kgbw (males and females combined). Signs of toxicity were indicative of mild CNS depression between one and 6 hours after treatment. These effects were fully reversible (BP, 1981a). Oral doses of 2 ml/kgbw (1,800 mg/kgbw) were without effect (BP, 1983a).

Dermal

Rabbit: LD_{50} 9 ml/kgbw (estimated) (8,100 mg/kgbw) by percutaneous absorption under occlusive dressing. Sign of toxicity following administration of large doses was marked CNS depression. Death occurred within 48 hours. There was no appreciable skin irritation (Dow, 1947 cited by Gingell *et al*, 1994).

Inhalation

Rat: No mortality following 4 hours exposure, whole-body to atmospheres containing 3,337 ppm (14,460 mg/m^3) or nose-only to 2,213 ppm (9,590 mg/m^3). Signs of toxicity were CNS depression at both concentrations, salivation and Lachrymation at the higher concentration. All effects were reversible (BP, 1981a, 1983b).

Rat: Exposure to 10,000 ppm (43,000 mg/m^3) for 4 hours showed signs of marked irritation and CNS depression (Gingell *et al*, 1994).

Mouse: No evidence of effect on respiratory rate, and by implication sensory irritation, in mice exposed nose-only to vapour concentrations of 2,800 to 7,200 mg/m^3 (650 - 1,660 ppm) (BP, 1983c).

4.34.4.2 Irritation and sensitisation

Skin irritation

2PG1EE was mildly irritant to rabbit skin (24-h occluded or 4-h semi-occluded) (BP, 1981b and 1984a).

Eye irritation

2PG1EE was not severely irritant to the rabbit eye (discomfort, conjunctival irritation and corneal reactions only). The effects were fully reversible within 7 days (BP, 1981b and 1984b).

Sensitisation

No specific data are available.

A guinea pig maximisation test on the acetate 2PG1EEA was negative suggesting that the parent glycol would also be unlikely to possess skin sensitising potential (BP, 1986g) (Section 4.35.4.2).

4.34.4.3 Repeated-dose toxicity (Table 4.34.1)

Subacute toxicity

SD rats were given 10 consecutive oral doses of 2 ml 2PG1EE/kgbw (1,800 mg/kgbw). The treatment was well tolerated by all animals although the body weight gain of the males was slightly reduced compared to controls. A slight increase in liver weight was seen in both sexes and minor haematological changes reported in the males only (BP, 1983d).

SD rats were exposed (nose-only), to atmospheres containing 2PG1EE vapour at 0, 1,400 or 8,900 mg/m^3 for 9 days. The test animals grew at a similar rate to controls. Initial exposure resulted in sedation, the effect becoming less marked as the study progressed. The only other effect that could be related to treatment was a small increase in liver weight in both sexes exposed to the highest concentration. The livers were histologically normal (BP, 1983e).

Subchronic toxicity

SD rats were exposed (whole-body) to atmospheres containing 0, 100, 300 or 2,000 ppm 2PG1EE for 13 weeks. Irritation of the eyes and nose were observed at the highest concentration. These effects were minimal and readily reversible. There was evidence of a slight increase in liver weight in the females exposed to the highest concentration. The livers were histologically normal. An increase in urine volume was observed in both male and female rats exposed to the high concentration during week 1 and to the high and intermediate concentrations during week 12. There were no detectable changes in the composition of either the urine or serum, or histological changes indicative of a toxic effect on the kidney. There was a small increase in focal macrophage aggregation in the lungs of the male and female rats exposed to the highest concentration (not considered to be of toxicological significance). There were no effects from this study to indicate any adverse effects on either the testes, haematopoietic tissues or blood (BP, 1986b).

4.34.4.4 Genotoxicity (Table 4.34.2)

In vitro

2PG1EE has been examined for its mutagenic potential in *Salmonella typhimurium* (Ames test), in the presence and in the absence of a metabolic activation system. There was no evidence of mutation induction in any of the strains tested, up to 5,000 µg/plate (BP, 1988a).

Cultured human lymphocytes were treated with 2PG1EE, both in the presence and absence of metabolic activation, at doses up to 5,000 µg/ml. The frequency of chromosome aberrations in cells treated with 2PG1EE was similar to those in the solvent controls and within the range of the historical solvent controls (BP, 1988b).

4.34.4.5 Chronic toxicity and carcinogenicity

No data available.

4.34.4.6 Reproduction and developmental toxicity (Table 4.34.3)

SD rats were exposed (whole-body) to vapour concentrations of 0, 100, 450 or 2,000 ppm 2PG1EE from day 6 to 15 of gestation. Signs of maternal toxicity consisting of reduced body weight gain and food consumption were observed in animals exposed to 2,000 ppm. The body weight gain of the dams exposed to 450 ppm was also slightly reduced compared to controls and signs of possible irritation were present. There was no evidence of maternal toxicity at 100 ppm. At all exposure concentrations, litter size and weight, the number of pre- and post-implantation losses and mean foetal weights were indistinguishable from control values. There was no evidence of any effects on foetal development as assessed by incidences of malformations, anomalies or skeletal variants (BP, 1986c).

NZW rabbits were exposed (whole-body) to vapour concentrations of 0, 100, 350 or 1,200 ppm 2PG1EE from day 6 to 18 of gestation. Slight maternal toxicity was evident among the animals exposed to the highest vapour concentration, manifested as slight reduction in mean food consumption and a retardation in body weight gain between days 6 and 10 of pregnancy. There was no evidence of maternal toxicity in the dams exposed to 100 or 350 ppm and no evidence of any treatment related effects on litter size and weight, pre- and post- implantation losses and mean foetal weights. Although the overall incidences of malformations were slightly higher in the treated animals compared to the controls they were not dose-related and all incidences were within the range of the historical control incidences (BP, 1986d).

4.34.4.7 Kinetics and metabolism

No specific data available.

In common with other glycol ethers, 2PG1EE would be expected to be readily absorbed across the skin and alveolar membrane. By analogy with propylene glycol methyl ether, metabolism to propylene glycol and CO_2 is likely to be the principal pathway of the secondary isomer (Section 4.28).

4.34.4.8 Neurotoxicity

No specific data are available.

Oral (BP, 1983a) and inhalation subacute (BP, 1983e) studies have indicated signs of reversible depression of the CNS by 2PG1EE. A 90-day inhalation study in rats provided no evidence of any effects on CNS and peripheral nervous system as could be detected from daily routine observations and from conventional histopathological examination of the brain and sciatic nerves at exposure concentrations up to 2,000 ppm (BP, 1986b) (Table 4.34.1).

4.34.4.9 Immunotoxicity

No specific data are available.

The results of studies on the effects of repeated exposure have not suggested any effects on lymphoid tissue or other elements of the immune system.

4.34.5 Human effects data

No data available.

Table 4.34.1: Systemic toxicity of 2PG1EE in SD rats

Route / Number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral	**(ml/kgbw)**	**(mg/kgbw)**			
6 M, 6 F	0	0	10 d	No effects	BP, 1983d
	2	1,800		Slight ↑ liver weight, ↓ bw. Minor blood effect (M)	
Inhalation	**(ppm)**	**(mg/m^3)**			
6 M, 6 F	(0	0	6 h/d (nose-only), 9 d	No effects	BP, 1983e
	300	1,300		Sedation	
	2,000)	8,700		Slight ↑ liver weight	
15 M, 15 F	(0, 100	0, 430	6 h/d (whole-body), 5 d/wk, 13 wk	No effects	BP, 1986b
	300	1,300		Slight ↑ in urine volume	
	2,000)	8,700		Irritation of eyes and nose. Slight ↑ in urine volume. F: slight ↑ liver weight , focal macrophage aggregation in lungs	

Table 4.34.2: Genotoxicity of 2PG1EE in vitro

Endpoint / Species	Strain or type / Target	Concentration	Result	Remark	Reference
Gene mutation		**µg/plate**			
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	Up to 5,000	**–ve**	+/– S9	BP, 1988a
Chromosome aberration					
Human lymphocyte		Up to 5,000	**–ve**	+/– S9	BP, 1988b

Table 4.34.3: Reproductive and developmental toxicity of 2PG1EE by inhalation

Route / Species, strain, number and sex/group	Concentration (mg/m^3)	Exposure regime	Result	Reference
Rat, SD, 25 F	0, 100 450 2,000	6 h/d, g.d. 6 - 15	No effects Slight ↓ maternal weight gain ↓ maternal weight gain and food consumption	BP, 1986c
Rabbit, NZW, 22 F	0, 100, 350 1,200	6 h/d, g.d. 6 - 18	No effects ↓ maternal weight gain and food consumption	BP, 1986d

4.35 Substance profile: 2PG1EEA

4.35.1 Identity

Name:	2-Propylene glycol 1-ethyl ether acetate
IUPAC name:	1-Ethoxy-2-acetoxypropanol
CAS registry No.	54839-24-6
Molecular formula:	$C_7H_{14}O_3$
Structural formula:	$CH_3–\underset{\displaystyle O–CO–CH_3}{\underset{\vert}{CH}}–CH_2–O–C_2H_5$
Molecular weight:	146.2
Other components:	2-Ethoxy-1-acetoxypropanol (< 10%)

4.35.2 Physico-chemical properties

Melting point:	–89°C
Boiling point:	158°C
Vapour pressure:	2.03 hPa
Solubility in water:	Soluble
Relative density:	$D_4^{20} = 0.941$

4.35.3 Conversion factors

1 ppm = 6.078 mg/m^3
1 mg/m^3 = 0.165 ppm

4.35.4 Toxicological data

4.35.4.1 Acute toxicity

Oral

Rat:	LD_{50} > 5 ml/kgbw (4,700 mg/kgbw). Signs of toxicity were non-specific including lethargy, salivation and pallor of the extremities. All animals were reported as normal by day 4 (BP, 1985a).

Dermal

No data are available.

Inhalation

Rat: No mortality (5 M and 5 F) following 4-hours exposure to air saturated with 2PG1EEA vapour at 6.99 mg /l (1,150 ppm), the highest achievable droplet-free vapour concentration. The only effects observed were indicative of irritation of the eyes and nose (BP, 1985b).

4.35.4.2 Irritation and sensitisation

Skin irritation

2PG1EEA has a low potential of irritancy to the rabbit skin following OECD guidelines (BP, 1986e).

Eye irritation

2PG1EEA was mildly irritant to the rabbit eye (initial hyperaemia of the conjunctival membranes) recovered by day 2. There were no effects on either the cornea or iris (BP, 1986f).

Sensitisation

There was no evidence of delayed contact hypersensitivity of 2PG1EEA in guinea pigs by the method of Magnusson and Kligman (BP, 1986g).

4.35.4.3 Repeated-dose toxicity (Table 4.35.1)

Subacute toxicity

SD rats were exposed to atmospheres containing 0, 102, 292 or 1,176 ppm 2PG1EEA for 28 days. The concentration of 1,176 ppm was the highest droplet free vapour concentration achievable. The study was carried out following OECD guidelines. The only effect observed was

a reduced response to external stimuli in those animals exposed to 292 ppm and 1,176 ppm. At the end of the study, comprehensive gross post mortem and histopathological examination did not reveal any evidence of local or systemic toxicity (BP, 1986h).

Subchronic toxicity

No specific study has been carried out on 2PG1EEA.

A 90-day inhalation toxicity study in rats of the parent glycol ether 2PG1EE provided evidence of sedation, local irritation of the respiratory tract and slight liver enlargement, in the absence of histological changes, in animals exposed to 2,000 ppm only (BP, 1986b) (Section 4.34.4.3).

4.35.4.4 Genotoxicity (Table 4.35.2)

In vitro

2PG1EEA has been tested for its mutagenic potential in *Salmonella typhimurium* (Ames test), in the presence or absence of metabolic activation. There was no increase in histidine revertants in any of the strains tested at doses up to 5,000 µg/plate (BP, 1985c).

Cultured CHO cells were exposed to 2PG1EEA concentrations up to 2,300 µg/ml with or without metabolic activation. There was no evidence of an increase in the proportion of metaphase figures containing aberrant chromosomes when compared to concurrent controls at any dose level (BP, 1985d).

The parent glycol ether 2PG1EE has also been tested for gene mutation in *Salmonella typhimurium* (Ames test) and for chromosome damage in CHO cells; both tests were negative (BP, 1988a,b) (Section 4.34.4.4).

4.35.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.35.4.6 Reproduction and developmental toxicity

No specific studies have been carried out.

Teratology studies in rats and rabbits carried out on the parent glycol ether 2PG1EE have provided evidence that the material is not selectively toxic to the foetus. In view of the likely ready metabolism of 2PG1EEA to 2PG1EE, the former is not expected to adversely effect the developing embryo (BP, 1986b,c) (Section 4.34.4.6).

4.35.4.7 Kinetics and metabolism

No specific data are available.

4.35.4.8 Neurotoxicity

There have been no studies carried out to specifically examine the neurological effects of 2PG1EEA.

The subacute studies reported above and the 90-day study on 2PG1EE do not suggest any effect of this material on the CNS, other than reversible depression at high concentrations.

4.35.4.9 Immunotoxicity

No tests to specifically examine the effects of 2PG1EEA on the immune system have been carried out. The results of studies on the effects of repeated exposure have not suggested any effects on lymphoid tissue or other elements of the immune system.

4.35.5 Human effects data

No data are available.

Table 4.35.1: Systemic toxicity of 2PG1EEA in SD rats by inhalation

Number and sex/group	Concentration		Exposure regime	Result	Reference
	(ppm)	(mg/m^3)			
5 M, 5 F	0, 102, 292 1,176	(0, 620, 1,770 7,150)	6 h/d, 5 d/wk, 28 d	No effects Sedation	BP, 1986h

Table 4.35.2: Genotoxicity of 2PG1EEA in vitro

Endpoint / Species	Strain or type / Target	Concentration	Result	Remark	Reference
Gene mutation		**µg/plate**			
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	50 - 5,000	**–ve**	+/– S9	BP, 1985c
Chromosome aberration					
CHO cells		230 - 2,300	**–ve**	+/– S9	BP, 1985d

4.36 Substance profile: DPGEE

4.36.1 Identity

Name:	Dipropylene glycol (mono) ethyl ether
IUPAC name:	Propanol, 2 ethoxy-methylethoxy
CAS registry No.	30025-38-8
Molecular formula:	$C_8H_{18}O_3$
Structural formula:	$C_2H_5–(O–\underset{\displaystyle CH_3}{\underset{\vert}{C}}H–CH)_2–OH$
Molecular weight:	145.2
Other components:	1-Ethoxy-2-propanol

4.36.2 Physico-chemical properties

Melting point:	< –50°C
Boiling point:	188°C
Vapour pressure:	56.67 hPa
Solubility in water:	Completely soluble
Relative density:	$D_4^{20} = 0.942$

4.36.3 Conversion factors

1 ppm = 6.036 mg/m^3
1 mg/m^3 = 0.166 ppm

4.36.4 Toxicological data

4.36.4.1 Acute toxicity

Oral

Rat:	LD_{50} 5 ml/kgbw (4.7 g/kgbw) (males and females combined). Clinical signs included lethargy, ataxia and irregular breathing with death occurring in the first 24 hours. Animals that survived completely recovered by the third day. Mortality was observed at doses as low as 2.76 ml/kgbw in females and 5.4 ml/kgbw in males (5.1 g/kgbw) (BP, 1986a).

Dermal

Rat: LD_{50} > 2,000 mg/kgbw (BP, 1990a).

Inhalation

Rat: Rats exposed to saturated vapour concentration (estimated to be 400 ppm or 2,410 mg/m^3) for 7 hours developed irritation of the nares and eyes with transient weight loss. One of the 12 exposed rats died. Complete recovery was observed in surviving rats 24 hours later (Rowe, 1947).

4.36.4.2 Irritation and sensitisation

Skin irritation

DPGEE was slightly irritant to rabbit skin (4 h, semi-occluded) (BP, 1985e).

Eye irritation

Application of DPGEE to the rabbit eye produced conjunctival irritation and diffuse corneal opacity that did not meet the EC criteria of an eye irritant (BP, 1985f).

Sensitisation

DPGEE was not sensitising in guinea pigs by the Magnusson and Kligman method (BP, 1985g).

4.36.4.3 Repeated-dose toxicity (Table 4.36.1)

Subacute toxicity

In a study conducted in accordance with OECD Guidelines, SD rats were given oral doses of DPGEE at 0, 50, 225 or 1,000 mg/kgbw/d for 4 weeks. No significant clinical signs of toxicity were observed during the study. At the end of the study increased absolute and relative liver weights in the absence of histopathological alterations were observed in both sexes receiving the highest dose level of 1,000 mg/kgbw. Minimal to slight hyaline droplet formation was noted in

the proximal tubular cells of the kidneys of all male rats at 1,000 mg/kgbw/d and in 2 of 5 male rats at 225 mg/kgbw (BP, 1990b).

Subchronic toxicity

In a study conducted in accordance with OECD guidelines, DPGEE was administered to SD rats at doses of 0, 50, 225 or 1,000 mg/kgbw/d for 13 weeks. Additional rats were administered either the vehicle or 1,000 mg/kgbw/d DPGEE, followed by a 4-week recovery period. The only sign of reaction to treatment was a transient post-dose salivation in both sexes at 1,000 mg/kgbw and in 2 males at 225 mg/kgbw during the final week of treatment. There was no effect of treatment upon performance in a FOB or motor activity test, nor were there any effects upon haematological parameters or gross pathological examination. There was an increase in the weight of the liver (both sexes) and kidney (males only) at the highest dose level, and this was accompanied by histological changes (minimal centrilobular hepatocyte hypertrophy in males and females, increased degree and incidence of renal cortical eosinophilic inclusions in males) at 225 or 1,000 mg/kgbw in these organs . There was a slight increase in blood cholesterol level and reduction in triglycerides in males and females, but this only reached statistical significance in the highest dose group. However, these effects were reversible during the 4-week recovery period (BP, 2001).

4.36.4.4 Genotoxicity (Table 4.36.2)

In vitro

DPGEE was not mutagenic when tested in histidine-dependent strains of *Salmonella typhimurium* (Ames test), in the presence and absence of metabolic activation, up to 5,000 μg/plate (BP, 1985h).

DPGEE did not induce chromosomal aberrations in cultured human lymphocytes with or without metabolic activation up to 1,000 μg/ml (BP, 1990c). This study is limited because the top concentration showed no evidence of toxicity in the absence of S9, the exposure time was short (3 h), and there was only a single cell-harvest time point. These concerns were addressed in a follow-up study using CHO cells, in which the highest concentration was 5,000 mg/ml. Under the conditions of this study, DPGEE caused no increases in the frequency of aberrations, and was concluded to be not clastogenic to CHO cells (Wright, 1997).

DPGEE was tested in the mouse lymphoma cell TK assay at a range of concentrations up to 5,000 μg/ml. The negative (solvent) control resulted in mutant frequencies within the expected

range for the L5178Y at the TK+/– locus. DPGEE did not cause mutations at any concentration either in the absence or presence of metabolic activation (BP, 1993).

4.36.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.36.4.6 Reproductive and developmental toxicity (Table 4.36.3)

DPGEE was tested for possible effects on reproduction in an OECD 415 one-generation study. The test substance was administered to SD rats by oral gavage at doses of 0, 50, 225 or 1,000 mg/kgbw/d for 10 weeks. The only findings in the parental animals were pre-dose salivation at 1,000 mg/kgbw, and an increase in the incidence and severity (minimal or slight) of basophilic renal tubules in males at 225 and 1,000 mg/kgbw. There was an apparent slight decrease in offspring viability in the high-dose group at parturition, which was related to a larger group mean litter size resulting in increased offspring deaths. The mean litter sizes at parturition were 15.8, 15.4, 15.1, and 16.7 for the control, low-, mid-, and high-dose groups respectively. On postnatal day 1, litter sizes in the treatment groups were similar to control, and remained so throughout lactation. No other effects were noted in the offspring, including landmarks of physical development (pinna unfolding, tooth eruption, eye opening) and reflexological responses. The NOAEL for reproductive toxicity in both parental animals and offspring was 1,000 mg/kgbw/d (Coles and Brooks, 1993).

4.36.4.7 Kinetics and metabolism

No specific data are available; however, as with other glycol ethers, percutaneous absorption is likely.

A single dose of 2,000 mg DPGEE/kgbw applied to the shaved skin of rats was not absorbed in amounts sufficient to produce signs of systemic toxicity. Metabolism is likely to proceed in a manner similar to DPGME with formation of PGEE, dipropylene glycol, propylene glycol and their conjugates (BP, 1990a).

4.36.4.8 Neurotoxicity

Specific studies to examine the neurological effects of DPGEE are not available.

Single oral of approximately 5 ml/kgbw (4.7 mg/kgbw) produced signs consistent with CNS depression in rats (BP, 1986a). Repeated oral doses up to 1,000 mg/kgbw in rats did not induce overt clinical effects on the central or peripheral nervous system (BP, 1990b).

In a 90-day study, DPGEE was administered to groups of 10 male and 10 female SD rats at doses of 50, 225 or 1,000 mg/kgbw/d by oral gavage. A control group received the dose vehicle (water) alone. There was no effect of treatment upon performance in a FOB or motor activity test in either sex (BP, 2001).

4.36.4.9 Immunotoxicity

Specific tests to evaluate the immunological effects of DPGEE are not available.

Results from repeated-dose studies do not suggest any effects on lymphoid tissue or other elements of the immune system (BP, 1990b, 2001).

4.36.5 Human effects data

No data are available.

Table 4.36.1: Systemic Toxicity of DPGEE in SD rats by oral gavage

Number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
5 M, 5 F	0, 50	1 x/d, 7 d/wk, 4 wk	No effects	BP, 1990b
	225		Hyaline droplet accumulation in kidney (M)	
	1,000		↑ liver weight. Hyaline droplet accumulation in kidney (M)	
10 M, 10 F; 5 M, 5 F (recovery)	0, 50	1 x/d, 7 d/wk, 13 wk	No effects	BP, 2001
	225		Transient post-dose salivation in 2 M. Histological changes in liver (M and F, centrilobular hepatocyte hypertrophy) and kidney (M, renal cortical tubular eosinophilic inclusions)	
	1,000		Transient post-dose salivation in M and F. Histological changes in liver as above. ↑ liver weight (M and F) and ↑ kidney weight (M). ↑ blood cholesterol level and reduction in triglycerides in M and F. Effects reversible on 4-wk recovery	

Table 4.36.2: Genotoxicity of DPGEE in vitro

Endpoint / Species	Strain or type / Target	Concentration	Result	Remark	Reference
Gene mutation					
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	Up to 5,000 µg/plate	**–ve**	+/– S9	BP, 1985h
Mouse	Lymphoma cell L5178Y, TK+/–	Up to 5,000 µg/ml	**–ve**	+/– S9	BP, 1993
Chromosome aberration		**(µg/ml)**			
Human	Lymphocyte	50 - 1, 000	**–ve**	+/– S9	BP, 1990c
CHO cells		Up to 5,000	**–ve**	+/– S9	Wright , 1997

Table 4.36.3: Reproductive and developmental toxicity of DPGEE in SD rats

Route / Number and sex/group	Concentration	Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**			
32 M, 32 F	0, 50 225 1,000	Single generation OECD 415	No effects M ↑ basophilic renal tubules M ↑ basophilic renal tubules. ↓ offspring viability. NOAEL reprotoxicity (M, F)	Coles and Brooks, 1993

4.37 Substance profile: PGPE

4.37.1 Identity

Name:	Propylene glycol *n*-propyl ether
IUPAC name:	2-Propanol, 1-propoxy
CAS registry No.	1569-01-3
Molecular formula:	$C_6H_{14}O_2$
Structural formula:	$C_3H_7–O–CH_2–\underset{\displaystyle OH}{\underset{\vert}{C}}H–CH_3$
Molecular weight:	118.2
Other components:	2-Propoxy-1-propanol, propylene glycol, dipropylene glycol, dipropylene glycol monopropyl ether

4.37.2 Physico-chemical properties

Melting point:	< –70°C
Boiling point:	150°C approximately
Vapour pressure:	2.27 hPa
Solubility in water	Complete (in all proportions)
Relative density:	D_4^{20} = 0.8886

4.37.3 Conversion factors

1 ppm = 4.915 mg/m^3
1 mg/m^3 = 0.2035 ppm

4.37.4 Toxicological data

4.37.4.1 Acute toxicity

Oral

Rat:	LD_{50} 4,390 mg/kgbw (males, fasted); 2,520 mg/kgbw (females, fasted) (Union Carbide, 1986).
	LD_{50} 2,890 mg/kgbw (males, fed) (Union Carbide, 1964).

Dermal

Rabbit: LD_{50} 3,820 mg/kgbw (males); 4,380 mg/kgbw (females) (Union Carbide, 1986).

LD_{50} 3,560 mg/kgbw (males, occluded) (Union Carbide, 1964).

Rat: LD_{50} 2,820 mg/kgbw (males, occluded) (Myers *et al*, 1977).

Inhalation

Rat: LC_0 saturated vapour atmosphere (6 h) (Union Carbide, 1986).

LC_0 substantially saturated atmosphere (8 h) (Myers *et al*, 1977).

LC_0 11.7 mg/l (11,700 mg/m^3) (8 h) (Union Carbide, 1964).

4.37.4.2 Irritation and sensitisation

Skin irritation

PGPE was slightly irritant to rabbit skin (24 h, occluded) (Union Carbide, 1986).

Eye irritation

PGPE (0.1 ml) was irritant to the rabbit eye (Union Carbide, 1986).

Sensitisation

No data are available.

4.37.4.3 Repeated-dose toxicity (Table 4.37.1)

Subacute toxicity

F344 rats were exposed (whole-body) to PGPE vapour at concentrations of up to 2,000 ppm over an 11-day period (9 exposures) in 3 studies. In the first study, dose-related increases in relative liver- and kidney weights were seen. Gross pathological lesions were limited to the eye, with corneal opacity visible at 2,000 ppm and accompanying histopathological changes, also at 500 and 1,000 ppm (Union Carbide, 1987a). In the second study, severe eye irritation (including discharge, redness and corneal opacities) was noted consistently during the first week of exposure (500 and 2,000 ppm), with tissue changes (including ulceration/necrosis) visible on microscopic examination. There was incomplete resolution of these lesions following a 4-week recovery period (Union Carbide, 1987b). Finally, SD rats appeared less susceptible with sporadic, equivocal changes noted after exposure to 300 or 600 ppm, whereas a NOAEL of 100 ppm was apparent for F344 rats (Klonne *et al*, 1989a).

Similar evidence of an effect on the eye was reported in rabbits, whereas guinea pigs were resistant (Union Carbide, 1987b). Mechanistic studies (Klonne *et al*, 1989b) suggested that these changes were linked to a high incidence of corneal dystrophy (i.e. presence of diffuse corneal crystals) in the rats used in these investigations. Pre-selection of animals (that is, exclusion of individuals exhibiting this spontaneous lesion prior to first exposure) resulted in a NOAEL of at least 600 ppm (the highest exposure tested) in F344 and SD rats (Klonne *et al*, 1989b).

With the exception of a direct effect of high concentrations of PGPE vapour on the eye, ataxia, prostration and narcosis (LOAEL = 2,000 ppm) and increased liver and kidney weights (LOAEL = 2,000 ppm, no histopathological involvement) were the only other treatment-related findings from these studies.

Subchronic toxicity

No exposure related clinical signs, morbidity or mortality were seen in F344 or SD rats exposed (whole-body) to target concentrations of 0, 30, 100 or 300 ppm PGPE for 14 weeks. The study design included a 3-month recovery period. Observational and functional parameters (ophthalmic observations, body and organ weights, haematology, serum chemistry, urinalysis) revealed no consistent, treatment-related changes. Macroscopic and microscopic examinations revealed no abnormality in any tissue, including the eye, in either the main or recovery phase animals (Dodd *et al*, 1990).

4.37.4.4 Genotoxicity (Table 4.37.2)

In vitro

PGPE was not mutagenic when test in *Salmonella typhimurium* (Ames test) or *Escherichia coli*, both in the presence and absence of metabolic activation, up to 5,000 µg/plate (Lawlor, 1996).

PGPE did not increase the occurrence of chromosomal aberrations in rat lymphocyte assays with independent repeat, in the presence and absence of metabolic activation, up to 5,000 µg/ml (Linscombe *et al*, 1996).

4.37.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.37.4.6 Reproductive and developmental toxicity (Table 4.37.3)

No maternal or foetal toxicity was reported when pregnant female SD rats were exposed (whole-body) to PGPE vapour at target concentrations of 0, 100, 750 or 1,500 ppm on day 6 to 15 of gestation and killed on day 21 of gestation. Periocular encrustation was noted in dams from the high dose group, with one showing microscopic evidence of a corneal ulcer. The incidence of poor ossification of some of the proximal phalanges of the hind limb was increased in high dose litters. However, apart from this indication of slight foetotoxicity, no other treatment-related variations were recorded in the study. Based on these findings it was concluded that PGPE was not selectively toxic to the foetus, with maternal and foetal NOAELs of at least 1,500 ppm (Neeper-Bradley, 1992a).

Clear evidence of maternal toxicity was seen when pregnant NZW rabbits were whole-body exposed to 0, 100, 750 or 1,500 ppm PGPE vapour on day 6 to 18 of gestation and killed on day 29 of gestation. Treatment-related clinical signs were noted in high dose animals, and included hypoactivity, ataxia, prostration and emaciation. There was significant maternal toxicity in high dose animals, with a high degree of mortality (27.3%) and one abortion, together with a reduction in body weight gain and reduced food and water consumption during the exposure period. Ophthalmic observations revealed treatment-related keratitis in animals from the 1,500 ppm group, but no microscopic lesions were present at sacrifice on day 29 of gestation. Pregnancy and foetal parameters were unaffected by treatment. A maternal NOAEL of 750 ppm, and a foetal NOAEL of at least 1,500 ppm, was obtained from this study (Neeper-Bradley, 1992b).

4.37.4.7 Kinetics and metabolism

No data are available.

4.37.4.8 Neurotoxicity

No data are available.

4.37.4.9 Immunotoxicity

No data are available.

4.37.5 Human effects data

No data are available.

Table 4.37.1: Systemic Toxicity of PGPE by inhalation

Species, strain, number and sex/group	Concentration		Exposure regime	Result	Reference
	(ppm)	(mg/m^3)			
Rat, F344, 10 M, 10 F	0, 500, 1,000, 2, 000	(0, 2,460, 4,915, 9,830)	9 x 6 h/d, 5 d/wk, 11 d	No deaths; clinical signs (ataxia, prostration) and ↓ bw gain in high dose group only; no effect on clinical chemistry or haematology; dose-related ↑ in relative liver- and kidney weight; gross pathological lesions of the eye (corneal opacity in high dose group) with histopathological changes (keratitis, superficial ulcers, vascularisation and stromal mineralisation) at all concentrations, most marked at 2,000 ppm	Union Carbide, 1987a
Rat, F344, 6 M, 6 F + 3/sex for interim + 6/sex control and high dose for recovery	0, 100, 500, 2, 000	(0, 490, 2,460, 9,830)	9 x 6 h/d, 5 d/wk, 11 d	No mortality; clinical signs in high dose (ataxia, narcosis, lachrymation); ↓ bw gain in high dose; ↑ absolute and/or relative liver weight, ↑ relative kidney weight ; gross lesions limited to the eye, characterised by conjunctivitis, keratitis, minor-moderate corneal ulceration and opacity present predominately in high dose rats from day 4; corneal necrosis, mineralisation and fibrosis present microscopically; residual eye lesions present in recovery group; ultra-structural changes in high dose group (seen with EM)	Union Carbide, 1987b

Table 4.37.1: Systemic Toxicity of PGPE by inhalation (cont'd)

Species, strain, number and sex/group	Concentration		Exposure regime	Result	Reference
	(ppm)	(mg/m^3)			
Rat, F344, 10 M, 10 F + 10/sex for recovery	0, 10, 100, 300, 600	(0, 50, 490, 1,475, 2,950)	9 x 6 h/d, 5 d/wk, 11 d	No deaths; no clinical or adverse effect on food/water intake, bw/bw gain; erythrocyte fragility, haematology, serum chemistry and urinalysis data comparable to controls; no biologically significant macroscopic lesions at necropsy; reversible ↑ in liver weight in high dose animals; histopathological changes limited to the eye, with mild dose-related ↑ in incidence and severity of fibroblastic proliferation, suppurative keratitis and corneal degeneration	Klonne *et al*, 1989a
Rat, SD, 10 M, 10 F + 10/sex for recovery	0, 10, 100, 300, 600	(0, 50, 490, 1,475, 2,950)	9 x 6 h/d, 5 d/wk, 11 d	As above, but less marked, equivocal histopathological changes	Klonne *et al*, 1989a
Rat, F344, 10 M control 15 M treated + 5 control, 10 or 15 M for recovery	0, 5, 50, 100	(0, 25, 246, 490)	9 x 6 h/d, 5 d/wk, 11 d	No deaths; no clinical signs or adverse effect on bw/bw gain or clinical chemistry parameters; no change in gross or microscopic lesions in any tissue (including eyes) at end of main or recovery phases of study	Klonne *et al*, 1989b
Rat, SD, 10 M control, 15 M treated + 5 control, 10 or 15 M for recovery	0, 5, 50, 100	(0, 25, 246, 490)	9 x 6 h/d, 5 d/wk, 11 d	As above	Klonne *et al*, 1989b

Table 4.37.1: Systemic Toxicity of PGPE by inhalation (cont'd)

Species, strain, number and sex/group	Concentration		Exposure regime	Result	Reference
	(ppm)	(mg/m^3)			
Rat, F344, 10 M, 10 F + 10/sex for recovery	0, 30, 100, 300	(0, 150, 490, 1,475)	6 h/d, 5 d/wk for 14 wk	No treatment-related deaths; no clinical signs or effect on food or water intake; bw gain consistently lower in 300 ppm F344 F during main study and wk 1-3 of recovery phase but M unaffected; no consistent, treatment related effect on urinalysis, serum chemistry or haematology; organ weights and incidence of macroscopic and microscopic lesions (including eyes) comparable to control	Dodd *et al*, 1990
Rat, SD, 10 M, 10 F + 10/sex/ for recovery	0, 30, 100, 300	(0, 150, 490, 1,475)	6 h/d, 5 d/wk for 14 wk	No treatment-related deaths; no clinical signs or effect on food or water intake; bw gain consistently lower in SD rats unaffected; no consistent, treatment-related effect on urinalysis, serum chemistry or haematology; organ weights and incidence of macroscopic and microscopic lesions (including eyes) comparable to control	Dodd *et al*, 1990
Guinea pig, Hartley, 6 M, 6 F	0, 100, 500, 2, 000	(0, 490, 2,460, 9,830)	9 x 6 h/d, 5 d/wk, 11 d	No mortality; no clinical signs of effects	Union Carbide, 1987b
Rabbit, NZW, 6 M, 6 F	0, 100, 500, 2, 000	(0, 490, 2,460, 9,830)	9 x 6 h/d, 5 d/wk, 11 d	Three high dose rabbits died after 4 or 5 exposures; clinical signs in high dose (ataxia, narcosis, lachrymation); ↓ bw gain in high dose; ↑ relative kidney weight; gross lesions limited to the eye, characterised by conjunctivitis, keratitis, minor-moderate corneal ulceration and opacity present predominantly in high dose from day 4; corneal necrosis, mineralisation and fibrosis present microscopically; residual eye lesions present in recovery group	Union Carbide, 1987b

Table 4.37.2: Genotoxicity of PGPE in vitro

Endpoint / Species	Strain or type / Target	Concentration	Result	Remark	Reference
Gene mutation		**(µg/plate)**			
Salmonella typhimurium	TA98, A100, TA1535, TA1537	100 - 5,000	**–ve**	+/– S9, liquid pre-incubation	Lawlor, 1996
Escherichia coli	WP2uvrA+	100 - 5,000	**–ve**	+/– S9, liquid pre-incubation	Lawlor, 1996
Chromosome aberration		**(µg/ml)**			
Rat lymphocyte		167 - 1,667 500 - 5,000	**–ve**	– S9, 24 h + S9, 24 h	Linscombe *et al*, 1996
Rat lymphocyte		167 - 3,000 500 - 5,000	**–ve**	– S9, 24 h and 48 h + S9, 24 h and 48 h	Linscombe *et al*, 1996

Table 4.37.3: Reproductive and developmental toxicity of PGPE by inhalation

Route / Species, strain, number and sex/group	Concentration (ppm)	Concentration (mg/m^3)	Exposure regime	Result	Reference
Rat, SD, 25 F	0, 100, 750, 1,500	(0, 490, 3,690, 7,370	6 h/d, g.d. 6 - 15	Negligible maternal toxicity (periocular encrustation and single corneal ulcer) in high dose dams, no effect on gestation parameters, or incidence of external, visceral or skeletal malformations; poor ossification of some of the proximal phalanges (variation) indicative of slight foetotoxicity in high dose litters; maternal and foetal NOAEL 1,500 ppm	Neeper-Bradley, 1992a
Rabbit, NZW, 22 F	0, 100, 750, 1,500	0, 490, 3,690, 7,370	6 h/d, g.d. 6 - 18	Maternal toxicity (mortality, abortion, ↓ bw gain and food and water intake) during exposure; no effect on gestation parameters, or incidence of external, visceral or skeletal malformations or variations; maternal NOAEL = 750 ppm, foetal NOAEL 1,500 ppm	Neeper-Bradley, 1992b

4.38 Substance profile: DPGPE

4.38.1 Identity

Name:	Dipropylene glycol (mono) propyl ether
IUPAC name:	1(1-Methoxy-2-propoxyethoxy)-2-propanol
CAS registry No.	29911-27-1
Molecular formula:	$C_9H_{20}0_3$
Structural formula:	$C_3H_7–(O–CH_2–\underset{\displaystyle CH_3}{\underset{\vert}{C}}H)_2–OH$
Molecular weight:	176.3
Other components:	Several isomers

4.38.2 Physico-chemical properties

Melting point:	< –75°C
Boiling point:	212°C
Vapour pressure:	0.11 hPa
Solubility in water:	190 mg/l

4.38.3 Conversion factors

1 ppm = 7.329 mg/m^3
1 mg/m^3 = 0.136 ppm

4.38.4 Toxicological data

4.38.4.1 Acute toxicity

Oral

Rat:	LD_{50} 2,032 mg DPGPE/kgbw for males and 1,500 mg/kgbw for females (Gilbert and Stebbins, 1995). LD_{50} (male and female) > 2,000 mg/kgbw (Pels Rijken,1995).

Dermal

Rabbit — 24-hour LD_{50} > 2,000 mg DPGPE/kgbw for males and females. Skin erythema (resolved by day 9) and oedema (until day 3) were seen. One rabbit had erythema for 14 days, and developed scales on day 8 (Gilbert, 1995a).

Inhalation

No data are available.

The vapour pressure of DPGPE is low. Given the low toxicity by the oral and dermal routes, it is anticipated that the acute inhalation toxicity would also be low.

4.38.4.2 Irritation and sensitisation

Skin irritation

DPGPE (0.5 ml) was irritant following occluded application to the intact rabbit skin for 4 hours. Slight erythema was still present in one of 6 rabbits 72 hours after test material removal, but was totally resolved by day 7 (Gilbert, 1995b).

Eye irritation

DPGPE (0.1 ml) was irritant to the rabbit eye. All ocular effects were resolved by day 7 post dosing (Gilbert, 1995c).

Sensitisation

No data are available.

4.38.4.3 Repeated-dose toxicity (Table 4.38.1)

Subacute toxicity

F344 rats were administered DPGPE in drinking water at dose levels of 0, 100, 300 or 1,000 mg/kgbw/d for 2 weeks. Parameters evaluated were clinical appearance, ophthalmologic examination, body weight, feed and water consumption, clinical chemistry, prothrombin time, haematology, urinalysis, selected organ weights, gross and histopathologic examination. Body weight gain of males and females dosed at 1,000 mg/kgbw was suppressed by 18% and 47%, respectively, corresponding to significant decreases in feed and water consumption (drinking water might have been unpalatable). A significant increase in mean urine specific gravity seen in males and females of the high-dose group was associated with reduced water consumption. There were significant increases in mean relative liver weights of the males at 300 and 1,000 mg/kgbw, and in mean absolute liver weight of the males at 1,000 mg/kgbw. There were no treatment-related gross or histopathologic alterations in males or females of the high dose group. The dose of 300 mg/kgbw was considered to be a NOAEL for males, because there were no toxic alterations associated with the elevated mean relative liver weight (Stebbins and Baker, 1999).

Subchronic toxicity

F344 rats were administered DPGPE in drinking water at doses of 0, 50, 100 or 500 mg/kgbw/d for 13 weeks. Treatment-related effects seen at 500 mg/kgbw consisted of an increase in absolute and relative liver weights of males, decreased water consumption in females, decreased urine volume in males and females, increased urine specific gravity in females, and an increase in blood cholesterol level of males. The differences in liver weight and cholesterol level were likely due to the induction of organelles required for the metabolism of DPGPE and altered lipid metabolism, respectively, and were not considered to be toxicologically significant. The NOAEL for male rats was 150 mg/kgbwd, and for females 500 mg/kgbw (Yano and Baker, 2000).

4.38.4.4 Genotoxicity (Table 4.38.2)

In vitro

DPGPE was not mutagenic when tested in pre-incubation assays using *Salmonella typhimurium* (Ames test) or *Escherichia coli* strains, in the presence and absence of an externally supplied metabolic activation system, at concentrations from 100 to 5,000 μg/plate. The results were confirmed in an independent repeat assay (Lawlor and Linscombe, 1997).

DPGPE did not cause chromosomal aberrations in rat lymphocytes, in the absence and presence of metabolic activation, up to 1,760 μg/ml. Based on the results of this assay, DPGPE was not clastogenic (Linscombe *et al*, 2002).

4.38.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.38.4.6 Reproductive toxicity and teratogenicity

No data are available.

4.38.4.7 Kinetics and metabolism

No data are available.

4.38.4.8 Neurotoxicity

No data are available.

4.38.4.9 Immunotoxicity

No data are available.

4.38.5 Human effects data

No data are available.

Table 4.38.1: Systemic toxicity of DPGPE in F344 rats by oral ingestion (drinking water)

Route / Number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
5 M, 5 F	0, 100 300 1,000	*Ad libitum*, 2 wk	No effects. NOAEL for M and F since ↑ mean relative liver weights without toxic alterations ↓ bw gain M 18%, F 47%. M and F ↓ feed/water consumption, ↑ mean urine specific gravity. M ↑ mean relative and absolute liver weight. M and F no gross or histopathologic alterations	Stebbins and Baker, 1999
10 M, 10 F	0, 50, 150 500	*Ad libitum*, 13 wk	No effects. NOAEL for M F ↓ water consumption , M, F: ↓ urine volume, F: ↑ urine specific gravity (not toxicologically relevant). M ↑ absolute and relative liver weights; M ↑ cholesterol. NOAEL for F	Yano and Baker, 2000

Table 4.38.2: Genotoxicity of DPGPE in vitro

Endpoint / Species	Strain or type / Target	Concentration	Result	Remark	Reference
Gene mutation		**(µg/plate)**			
Salmonella typhimurium	TA98, 100, 1535, 1537	100 - 5,000	**–ve**	+/– S9, pre-incubation	Lawlor and Linscombe, 1997
Escherichia coli	WP2uvrA	100 - 5,000	**–ve**	+/– S9, pre-incubation	Lawlor and Linscombe, 1997
Chromosome aberration		**(µg/ml)**			
Rat lymphocyte		440, 880, 1,760	**–ve**	+/– S9, 48 h	Linscombe *et al*, 2002

4.39 Substance profile: 2PG1PhE

4.39.1 Identity

Name:	2-Propylene glycol 1-phenyl ether
IUPAC name:	1-Phenoxy-2-propanol
CAS registry No.	770-35-4
Molecular formula:	$C_9H_{12}O_2$
Structural formula:	$CH_3–\underset{\displaystyle OH}{\underset{\vert}{C}}H–CH_2–O–C_6H_5$
Molecular weight:	152.2
Other components:	Dipropylene glycol phenyl ether (< 7%)

4.39.2 Physico-chemical properties

Melting point:	13°C approximately
Boiling point:	242.7°C
Vapour pressure:	0.05 hPa at 25°C
Solubility in water:	10 g/l
Relative density:	$D_4^{20} = 1.0622$

4.39.3 Conversion factors

1 ppm = 6.327 mg/m^3
1 mg/m^3 = 0.158 ppm

4.39.4 Toxicological data

4.39.4.1 Acute toxicity

Oral

Rat:	LD_{50} in males: 2,830 mg/kgbw, in females: 3,730 mg/kgbw. The major effect was CNS depression and death occurred within 48 hours (Norris and Olson, 1968).

Dermal

Rabbit: LD_{50} > 2,000 mg/kgbw. No signs of toxicity were observed at this dose level (Norris and Olson, 1968).

Inhalation

Rat: No deaths after 7 hours of exposure to saturated vapour concentrations. Signs of nasal, respiratory and eye irritation were observed (Norris and Olson, 1968).

4.39.4.2 Irritation and sensitisation

Skin irritation

2PG1PhE was slightly irritant to rabbit skin after repeated, prolonged contact to undiluted material (Norris and Olson, 1968; Philipps *et al*, 1985).

Eye irritation

2PG1PhE caused slight conjunctival irritation and slight, transient corneal injury in rabbit eyes (undiluted material). Recovery within a few days to one week (Norris and Olson, 1968).

Sensitisation

No data are available.

4.39.4.3 Repeated-dose toxicity (Table 4.39.1)

Subacute toxicity

NZW rabbits received 14 daily dermal applications of 1,000 mg 2PG1PhE/kgbw. No signs of systemic toxicity were observed (Phillipps *et al*, 1985).

Subchronic toxicity

In a 28-day study, NZW rabbits received daily dermal applications of 0, 100, 300 or 1,000 mg/kgbw/d. Adverse in-life effects were limited to a mild and transient irritation of the application site. Clinical chemistry, necropsy observations, organ weights and histopathology were without evidence of adverse effects (Calhoun *et al*, 1986c).

4.39.4.4 Genotoxicity (Table 4.39.2)

In vitro

2PG1PhE did not cause histidine reverse mutations when tested in *Salmonella typhimurium* (Ames test) at concentrations of up to 5,000 µg/plate, in the presence and absence of a metabolic activation system (Bootman and May, 1985). 2PG1PhE induced no increases in chromosome aberrations in peripheral human lymphocytes at concentrations up to 400 µg/ml, both in the presence and absence of metabolic activation (Bootman, 1986).

In vivo

CD-1 mice were administered 2PG1PhE on 2 consecutive days at dose levels of 0, 500, 1,000 and 2,000 mg/kgbw/d. A statistically significant increase in the frequency of micronucleated PCE occurred in the high dose group of 2,000 mg/kgbw, a dose that also induced a remarkable decrease (approximately 10°C) in body temperature, indicating excessive toxicity. Thus, the increase of micronucleated PCE was interpreted to be the result of treatment-induced hypothermia and not due to a direct action of 2PG1PhE and/or its metabolites on cellular targets responsible for micronucleus induction (Day, 2000).

4.39.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.39.4.6 Reproductive and developmental toxicity

No data are available.

4.39.4.7 Kinetics and metabolism

Absorption, metabolism and excretion of 2PG1PhE were investigated in F 344 rats following oral administration of a single oral dose of 10 or 100 mg/kgbw ^{14}C-2PG1PhE. Urine was collected at 12, 24 and 48 hours and faeces at 24 and 48 hours post-dosing, and the radioactivity was determined. The administered doses were rapidly absorbed from the gastro-intestinal tract and excreted, mainly via the urine (93% of the low and 96% of the high dose) and mostly within 12 hours after dosing (85% of the low and 90% of the high dose). Total faecal excretion remained < 10%. Rats eliminated the entire administered dose within 48 hours after dosing. Metabolites tentatively identified in urine were conjugates of phenol (sulphate, glutathione) with low levels (< 2%) of hydroquinone (glucuronide), conjugates of 2PG1PhE (glucuronide, sulphate) and a ring-hydroxylated metabolite of 2PG1PhE. There was no free 2PG1PhE or phenol in non-acid-hydrolysed urine. In acid-hydrolysed urine, 61% of the dose was identified as phenol and 13% as 2PG1PhE. Although 2PG1PhE was stable to acid hydrolysis, some of the phenol in acid hydrolysed urine may have arisen from degradation of acid-labile metabolite(s) as well as hydrolysis of phenol conjugates (Saghir *et al*, 2003).

4.39.4.8 Neurotoxicity

No data are available.

4.39.4.9 Immunotoxicity

No data are available.

4.39.5 Human effects data

2PG1PhE has bactericidal properties and it has been used as a preservative in medical disinfectants and in cleansing and cosmetic formulations. 2PG1PhE has been mentioned as an antibacterial agent in pharmaceutical preparations for acne treatment. No other data are available (Roberts, 1986).

Table 4.39.1: Systemic dermal toxicity of 2PG1PhE in NZW rabbits

Route / Number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
10 F	0,1,000	7 d/wk. 2 wk	No effects	Phillips *et al*, 1985
5 M, 5 F	0, 100, 300, 1,000	5 d/wk. 4 wk	No effects	Calhoun *et al*, 1986c

Table 4.39.2: Genotoxicity of 2PG1PhE

Endpoint / Species	Strain or type / Target	Concentration	Result	Remark	Reference
In vitro					
Gene mutation		**(µg/plate)**			
Salmonella typhimurium	TA98, TA100, TA1535, TA1537	50 - 5,000	**–ve**	+/– S9	Bootman and May, 1985
Chromosome aberration		**(µg/ml)**			
Human lymphocyte	Peripheral	100 - 400	**–ve**	+/– S9	Bootman, 1986
In vivo					
Micronucleus frequency		**(mg/kgbw)**			
Mouse	CD-1 Bone marrow	2 x oral gavage 0, 500, 1,000	**–ve**	Single harvest 24 h after second dose	Day, 2000
		2,000	**+ve**	Attributed to treatment-induced hypothermia	

Table 4.39.3: Absorption (uptake), distribution, metabolism and elimination of 2PG1PhE in F344 rats

Route / Number and sex/group	Dose	Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**			
3 M	10, 100	1 x	Major route of excretion via urine (93 and 96%, respectively). Most urinary excreted radioactivity within 12 h after dosing (85 and 90%, respectively). Entire dose eliminated within 48 h. Tentative metabolites: conjugates of phenol, with low (< 2%) of hydroquinone and 2PG1PhE conjugates, and ring-hydroxylated metabolite of 2PG1PhE	Saghir *et al*, 2003

4.40 Substance profile: 2PG1BE

4.40.1 Identity

Name:	2-Propylene glycol 1-*n*-butyl ether
IUPAC name:	1-Butoxy-2-propanol
CAS registry No.	5131-66-8
	29387-86-8 (mixture of isomers)
Molecular formula:	$C_7H_{16}O_2$
Structural formula:	$C_4H_9–O–CH_2–\underset{\displaystyle CH_3}{\underset{\vert}{CH}}–OH$
Molecular weight:	132.2
Other components:	2-Butoxy-1-propanol (< 5%)

4.40.2 Physico-chemical properties

Melting point:	< –75°C
Boiling point:	170.2°C
Vapour pressure:	1.13 hPa
Solubility in water:	60 g/l
Relative density:	D_4^{20} = 0.882

4.40.3 Conversion factors

1 ppm = 5.496 mg/m^3
1 mg/m^3 = 0.182 ppm

4.40.4 Toxicological data

4.40.4.1 Acute toxicity

Oral

Rat:	LD_{50} 3,300 mg/kgbw. Signs of toxicity were lethargy, CNS depression, coma, hypopnoea and dacryorrhea (Reijnders *et al*, 1987).

Dermal

Rat: LD_{50} > 2,000 mg/kgbw. There were no signs of systemic toxicity and no abnormalities of the treated skin surface (Reijnders and Verschuuren, 1987).

Inhalation

Rat: No signs of toxicity during 4-hour exposure to saturated vapour atmosphere (Corley *et al*, 1989a).

4.40.4.2 Irritation and sensitisation

Skin irritation

2PG1BE was moderately irritant to rabbit skin when applied for 4-hours in undiluted form or as a 75% dilution in water; a 50% dilution in water was slightly irritant. 2PG1BE was not irritant following 4-h application of a 25% dilution in water (Weterings *et al*, 1987a,b).

Eye irritation

Undiluted 2PG1BE was moderately irritant to rabbit eyes. Transient corneal opacity was observed (Weterings *et al*, 1987c).

Sensitisation

2PG1BE (40% in propylene glycol) was not sensitising in guinea pigs in the modified 3-induction epicutaneous Buehler test (Vankerkom and Verschuuren, 1987).

4.40.4.3 Repeated-dose toxicity (Table 4.40.1)

Subacute toxicity

Daily oral dosing of 2PG1BE to SD rats at levels of 0, 100, 200 and 400 mg/kgbw/d for 14 consecutive days caused neither haematological nor other adverse effects (Debets and Verschuuren, 1987).

F344 rats exposed (nose-only) to the maximum attainable vapour concentration of 700 ppm 2PG1BE for 2 weeks did not show any haematological or other adverse effects. An increase in liver weight seen at 700 ppm was without histopathologic lesions or changes in clinical chemistry parameters (Corley *et al*, 1989b).

Male and female rats were exposed (whole-body) to 600 ppm 2PG1BE for 31 days. The only effect seen was an increase in liver weight of the females (Pozzani and Carpenter, 1965).

F344 and SD rats were exposed (whole-body) to 2PG1BE vapour concentrations of 0, 10, 100, 300 or 600 ppm over an 11-day period (9 exposures). The only exposure-related effects reported in male and female F344 rats were an increase in liver weights without histopathologic liver lesions in the 600 ppm group) and a low incidence of mild eye lesion in the 300 and 600 ppm groups. There were no effects seen in SD rats (Klonne *et al*, 1989c).

Subchronic toxicity

Oral

2PG1BE was administered to F344 rats in the drinking water to yield dose levels of 0, 100, 350 and 1,000 mg/kgbw/d for 13 weeks. At 1,000 mg/kgbw, the drinking water became unpalatable resulting in reduced water and food consumption and decreased body weight, and secondary alterations in clinical chemistry, electrolytes, haematology and urinalysis. At 1,000 mg/kgbw, absolute and relative liver weights were higher in male rats and absolute and relative kidney weights were higher in female rats. No gross or histopathologic alterations were noted that could be associated with 2PG1BE administration. Therefore, the organ weight changes were not considered as adverse effects. No effects attributable to test material 2PG1BE were evident in male and female rats administered 100 or 350 mg/kgbw/d. The NOAEL was 1,000 mg/kgbw (Grandjean *et al*, 1992).

Dermal

2PG1BE was applied to the skin of Wistar rats using various dilutions in propylene glycol to provide dose levels of 0, 88, 264, 880 mg/kgbw/d for 13 weeks. Skin reactions at the application site, consisting of erythema, oedema, scaliness, small wounds, incrustations and/or occasionally superficial scar tissue were observed in all groups, including the control group. The findings were generally more pronounced in the treatment groups. Otherwise, no clinical signs were observed that were related to the test substance. No compound-related changes were observed in the ophthalmologic examination, in body weights, food intake, clinical pathology or upon gross or

microscopic examination. The microscopic findings for skin confirmed the clinical observations. It was concluded that 880 mg 2PG1BE/kgbw was the systemic NOAEL (Jonker *et al*, 1988).

2PG1BE was applied (non-occlusively) to the intact skin of NZW rabbits for 13 weeks with decreasing dilutions equivalent to doses of 0, 11.4, 114 or 1,140 mg 2PG1BE/kgbw/d. During exposure, animals were restrained to prevent oral ingestion. Evaluation of signs, body weight changes, organ weights, haematology, and gross and microscopic pathology data did not reveal any 2PG1BE-related effects other than mild to moderate irritation at the site of application. The test site skin revealed erythema (114 and 1,140 mg/kgbw), oedema, atonia, desquamation and fissuring (1,140 mg/kgbw). Microscopic evaluation of treated skin (1,140 mg/kgbw) exhibited acanthosis and hyperkeratosis. The results demonstrate that subchronic percutaneous exposure to 2PG1BE does not produce systemic toxicity (Innis *et al*, 1990).

4.40.4.4 Genotoxicity and cell transformation (Table 4.40.2)

In vitro

2PG1BE was not mutagenic when tested in *Salmonella typhimurium* strains (Ames test) using a pre-incubation modification of the standard method, in the presence or absence of a metabolic activation system. The test material was tested twice in each strain up to a maximum concentration of 5,000 μg/plate (Bruce *et al*, 1987).

2PG1BE did not cause chromosomal aberrations in CHO cells, in the presence or absence of metabolic activation, up to 5,000 μg 2PG1BE/ml (Gollapudi *et al*, 1988a).

In a series of tests (comparing several glycol ethers, their acetic acid and aldehyde metabolites) including the endpoints SCE, chromosomal aberration, micronucleus induction, and aneuploidy (all in V79 cells) 2PG1BE was negative (Elias *et al*, 1996). Details, such as on the concentrations employed and on the methodology, are lacking.

In addition, 2PG1BE was negative in a test on inhibition of metabolic cooperation between V79 cells and a cell transformation assay with SHE cells (Elias *et al*, 1996).

4.40.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.40.4.6 Reproductive and developmental toxicity (Table 4.40.3)

Pregnant Wistar rats were treated dermally with 2PG1BE at dose levels of 0, 264 or 880 mg/kgbw on day 6 to 16 of gestation. Minor skin reactions were noted in the maternal animals; no compound-related embryo or foetal toxicity or teratogenicity was seen at any dose level (Waalkens-Berendsen *et al*, 1989).

Pregnant NZW rabbits were exposed dermally to 2PG1BE at dose levels of 0, 10, 40 or 100 mg/kgbw/d on day 7 to 18 of gestation. No significant maternal toxicity was noted in the dams, except for erythema at the site of application of the test material. There were no malformations or variations in any of the foetuses from any of the litters that could be attributed to treatment with 2PG1BE. The NOAEL for this study was 100 mg/kgbw/d for rabbits by dermal exposure (Gibson *et al*, 1989).

4.40.4.7 Kinetics and metabolism

Skin permeation was calculated using the Franz cell method with human skin. 2PG1BE was tested in pure form and with 70 % acetone. In pure form the lag time was 37 minutes, flux at steady state was 0.017 mg/cm^2/h, and permeation 0.019 x 10^{-3} cm/h. In mixture with acetone the respective values were 44 minutes, 0.026 mg/cm^2/h and 0.027 x 10^{-3} cm/h (Larese *et al*, 1999).

Male F344 rats were administered 2PG1BE dissolved in 0.5% Methocel by gavage at dose levels of 0 (vehicle controls) or 1,000 mg/kgbw/d for 7 consecutive days, to investigate the potential association of 2PG1BE-induced increases in rat liver weights with the induction of specific CYP-dependent MFOs. Liver weight and the activity of several MFOs (CYP1A1, CYP1A2, CYP2B1/2 and CYP2E1) and the Phase II enzymes, UDP-glucuronosyl transferase (UGT) and sulphotransferase (ST), were measured. The relative liver weights of rats administered PGBE were significantly elevated by approximately 16%. A statistically significant 3.1 to 3.5-fold increase in pentoxyresorufin O-dealkylase (PROD) activity was observed with the test material relative to controls indicating a modest induction of CYP2B1/2 dependent MFO. In addition, *p*-nitrophenol (pNPH) activity was elevated by 55% in 2PG1BE treated rats, suggesting a modest increase in CYP2E1-dependent MFO activity. These changes are consistent with an adaptive response of treated rats to metabolise relatively high dosages of 2PG1BE (Stott and Kan, 2000).

4.40.4.7 Neurotoxicity

No data are available.

4.40.4.8 Immunotoxicity

No data are available.

4.40.5 Human effects data

No data are available.

Table 4.40.1: Systemic toxicity of 2PG1BE

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw/d)**				
Rat, SD, 6 M, 6 F	0, 100, 200, 400		1 x/d, 14 d	No effects	Debets and Verschuuren, 1987
Oral, drinking water					
Rat (F344), 10 m/10 f	0, 100, 350 1,000		*Ad libitum*, 13 wk	No effects. Unpalatability of water, ↓ water and food consumption and bw, secondary alterations in clinical parameters, ↑ liver (M) and kidney (F) weights without histopathologic alterations, therefore NOAEL	Grandjean *et al*, 1992
Dermal	**(ml/kgbw)**	**(mg/kgbw)**			
Rat, Wistar, 10 M, 10 F	0, 0.1, 0.3, 1.0	0, 88, 264, 880	5 x/wk, 13 wk	Only local effects. No systemic effects.	Jonker *et al*, 1988
	% (w/v)	**(mg/kgbw)**			
Rabbit, NZW 5M, 5 F	0, 0.57, 5.7, 57 in 2 ml/kgbw	0, 11.4, 114, 1,140	7 h/d, 5 d/wk, 13 wk	Only local effects. No systemic effects.	Innis *et al*, 1990
Inhalation	**(ppm)**	**(mg/m^3)**			
Rat, F344, 5 M, 5 F	0, 50, 200 700	(0, 275, 1,100 3,800)	6 h/d, 9 x, 2 wk	No effects ↑ Liver weight without histopathologic lesions or changes in clinical chemistry parameters	Corley *et al*, 1989b

Table 4.40.1: Systemic toxicity of 2PG1BE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation (cont'd)	**(ppm)**	**(mg/m^3)**			
Rat, NS, 6 M, 6 F	0 600	(0 3,300)	7 h/d, 5 d/wk, 31 d	No effects No effects in M. ↑ liver weight (F)	Pozzani and Carpenter, 1965
Rat, F344, 10 M, 10 F	0, 10, 100 300 600	(0, 55, 550 1,600 3,300)	9 x 6 h/d, 5 d/wk, 11 d	No effects Low incidence of mild eye lesions ↑ Liver weight without histopathologic lesions	Klonne *et al*, 1989c
Rat, SD, 10 M, 10 F	0, 10, 100, 300, 600	(0, 55, 550, 1,600, 3,300)	9 x 6 h/d, 5 d/wk, 11 d	No effects.	Klonne *et al*, 1989c

Table 4.40.2: Genotoxicity of 2PG1BE in vitro

Endpoint / Species	Strain or type / Target	Concentration	Result	Remark	Reference
Gene mutation		**(µg/plate)**			
Salmonella typhimurium	TA98, TA100, TA1535, TA1537	5 - 5,000	**–ve**	+/– S9, pre-incubation	Bruce *et al*, 1987
Chromosome aberration		**(µg/ml)**			
CHO cells		500, 1,667, 5,000	**–ve**	+/– S9	Gollapudi *et al*, 1988a
Human lymphocyte		NS [a]	**–ve**		Elias *et al*, 1996
V79 cells		NS [a]	**–ve**		Elias *et al*, 1996
Sister chromatid exchange					
V79 cells		NS [a]	**–ve**		Elias *et al*, 1996
Micronucleus induction					
V79 cells		NS [a]	**–ve**		Elias *et al*, 1996
Aneuploidy					
V79 cells		NS [a]	**–ve**		Elias *et al*, 1996

[a] Concentrations partly presented in graphs

Table 4.40.3: Reproductive and developmental toxicity of 2PG1BE

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Dermal					
Rat, Wistar, 25 F	0, 0.3 1.0	(0, 264 880)	g.d. 6 - 16	No effects Local dermal effects. No foetotoxic or teratogenic effects	Waalkens-Berendsen *et al*, 1989
Rabbit, NZW, 20 F		0, 10, 40, 100	g.d. 7 - 18	No effects	Gibson *et al*, 1989

4.41 Substance profile: DPGBE

4.41.1 Identity

Name:	Dipropylene glycol (mono) *n*-butyl ether
IUPAC name:	1-(2-butoxy-1-methylethoxy)-2-propanol
CAS registry No.	29911-28-2 (major isomer)
Molecular formula:	$C_{10}H_{22}O_3$
Structural formula:	$C_4H_9–(O–CH_2–\underset{\displaystyle CH_3}{\underset{\vert}{C}}H)_2–OH$
Molecular weight:	190.3
Other components:	Various isomers

4.41.2 Physico-chemical properties

Melting point:	< –75°C
Boiling point:	229°C
Vapour pressure:	0.08 hPa
Solubility in water:	50 g/l
Relative density:	$D_4^{20} = 0.910$

4.41.3 Conversion factors

1 ppm = 7.911 mg/m^3
1 mg/m^3 = 0.126 ppm

4.41.4 Toxicological data

4.41.4.1 Acute toxicity

Oral

Rat:	LD_{50} 2 to 3 g DPGBE/kgbw (Reijnders *et al*, 1987).
	LD_{50} 4,400 mg/kgbw for male rats, 3,700 mg/kgbw for female rats (Reijnders *et al*, 1987).

LD_{50} 2,830 µl/kgbw (2,575 mg/kgbw) for males, 1,620 µl/kgbw (1,475 mg/kgbw) for females (Reijnders *et al*, 1987).

Dermal

Rat: LD_{50} > 2,000 mg/kgbw (Reijnders and Verschuuren, 1987).

Rabbit: LD_{50} 7,130 µl/kgbw (6,490 mg/kgbw) for males, 5,860 µl/kgbw (5,330 mg/kgbw) for females (Reijnders and Verschuuren, 1987).

DPGBE does not appear to be readily absorbed through the skin.

Inhalation

Rat: Survived exposure for 6 hours to saturated atmosphere; no clinical signs (Myers and Tyler, 1992).

Survived 4-hour inhalation of saturated vapour; no clinical signs (42.1 ppm; 333 mg/m^3) (Gushow *et al*, 1987).

No deaths when exposed (nose-only) to an aerosol of DPGBE (2.04 mg/l; 2,040 mg/m^3); 48% of the particles by weight were ≤ 3 µm in size. There were no gross pathologic, treatment-related changes 2 weeks after exposure (Cieszlak *et al*, 1990).

4.41.4.2 Irritation and sensitisation

Skin irritation

No acute skin irritation data are available.

Single 4-hour application of neat and diluted DPGBE to rabbit skin under semi-occlusive dressing, followed by 14 days of observation, resulted in irritation only (Weterings *et al*, 1987a,b).

Eye Irritation

DPGBE (0.1 ml) instilled into the eyes of 3 female rabbits produced conjunctival irritation not associated with corneal injury. Slight to obvious redness and slight to moderate conjunctival swelling were observed 1 hour after dosing and resolved in 7 days in 2 rabbits and by 14 days in the third rabbit. With the exception of corneal epithelial damage in the first few days, no adverse effects on the cornea or iris were observed (Weterings *et al*, 1987c).

Sensitisation

When DPGBE (0.3 ml [273 mg] dissolved in propylene glycol) was applied under occlusive patch following a modified Buehler procedure, it did not induce contact hypersensitivity in guinea pigs (Vankerkom, 1987).

4.41.4.3 Repeated-dose toxicity (Table 4.41.1)

Subacute toxicity

Gavage

SD rats received daily oral doses of DPGBE at levels of 0, 100, 200 or 400 mg/kgbw for 14 consecutive days. No mortalities occurred, moreover no evident signs of systemic toxicity were observed in any of the treatment groups. In addition, no test substance related effects on body weight gain and food consumption were reported. Organ weights, organ/body weight ratios, clinical chemistry and haematology data (including RBC osmotic fragility), and gross and microscopic pathology revealed no test substance related changes. Based on these findings it was concluded that repeated daily oral dosing of DPGBE for 14 days at a level of 400 mg/kgbw caused neither a haemolytic effect nor any other adverse treatment-related effects (Debets and Verschuuren, 1987).

Diet

SD rats were fed dietary concentrations leading to a test substance uptake of approximately 0, 250, 500 or 750 mg DPGBE/kgbw/d for 14 days. There were no palatability problems. Food consumption, body weights and organ weights were comparable to the control group. No mortality was observed and no other clinical signs were detected. There were no toxicologically significant changes in haematological parameters or macroscopic alterations. No findings were

attributable to the test substance and no target organs were identified. In this study 750 mg/kgbw was the NOAEL (Thévenaz *et al*, 1988a).

Inhalation

F344 rats were exposed (9 x, nose-only) to concentrations of 0, 20 or 40 ppm DPGBE vapour. The highest exposure concentration was the maximum that could be practically attained. Male rats exposed to 20 or 40 ppm of DPGBE had mild haemo-concentration, probably due to reduced water intake; this was not judged to be a primary effect of DPGBE. Moreover, the RBC parameters were within normal ranges for historical control groups of male rats. Female rats exposed to 20 or 40 ppm had slightly increased mean relative liver weights; the increases did not follow a clear dose response relationship and were not associated with histopathologic changes in the liver or alterations in serum chemistry values. Therefore, they were not considered to be toxicologically significant. In all, repeated exposure to vapours of DPGBE at 20 or 40 ppm caused no significant adverse effects (Lomax *et al*, 1987).

DPGBE caused nasal irritation and liver enlargement in a 2-week aerosol inhalation study where F344 rats were exposed (nose-only) to aerosol concentrations of 0, 200, 810, 2,010 mg/m^3. At the highest exposure level, rats exhibited decreased activity, decreased body weight, increased liver cell size, and irritation of the membranes of the nose. The mid-concentration showed similar but less intense effects and 200 mg/m^3 was the NOEL (Cieszlak *et al*, 1991b).

Subchronic toxicity

Oral

DPGBE caused no testicular, blood or thymic injury in SD rats given oral doses of 0, 200, 450 or 1,000 mg/kgbw/d in the diet for 13 weeks. At the high dose, rats incurred slightly decreased body weights, minor changes in clinical chemistry, and slightly increased liver weights but there were no other treatment-related effects. In the mid-dose group, minimal increases in urinary magnesium and plasma urea concentrations, not considered toxic effects, were observed. The NOAEL was 450 mg/kgbw (Thévenaz *et al*, 1988b).

Dermal

Dermal treatment of Wistar rats dosed 0, 91, 273 or 910 DPGBE mg/kgbw/d for 13 weeks did not cause testicular, blood, or thymic damage. Body weights were decreased in the two highest dose groups and, in the high dose group only, rats had slightly increased liver weights and slight

alterations in clinical chemistry readings. No histopathology was detected in any of the treatment groups. The NOAEL was 91 mg/kgbw/d (Lina *et al*, 1988).

4.41.4.4 Genotoxicity (Table 4.41.2)

In vitro

In all *in vitr*o assays, DPGBE was investigated in the presence and absence of S9 metabolic activation systems.

DPGBE was not mutagenic in the Ames test using *Salmonella typhimurium* at concentrations up to 5,000 μg/plate (Van de Waart and Enninga, 1987). DPGBE was also without activity in the CHO/HGPRT forward gene mutation assay at dose levels ranging from 279 to 5,000 μg/ml (Linscombe *et al*, 1995).

DPGBE produced chromosomal aberrations in cultured CHO-K1 S1B cells treated with concentrations up to 5,000 μg/ml in three separate studies under varying conditions in one laboratory (Enninga, 1987; Waalkens and Enninga, 1987; Enninga and Van de Waart, 1989 as cited by Enninga and Verschuuren, 1990).

In another study in a separate laboratory, DPGBE was negative using cultured CHO-K1 CCL61 cells at concentrations up to 5,000 μg/ml. A follow-up study by the second laboratory at the same concentrations using the CHO-K1 S1B cell line confirmed the negative results in the CHO chromosome aberration test (Gollapudi *et al*, 1988b; Linscombe and Verschuuren, 1991).

A number of possibilities were put forward to explain the discrepancy in results between the two laboratories. These included differences in the culture conditions, the possible presence of peroxides, differential sensitivity of the two cell lines and the potential solvent effects of DMSO (one laboratory used DMSO to dissolve the material and another did not). Through the series of chromosomal aberration tests all of the above factors were ruled out as potential contributors to the positive results observed by the initial testing laboratory.

In vivo

A bone marrow micronucleus test was conducted in CD-1 mice given single doses of up to 2,500 mg DPGBE/kgbw by oral gavage. No treatment-related response (increased frequency of micronuclei of the bone marrow) was reported (McClintock *et al*, 1988).

The results of the *in vitro* assays were equivocal and propylene glycol ethers are rarely genotoxic. No treatment-related response was seen in the *in vivo* micronucleus assay. It may therefore be concluded that DPGBE does not present a genotoxicity hazard.

4.41.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.41.4.6 Reproductive and developmental toxicity (Table 4.41.3)

Dermal

DPGBE was not embryotoxic, foetotoxic or teratogenic when applied under occlusion to the skin of pregnant rats at doses of 273 or 910 mg/kgbw on day 6 to 16 of gestation. Minor skin irritation was seen at both dose levels in the maternal animals (Wilmer and Van Marwijk, 1988).

4.41.4.7 Kinetics and metabolism (Table 4.41.4)

The disposition and metabolic fate of ^{14}C-DPGBE was determined following a single oral administration of 76 or 837 mg DPGBE/kgbw in male F344 rats. Urine, faeces and expired air were collected for 48 hours, tissues were collected 48 hours after dosing and blood samples were drawn at various times over 48 hours. All samples were analysed for total ^{14}C-activity. Urinary metabolites of DPGBE were identified structurally to determine if the metabolic fate of DPGBE was comparable to that of 2PG1ME (Section 4.28) and DPGME (Section 4.32). Urine and expired CO_2 were the major routes of ^{14}C-activity elimination for both dose levels. There were no marked differences in the distribution of radioactivity in the tissues between the dose groups. The rank order of tissues, from highest to lowest amount of radioactivity per gram of tissue, was: liver, bone marrow, kidneys, skin and blood, muscle, carcass, and perirenal fat and brain (about equivalent). Peak blood levels of radioactivity occurred at 0.5 hours after dosing in rats given 76 mg/kgbw and at 4.0 hours after 837 mg/kgbw. The urinary profiles were not qualitatively different between the dose levels; however, some quantitative differences were evident (Zempel *et al*, 1991).

The metabolic fate of DPGBE has many similarities to that of 2PG1ME and DPGME suggesting a common route of metabolism. Despite differences in the overall disposition of DPGBE, DPGME and 2PG1ME, no major differences in their systemic toxicity would be anticipated, since they appear to be metabolised via a common route to the same general types of metabolites.

A study was undertaken to examine the potential association of DPGBE induced increases in liver weights of rats with the induction of specific CYP-dependent MFOs. Male F344 rats were administered 0 or 1,000 mg/kgbw/d via gavage for 7 consecutive days. Liver weight and the activity of several MFOs (CYP1A1, CYP1A2, CYP2B1/2 and CYP2E1) and the Phase II enzymes, UGT and ST, were measured. The relative liver weights of rats administered DPGBE were significantly elevated approximately 25%. A significant 3.1 to 3.5-fold increase in PROD activity was observed with the test material relative to controls indicating a modest induction of CYP2B1/2 dependent MFO. In addition, pNPH activity was increased by 33% in DPGBE-treated rats, suggesting a modest increase in CYP2E1 dependent MFO activity while the activity of the Phase II enzyme UGT was slightly elevated (25%) in DPGBE-treated rats. These changes were consistent with an adaptive response of treated rats to metabolise relatively high dosages of DPGBE (Stott and Kan, 2000).

4.41.4.8 Neurotoxicity

No data are available.

4.41.4.9 Immunotoxicity

No data are available.

4.41.5 Human effects data

There has been no adverse human experience reported implicating exposure to DPGBE.

DPGBE was tested for skin sensitisation in 82 human volunteers. Treatment consisted of 9 doses of 0.4 ml test material under an occlusive patch over a period of 3 weeks. No evidence of skin sensitisation was seen when challenge patches were applied 17 days later (Maclennon and Hedgecock, 1988).

Table 4.41.1: Systemic toxicity of DPGBE in rats

Route / Strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**			
SD, 6 M, 6 F	0, 100, 200, 400	1 x/d, 14 d (consecutive)	No effects at all dose levels	Debets and Verschuuren, 1987
Oral, diet	**(mg/kgbw)**			
SD, 5 M, 5 F	0, 250, 500, 750	1 x/d, 14 d (consecutive)	No effects at all dose levels	Thévenaz *et al*, 1988a
SD, 25 M, 25 F	0, 200, 450	1 x/d, 13 wk	No effects; ↑ magnesium in urine (M); slight ↑ in urea plasma level (M, F)	Thévenaz *et al*, 1988b
	1,000		Marginal ↓ bw (M); slight ↑ in gamma-transferase activity (M), ↑ urea, cholesterol, potassium (M, F), ↑ glucose (F); lower urinary pH, moderate ↑ in transitional epithelium cells, ↓ sodium excretion (M); marginal ↑ absolute and/or relative liver weights (M)	
Dermal, non-occluded	**(mg/kgbw)**			
Wistar, 10 M, 10 F	0, 91	1 x/d, 5 d/wk, 13 wk	No effects	Lina *et al*, 1988
	273		Skin reaction accompanied by histological changes;↓ bw (M), slight ↑ in liver weight (M, F)	
	910		Skin reaction accompanied by histological changes;↓ bw (M), slight ↑ in liver weight (M, F); ↑ GOT and GTP (M), ↑ plasma triglyceride concentration and ↓ glucose (F)	

Table 4.41.1: Systemic toxicity of DPGBE in rats (cont'd)

Route / Strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation, vapour	**(ppm)**	**(mg/m^3)**			
F344, 5/sex	0 20, 40	(0 160, 320)	9 x 6 h/d, nose-only	No effects ↑ liver weights in the absence of histopathological alteration	Lomax *et al*, 1987
Inhalation, aerosol	**(mg/l)**	**(mg/m^3)**			
F344, 5 M, 5 F	0, 0.2 0.81 2.01	(0, 200 810 2,010)	9 x 6 h/d, nose-only	No effects ↓ Bw (M, F); histopathological alterations in liver and nasal cavities (M, F); Considered secondary to weight loss and stress: lymphoid depletion of thymus and/or spleen ↓ Bw (M, F); histopathological alterations in liver and nasal cavities (M, F); Considered secondary to weight loss and stress: ↓ mean RBC count, Hb concentration and Hct, and lymphoid depletion of thymus and/or spleen	Cieszlak *et al*, 1991

Table 4.41.2: Genotoxicity of DPGBE

Endpoint / Organism	Strain or type / Target	Concentration	Result	Remark	Reference
In vitro					
Gene mutation					
Salmonella typhimurium	TA1535, TA100, TA1537, TA1538, TA98	Up to 5,000 µg/plate	**–ve**	+/– S9	Van de Waart and Enninga, 1987
CHO cell	HGPRT locus	279-5,000 µg/ml	**–ve**	+/– S9	Linscombe *et al*, 1995
Sister chromatid exchange					
V79 cell		3 - 9 mmol	**–ve**	– S9	Elias *et al*, 1996
Chromosome aberration		**(µg/ml)**			
CHO-K1 cell	S1B	4,500	**+ve**	– S9, BHT did not reduce clastogenicity	Enninga, 1987
		3,500		+ S9, BHT did not reduce clastogenicity	
CHO-K1 cell	S1B	1,000 - 4,000	**+ve**	– S9	Waalkens and Enninga, 1987
CHO-K1 cell	S1B	5,000	**+ve**	– S9	Enninga and Van de Waart, 1989
		3,000		+ S9	
CHO-K1 cell	CCL61	500 - 5,000	**–ve**	+/– S9	Gollapudi *et al*, 1988b
CHO-K1 cell	S1B	500 - 5,000	**–ve**	+/– S9	Linscombe and Verschuuren, 1991
V79 cell		NS [a]	**–ve**	– S9	Elias *et al*, 1996
Human lymphocyte		NS [a]	**–ve**	– S9	Elias *et al*, 1996

Table 4.41.2: Genotoxicity of DPGBE (cont'd)

Endpoint / Organism	Strain or type / Target	Concentration	Result	Remark	Reference
Micronucleus formation					
V79		NS [a]	**–ve**	– S9	Elias *et al*, 1996
Aneuploidy					
V79 cell		NS [a]	**–ve**	– S9	Elias *et al*, 1996
In vivo					
Micronucleus frequency		**(mg/kgbw/d)**			
Mouse	CD-1 Bone marrow	0, 250, 833, 2,500, 1 x oral gavage	**–ve**	Bone marrow collected at 24, 48 and 72 h	McClintock *et al*, 1988
Mouse	CD-1 (4 M, 4 F) Bone marrow	100, 200, 400 1 x i.p.	**–ve**	Bone marrow collected at 24, 48 and 72 h	McClintock *et al*, 1988

[a] Concentrations partly presented in graphs

Table 4.41.3: Reproductive and developmental dermal toxicity of DPGBE in Wistar rats

Route / number and sex/group	Dose		Exposure regime	Result	Reference
	(ml/kgbw)	(mg/kgbw)			
20 F	0, 0.3, 1.0	(0, 273, 910)	Dermal, non-occlusive application, dissolved in 1,2-propylene glycol, g.d. 6 - 16	No effects at both doses	Wilmer and Van Marwijk, 1988

Table 4.41.4: Absorption (uptake), distribution, metabolism and elimination of DPGBE in F344 rats

Route / Number and sex/group	Dose		Exposure regime	Result	Reference
	(mmol/ kgbw)	(mg/kgbw)			
Oral, gavage					
4 M	0.4	(76)	1 x	42% exhaled as CO_2. 42% eliminated in urine as sulphate and glucuronide conjugates of DPGBE, PGBE, DPGBE, dipropylene glycol and propylene glycol. > 1% in faeces	Zempel *et al*, 1991
	4.4	(837)	1 x	35% exhaled as CO_2. 51% eliminated in urine as sulphate and glucuronide conjugates of DPGBE, PGBE, DPGBE, dipropylene glycol and propylene glycol. 11% in faeces	

4.42 Substance profile: TPGBE

4.42.1 Identity

Name:	Tripropylene glycol (mono) *n*-butyl ether
IUPAC name:	1-(2-(2-Butoxy-1-methylethoxy)-1-methylethoxy)-2-propanol
CAS registry No.	55934-93-5
Molecular formula:	$C_{13}H_{28}O_4$
Structural formula:	$C_4H_9–(O–CH_2–\underset{\displaystyle CH_3}{\underset{\vert}{CH}})_3–OH$
Molecular weight:	248.4
Other components:	No data

4.42.2 Physico-chemical properties

Melting point:	< –75°C
Boiling point:	274°C
Vapour pressure:	< 0.01 hPa
Solubility in water:	30 mg/l
Relative density:	$D_4^{20} = 0.930$

4.42.3 Conversion factors

1 ppm = 10.326 mg/m^3
1 mg/m^3 = 0.097 ppm

4.42.4 Toxicological data

4.42.4.1 Acute toxicity

Oral

Rat:	Males LD_{50} of 3,100 mg/kgbw (undiluted TPGBE); females LD_{50} of 2,600 mg/kgbw (Debets, 1988) or 2,000 mg/kgbw (Wall, 1988). LD_{50} 3.3 ml/kgbw (3,100 mg/kgbw) (Rowe, 1954).

Dermal

Rat/rabbit: No evidence of systemic toxicity at 2,000 mg/kgbw (Debets, 1988; Reijnders and Van Garderen, 1987; Wall, 1988).

Inhalation

No data are available.

4.42.4.2 Irritation and sensitisation

Skin irritation

Application of TPGBE (0.5 ml) to the intact skin of 3 rabbits for 4 hours produced slight redness and swelling, and slight scaling (Weterings *et al*, 1988a). A single 24-hour exposure produced very slight, to slight erythema (Wall, 1988).

Five applications of 85% TPGBE to either intact or abraded rabbit skin produced slight redness (Weterings *et al*, 1988a).

Daily application of TPGBE (0.1-0.5 ml) for 3 to 5 days produced slight erythema and exfoliation when applied topically to either the intact or abraded skin of a male NZW rabbits (Wall,1988).

Eye irritation

Undiluted TPGBE applied to the rabbit eye caused severe lachrymation and discharge (for 2 days following application), slight conjunctival redness and moderate swelling; symptoms resolved in 7 to 14 days. Corneal effects were noted after 1 day but had resolved by 3 days (Weterings *et al*, 1988b).

TPGBE (0.1 ml) was instilled into the eyes of a female NZW rabbit. At either 30 seconds (one eye) or 1 hour (one eye) after exposure, the animal's eyes were washed with water. Ocular TPGBE exposure resulted in slight discomfort, moderate conjunctival redness and swelling, slight to moderate reddening of the iris, and moderate corneal injury. Signs of ocular irritation were no longer observed by 48 hours in the eye washed 30 seconds after exposure. In the eye washed 1 hour after exposure, corneal injury and ocular irritation were resolved within 7 days (Wall, 1988).

Sensitisation

Undiluted TPGBE was negative in the Buehler guinea pig skin sensitisation assay (Daamen and Verschuuren, 1989).

4.42.4.3 Repeated-dose toxicity (Table 4.42.1)

Subacute toxicity

F344 rats received by gavage TPGBE in corn oil at targeted dose levels of 0, 100, 350 or 1,000 mg/kgbw/d for 4 weeks. A comprehensive set of ante-mortem and post-mortem parameters were evaluated, including in-life observations, body weight, feed consumption, haematology, clinical chemistry, organ weights, gross pathology and histopathology. Pronounced lethargy was noted in high dose males and females on test days 1 to 3, which subsided within 4 hours post-dosing. The animals adapted to the treatment regimen and appeared clinically normal by test day 4. However, one high dose female was lethargic on test day 15. A dose-related increase in absolute and relative liver weights was observed in male and female rats administered 350 and 1,000 mg/kgbw. This observation was accompanied by increased hepatocellular size and altered staining in males and females given 1,000 mg/kgbw. Altered staining properties were also noted in the males given 350 mg/kgbw. No evidence of a treatment-related degenerative change accompanied these minor hepatic effects. The compensatory hypertrophy was attributed to metabolism of the test material and the changes were considered to be of no toxicological significance. There were no other treatment-related microscopic effects. The NOAEL for TPGBE in males and females was 350 mg/kgbw/d (Mizell *et al*, 1990).

Subchronic toxicity

TPGBE was administered in the drinking water of F344 at dose levels of 0, 100, 350 and 1,000 mg/kgbw/d for 13 weeks. A recovery group of rats was given TPGBE in the drinking water at 0 or 1,000 mg/kgbw/d for 13 weeks followed by 4 weeks of tap water. Dose-related decreases in water palatability were observed at all dose levels. Decreased water consumption was associated with decreased feed consumption and body weight gain in high-dose animals only. Minor histopathologic and organ weight alterations of the liver and kidney weights occurred in males given 1,000 mg/kgbw and in the livers of females given 1,000 mg/kgbw. The hepatic effects were interpreted to be an adaptive change, possibly associated with the metabolism of TPGBE. The renal changes were characterised as slight tubular degeneration/regeneration and represented a minimal difference in severity over the control. The body weight, organ weight and histopathologic effects were reversible during the 4-week recovery period. The NOAEL for male and female rats was 350 mg/kgbw (Kirk *et al*, 1992).

4.42.4.4 Genotoxicity (Table 4.42.2)

In vitro

TPGBE was not mutagenic in the Ames test using *Salmonella typhimurium* (Ames test), in the presence or absence of S9 metabolic activation, at concentrations up to 5,000 μg/plate (Samson *et al*, 1989).

In vivo

TPGBE was tested in the mouse bone marrow micronucleus test. TPGBE was mixed with corn oil and administered to CD-1 mice by oral gavage at dose levels of up to 1,875 mg/kgbw. Mice treated with 120 mg/kgbw cyclophosphamide and killed at 24 hours served as positive controls. There was no significant increase in micronuclei of the bone marrow in treated versus control groups. (McClintock *et al*, 1989).

4.42.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.42.4.6 Reproductive toxicity and teratogenicity

No data are available.

4.42.4.7 Kinetics and metabolism

No data are available.

4.42.4.8 Neurotoxicity

No data are available.

4.42.4.9 Immunotoxicity

No data are available.

4.42.5 Human effects data

No data are available.

Table 4.42.1: Systemic oral toxicity of TPGBE in F344 rats

Route / Number and sex/group	Dose (mg/kgbw)	Exposure regime	Result	Reference
Gavage				
5 M, 5 F	0, 100 350 1,000	1 x/d, 5 d/wk, 4 wk	No effects ↑ absolute and relative liver weights. Altered hepatocellular staining (M); NOAEL Lethargy (M, F). ↑ absolute and relative liver weights. Increased hepatocellular size and staining (M, F). No other treatment-related microscopic effects	Mizell *et al*, 1990
Drinking water	**(mg/kgbw)**			
10 M, 10 F	0 100 350 1,000	*Ad libitum*, 7 d/wk, 13 wk	No effects ↓ palatability ↓ palatability. NOAEL (M and F) ↓ palatability. ↓ Food consumption and bw. Histopathologic and organ weight alterations of liver and kidney (M) or liver (F). Bw, organ weight and histopathologic effects reversed after 4-wk recovery	Kirk *et al*, 1990

Table 4.42.2: Genotoxicity of TPGBE

Endpoint / Species	Strain or type / Target	Concentration	Result	Remark	Reference
In vitro					
Gene mutation		**(µg/plate)**			
Salmonella typhimurium	TA98, TA100, TA1535, TA1537	Up to 5,000	**–ve**	+/– S9	Samson *et al*, 1989
In vivo					
Chromosome aberration		**(mg/kgbw)**			
Mouse	CD-1 Bone marrow	0, 187.5, 625 or 1,875, 1 x oral gavage	**–ve**	Bone marrow collected at 24, 48 and 72 h after dosing.	McClintock *et al*, 1989

4.43 Substance profile: PGTBE

4.43.1 Identity

Name:	Propylene glycol *tert*-butyl ether
IUPAC name:	1(1,1-dimethylethoxy)-2-propanol
CAS registry No.	57018-52-7
Molecular formula:	$C_7H_{16}O_2$
Structural formula:	$CH_3–CH(OH)–CH_2–O–C(CH_3)_3$
Molecular weight:	132.2
Other components:	No data

4.43.2 Physico-chemical properties

Melting point:	< –82°C
Boiling point:	154°C
Vapour pressure:	3.74 hPa at 25°C
Solubility in water:	> 10%

4.43.3 Conversion factors

1 ppm = 5.496 mg/m^3
1 mg/m^3 = 0.182 ppm

4.43.4 Toxicological data

4.43.4.1 Acute toxicity

Oral

Rat:	LD_{50} 4,599 mg/kgbw (males); 3,275 mg/kgbw (females) 3,771 mg/kgbw (males and females) (Wingard, 1982a).

Dermal

Rabbit:	LD_{50} > 2,000 mg/kgbw (males and females) (Wingard, 1982b).

Inhalation

Rat: LC_{50} > 2.68 mg/l (4 h) (Hagensen *et al*, 1982).

4.43.4.2 Irritation and sensitisation

Skin irritation

Undiluted PGTBE was mildly irritant to rabbit skin (24 h, under occlusion) (Wingard, 1982c).

Eye irritation

Undiluted PGTBE was severely irritant to rabbit eye (Wingard, 1982d), but a 20% solution was not irritant (Bier, 1985).

Sensitisation

There was no evidence of delayed contact hypersensitivity in a guinea pig maximisation test (Kynoch *et al*, 1988).

4.43.4.3 Repeated-dose toxicity (Table 4.43.1)

Subacute toxicity

Male and female F344 rats and $B6C3F_1$ mice were exposed by inhalation to 0, 75, 150, 300, 600 and 1,200 ppm PGTBE for 16 to 17 days in a range-finding study for subsequent 14 weeks and carcinogenicity studies (below). All animals survived. Body weights of top dose female mice were lower than controls. Kidney weights of male rats were raised. Liver weights of both sexes were raised at doses of 600 and 1,200 ppm in rats and mice; liver weights were also increased in female mice at 300 ppm (Doi *et al*, 2004; NTP, 2004).

Subchronic toxicity

Male and female F344 rats were exposed by inhalation (whole-body) to 0, 25, 80, 250 or 750 ppm PGTBE for 13 weeks. The study included an interim kill after 4 weeks of exposure, plus a recovery group that was killed at week 16. In all groups, no clinical signs were observed and the

majority of parameters were unaffected by treatment. The exception was a slight increase of urinary occult blood in treated males after 13 weeks of treatment, and also after the 3-week recovery period. There were no histopathological or haematological findings that correlated with this observation, suggesting that this was an incidental finding, unrelated to treatment. Liver, kidney and spleen weights were increased in treated animals but there was no sign of any accompanying change in clinical chemistry parameters or microscopic evidence of histopathological damage. No treatment-related changes were present in any of the other tissues examined including bone marrow and thymus. A subchronic NOAEL of 750 ppm was obtained from this study (Lulham and Procter, 1985).

Male and female F344 rats were exposed by inhalation to 0, 75, 150, 300, 600 and 1,200 ppm PGTBE for 14 weeks. Survival was unaffected, no clinical signs were observed and no reductions in body weight occurred at any dose. No changes in clinical chemistry or haematology related to treatment were observed. Liver weights were increased in exposed males and females at 600 ppm and higher. Kidney weights of males at all doses and females at and above 300 ppm were raised. There were changes in urinalysis, increased renal tubule cell proliferation, pathological observations of renal tubule regeneration and medullary casts and dose-related hyaline droplets. The level of $\alpha_{2\mu}$-globulin was increased in male rats at all exposure concentrations (Doi *et al*, 2004; NTP, 2004).

Male and female $B6C3F_1$ mice were exposed to 0, 75, 150, 300, 600 and 1,200 ppm PGTBE for 14 weeks. Survival was unaffected. Body weight gain was lower in males at 150 ppm and above. Liver weights were increased in both sexes at and above 600 ppm with centrilobular hypertrophy being observed in males at this dose and at 1,200 ppm only in females. Nasal squamous metaplasia was slightly increased in both sexes at 1,200 ppm (Doi *et al*, 2004; NTP, 2004).

4.43.4.4 Genotoxicity (Table 4.43.2)

In vitro

PGTBE (up to 5,000 µg/ml) was not mutagenic in *Salmonella typhimurium* (Ames test) (Barfknecht *et al*, 1986; Jones *et al*, 1987).

PGTBE did not induce mutations at the thymidine-kinase locus in L5178Y mouse lymphoma cells (Lyondell, 2001a) or chromosomal aberrations in cultured peripheral human lymphocytes (ARCO Chemical, 1991).

Up to 1% PGTBE was also not clastogenic when tested in CHO cells in the presence or absence of metabolic activation (Allen *et al*, 1987).

All of the above tests were conducted after incubation in the presence or absence of an Arochlor 1254-induced S9 fraction to enhance metabolism.

4.43.4.5 Chronic toxicity and carcinogenicity (Table 4.43.1)

Male and female F344 rats and $B6C3F_1$ mice were exposed to concentrations of 0, 75, 300 or 1,200 ppm PGTBE by inhalation for 2 years. Survival, haematology and clinical chemistry were unaffected, and decreases in body weight occurred in both species at the top dose. Clinical signs were observed in mice at 1,200 ppm. The main target organs were the liver and kidney. Liver adenomas were increased in male rats: the incidence of hepatocellular adenoma/carcinoma increased in top dose male and female mice. Renal tubular degenerations were seen in male rats in all dose groups, with accompanying increases in $\alpha_{2\mu}$-globulin. Marginal increases in the incidences of renal tumours were reported at 300 and 1,200 ppm in male rats (Doi *et al*, 2004; NTP, 2004).

4.43.4.6 Reproductive and developmental toxicity (Table 4.43.3)

In a preliminary, one-generation study, male and female CD rats were given 0,100, 300 or 1,000 mg PGTBE/kgbw/d by oral gavage for 15 days prior to pairing, throughout mating, gestation and lactation and until study termination. Clinical signs were restricted to post-dosing salivation at 1,000 and 300 mg/kgbw/d, but no systemic effects were apparent in either sex either during the in-life phase of the study or at necropsy. Litter data revealed no effect on litter size at birth and subsequent survival to weaning, or initial body weight and subsequent growth. Overall there was no evidence of marked toxicity in either the parental or F1 generation exposed to a limit dose of 1,000 mg PGTBE/kgbw/d (Lyondell Chemical, 2000).

The same dose levels were used in the main study (Lyondell Chemical, 2001b). Again, there were no deaths, and clinical signs were limited to salivation after dosing in the mid and high dose groups. No systemic effects were apparent in either sex during treatment or at necropsy examination. Sperm parameters in high dose males were normal. Mean litter sizes were comparable, but offspring from dams exposed to 1,000 mg/kgbw/d showed slightly lower birth body weights, reduced weight gains from birth to weaning and a slight decrease in postnatal survival. All other litter- and individual off-spring parameters were normal. The parental NOAEL was 1,000 mg/kgbw/d, while 300 mg/kgbw/d was the NOAEL for growth and development in the offspring.

Pregnant NZW rabbits were exposed (whole-body) by inhalation to concentrations of 0, 230, 720 or 980 ppm PGTBE on day 7 to 19 of gestation. There was no evidence of maternal toxicity

during the in-life phase of the study, or during a macroscopic examination at necropsy. Foetal parameters (including litter size, body weights and morphology) were unaffected by treatment. A NOAEL of 980 ppm for developmental toxicity was obtained (Schardein, 1988a).

Foetal development was also unaffected in CDF rats following whole-body inhalation of concentrations of 0, 230, 725 or 990 ppm PGTBE on day 6 to 15 of gestation. Maternal toxicity was evident in high dose animals, however, with approximately half of the dams appearing pale through most of the exposure period and displaying significant increases in absolute and relative liver weights in the mid and high dose animals. A maternal NOAEL of 230 ppm, and a NOAEL for foetal development of > 990 ppm were obtained from this study (Schardein, 1988b).

4.43.4.7 Kinetics and metabolism (Table 4.43.4)

PGTBE is well absorbed in F344 rats following oral administration, and excreted mainly in the urine (as glucuronide and sulphate conjugates, accounting for 50 - 67% of total dose) and the lungs (CO_2, 20 - 25%) (Sipes and Carter, 1994). Dermal uptake was poor, accounting for 2.8% (F344 rats) or 7.8% ($B6C3F_1$ mouse) of the topical dose. A plasma half-life of 16 minutes and a mean clearance of 25.1 ml·min/kgbw were obtained in the rat after *i.v.* injection (Sipes and Carter, 1994).

4.43.4.8 Neurotoxicity

No data are available.

4.43.4.9 Immunotoxicity

No data are available.

4.43.5 Human effects data

No data are available.

Table 4.43.1: Systemic toxicity of PGTBE in F344 rats by inhalation

Route / Number and sex/group	Concentration (ppm)	Concentration (mg/m^3)	Exposure regime	Result	Reference
10 M, 10 F	0, 25, 80, 250, 750	(0, 140, 440, 1,370, 4,120)	6 h/d, 5 d/wk, 4 wk	No deaths or clinical signs. Dose-related ↑ in liver weight (M). No gross or microscopic changes in any other tissues. Affected dose levels not stated (summary only)	Lulham and Procter, 1985
10 M, 10 F	0, 25, 80, 250, 750	(0, 140, 440, 1,370, 4,120)	6 h/d, 5 d/wk, 13 wk 3 wk recovery	No deaths or clinical signs. Dose-related ↑ in liver weight (M, F), present also in recovery animals; ↑ kidney and spleen weight (M,F); no gross or microscopic changes present in any other tissues. Affected dose levels not stated (summary only)	Lulham and Procter, 1985

Table 4.43.2: Genotoxicity of PGTBE

Endpoint / Species	Strain or type / Target	Concentration	Result	Remark	Reference
In vitro					
Gene mutation					
Salmonella typhimurium	TA1535, TA1537, TA1538, TA98, TA100	50 - 5,000 µg/plate	**–ve**	+/– S9	Barfknecht *et al*, 1986
Salmonella typhimurium	TA1535, TA1537, TA1538, TA98, TA100	5 - 5,000 µg/plate	**–ve**	+/– S9	Jones *et al*, 1987

Table 4.43.2: Genotoxicity of PGTBE (cont'd)

Endpoint / Species	Strain or type / Target	Concentration	Result	Remark	Reference
Gene mutation (cont'd)					
Salmonella typhimurium	TA1535, TA1537, TA97, TA98, TA100	100 - 10,000 µg/plate	**–ve** **+ve**	+/– S9 (TA1537 only – S9, TA97 also + hamster S9) – S9	Doi *et al,* 2004; NTP, 2004
Mouse lymphoma cell	L5178Y TK+/–	10 - 5,000 µg/ml	**–ve**	+/– S9. Slight cytotoxicity at 5,000 µg/ml	Lyondell Chemical, 2001a
Chromosome aberration					
Human	Lymphocyte	1,000, 3,330, 5,000 µg/ml	**–ve**	+/– S9	Arco Chemical, 1991
CHO cell	K1-BH4	0.1%, 0.5% or 1%	**–ve**	+/– S9. Cytotoxicity at 1% in absence of S9	Allen *et al*, 1987
CHO cells		Up to 5,000 µg/ml	**–ve**	+/– S9	Doi *et al,* 2004; NTP, 2004
Sister chromatid exchange					
CHO cells		Up to 5,000 µg/ml	**–ve**	+/– S9	Doi *et al,* 2004; NTP, 2004
In vivo					
Micronucleus induction					
Mouse	$B6C3F_1$, M F	75 - 1,200 ppm, 14 wk whole-body	**–ve** Weakly **+ve**	After 3 months	Doi *et al,* 2004; NTP, 2004

Table 4.43.3: Reproductive and developmental toxicity of PGTBE

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**			
Rat, CD, 8 M, 8 F	0, 100, 300 or 1,000	Males: treatment begun 15 d prior to mating until termination (total 43 x) Females: treatment begun 15 d prior to mating and continued through gestation and lactation (total 56 x for 8 wk)	Adults: Clinical signs limited to salivation in mid- and high dose groups (both sexes); no adverse effect on bw gain, food intake, regularity and duration of oestrus cycle, mating performance, fertility or length of gestation period Litters: No effect on litter size or bw at birth, bw gain or subsequent pup survival	Lyondell Chemical, 2000
Rat, CD, 24 M, 24 F	0, 100, 300 or 1,000	Males: treatment begun 71 d prior to mating until termination (total 126 x for 18 wk) Females: treatment begun 15 d prior to mating and continued through gestation and lactation (total 56 x for 8 wk)	Adults: Dose-related salivation post-dosing at 300 and 1,000 mg/kgbw , no adverse effect on bw gain or food intake in either sex; regularity of oestrus cycle, mating performance and fertility, gestation and parturition were unaffected by treatment with high pregnancy rates in all treated groups; no effect on sperm motility; no effect on organ weights or macroscopic appearance Litters: Mean litter size at birth similar for all groups; slight ↓ birth bw and slight ↓ viability to post-natal d 4 in high dose litters (both not significant), performance thereafter unremarkable. Overall, no treatment-related effect on number of implantations, litter size, survival, bw or macroscopic observations at post-natal d 21	Lyondell Chemical, 2001b

Table 4.43.3: Reproductive and developmental toxicity of PGTBE (cont'd)

Route / Species, strain, number and sex/group	Dose or concentration		Exposure regime	Result	Reference
Inhalation	**(ppm)**	**(mg/m^3)**			
Rabbit, NZW, 16 F	0, 230, 720, 980	(0, 1,264, 3,960 or 5, 390)	Dams: 6 h/d, g.d. 7 - 19	Dams: No effect on survival, behaviour, clinical signs, bw, food and water intake, clinical chemistry, haematology or macroscopic observations at time of Caesarean section Offspring: No effect on foetal bw, external appearance; no malformations; no effect on incidence of visceral and skeletal variations, abnormalities etc.	Schardein, 1988a
Rat, CDF, 25 F	0 ,230, 725, 990	(0, 1,264, 3,980 or 5,440)	Dams: 6 h/d, g.d. 6 - 15	Dams: Around 50% of high dose dams appeared pale during exposure, but no effect on bw gain, or food or water intake; ↑ absolute and relative liver weights in mid and high dose groups (adaptive response) Offspring: No effect on foetal bw, external appearance; no malformations; no effect on incidence of visceral and skeletal variations, abnormalities etc.	Schardein, 1988b

Table 4.43.4: Absorption (uptake), distribution, metabolism and elimination of PGTBE

Route / Species, strain, number and sex/group	Dose or concentration	Exposure regime	Result	Reference
Oral, gavage	**(mg/kgbw)**			
Rat, F344, 3 M	3.8, 37.7 or 377	1 x	87-100% of ^{14}C dose eliminated within 72 h, majority within first 24 h; excretion via urine (50-67%), CO_2 (22-26%) and faeces (3-11%). Glucuronide (23-52%) and sulphate (7-13%) conjugates present in urine	Sipes and Carter, 1994
Dermal, occluded				
Rat, F344, 3 M	4.71 mg/8.4 cm^2	1 x	2.8% dermal uptake, as shown by presence of ^{14}C in urine (1.8%) and exhaled CO_2 (1%)	Sipes and Carter, 1994
Mouse, B6C3F_1, 3 M	4.71 mg/0.8 cm^2	1 x	7.8% dermal uptake, as shown by presence of ^{14}C in urine (2.0%), faeces (0.4%) and exhaled CO_2 (5.4%)	Sipes and Carter, 1994
Intravenous	**(mg/kgbw)**			
Rat, F344, 3 M	37.8	1 x in saline via jugular cannula	Half-life (plasma) 16 min, clearance 25.1 ml·min/kgbw. 40% of dose in bile as glucuronide, enterohepatic re-circulation reduces amount in faeces to 11% of dose	Sipes and Carter, 1994

4.44 Substance profile: DPGTBE

4.44.1 Identity

Name:	Dipropylene glycol *tert*-butyl ether
IUPAC name:	Propanol, [2-(1,1-dimethylethoxy)methylethoxy]
CAS registry No.	132739-31-2
Molecular formula:	$C_{10}H_{22}O_3$
Structural formula:	$(CH_3)_3–C(O–CH_2–CH–CH_3)_2–OH$
Molecular weight:	190.3
Other components:	Propanol, oxybis (approximately 0.5% w/w) Propane, 2,2'-[oxybis[(methyl-2,1-ethanediyl)oxy]]bis[2-methyl]- (approximately 0.2% w/w)

4.44.2 Physico-chemical properties

Melting point:	< –25°C
Boiling point:	213 - 219°C
Vapour pressure:	41.1 - 43.7 Pa at 25°C
Solubility in water:	165 g/l

4.44.3 Conversion factors

1 ppm = 7.911 mg/m^3
1 mg/m^3 = 0.126 ppm

4.44.4 Toxicological data

4.44.4.1 Acute toxicity

Oral

Rat:	LD_{50} 2,600 mg/kgbw (fasted) (Cerven, 1992a).

Dermal

Rabbits	LD_{50} > 2,000 mg/kgbw (Cerven, 1992b).

Inhalation

Rat: LD_{50} > 5.11 mg/l (Arco Chemical, 1997).

4.44.4.2 Irritation and sensitisation

Skin irritation

DPGTBE was not irritant to rabbit skin (Cerven, 1992c).

Eye irritation

DPGTBE (0.1 ml) was irritant to the rabbit eye with reversible corneal opacity present at 72 hours but resolved by day 7. The degree of irritation decreased after flushing the eye with lukewarm water 20 to 30 seconds after instillation (Cerven, 1992d).

Sensitisation

DPGTBE was not a dermal sensitiser in the guinea pig maximisation test (Arco Chemical, 1996a).

4.44.4.3 Repeated-dose toxicity (Table 4.44.1)

Subacute toxicity

DPGTBE was administered by gavage to Crl:CD BR rats at doses of 0, 100, 300 or 1,000 mg/kgbw/d for 14 days. Clinical signs, present from day 7 onwards, were limited to salivation in the mid- and high dose groups immediately after dosing, possibly reflecting unpalatability of the test substance. There were no unscheduled deaths. Liver and kidney weights were increased in the mid- and high-dose animals (both sexes) with minor centrilobular hepatocyte enlargement and eosinophilic droplets (tentatively identified as $\alpha_{2\mu}$-globulin) present in renal cortical tubular epithelia in males only. No histopathological changes were present in females. No NOAEL could be derived for treatment-related induction of eosinophilic droplets in renal cortical tissue in males, however it is probable that these changes were caused by *tert*-butanol (a predicted metabolite of DPGTBE), which is a known to trigger $\alpha_{2\mu}$-globulin nephropathy in male rats (Williams and Borghoff, 2001). As a consequence these changes are not considered predictive of a hazard to human health. The NOAEL for histopathological effects

(excluding kidney tissue) was 1,000 mg/kgbw/d in females, and 300 mg/kgbw/d in males (based on minor centrilobular hepatocyte enlargement) (Arco Chemical, 1996b).

Subchronic toxicity

DPGTBE was administered by gavage to Crl:CD BR rats at doses of 0, 62.5, 250 or 1,000 mg/kgbw/d for 13 weeks. A further group of 10 males and 10 females dosed at 1,000 mg/kgbw/d, along with an identical number of controls, were assigned to a recovery group. (As part of the same study, additional rats were used in a neurotoxicity investigation [Section 4.44.4.10]). Clinical signs were present only the high-dose animals, and were characterised by salivation after dosing, together with signs of CNS depression (unsteady gait, partially closed eyelids etc) in a small proportion of animals from weeks 8 to 12 (absent in recovery group during weeks 14 to 17). There were no unscheduled deaths. Increased plasma albumin and globulin levels were noted in high dose animals of both sexes at weeks 5 and 13. Liver weights were increased in both sexes given 1,000 mg/kgbw/d after 13 weeks of treatment, with minimal centrilobular hepatocyte hypertrophy seen on microscopic examination (liver parameters and microscopic structure normal after 4 weeks recovery). Kidney weights were increased at 13 weeks in both sexes given 1,000 mg/kgbw and in males given 250 mg/kgbw/d with microscopic changes (eosinophilic inclusions, minimal basophilic cortical tubules, cellular debris casts) present in males only. These effects on renal weights and histopathology had partially resolved in animals from the recovery group. High dose females showed increased mean adrenal weights, together with minimal increases in width of the zona fasiciculata, relative to controls. These changes were fully reversed after 4 weeks without treatment. The 1,000 mg/kgbw/d dose was a clear effect level in the rat, producing changes in the liver, adrenals and kidneys. Changes in male rat kidney were considered not to be relevant to humans (see also subacute study above). Based on macroscopic and/or microscopic changes in liver and adrenal gland, the subchronic NOAEL was 250 mg/kgbw/d (Arco Chemical, 1996c).

4.44.4.4 Genotoxicity (Table 4.44.2)

In vitro

DPGTBE was not mutagenic when tested, with and without metabolic activation, in a *Salmonella typhimurium* plate incorporation assay (Ames test) (Arco Chemical, 1996d).

In vivo

DPGTBE was negative in a mouse micronucleus test following injection of up to 800 mg/kgbw i.p. (Arco Chemical, 1996e).

4.44.4.5 Chronic toxicity and carcinogenicity

No data are available.

4.44.4.6 Reproductive and developmental toxicity

No data are available.

4.44.4.7 Kinetics and metabolism

No specific data are available.

The high water solubility (165 g/l) and low octanol-water partition coefficient (log P_{ow} = 1.68) (Arco Chemical, 1996f) will encourage excretion of DPGTBE by the body, with little potential for bioaccumulation expected. Although toxicity following oral administration was low, systemic effects did occur (particularly after repeated administration) suggesting that intestinal absorption and systemic distribution had occurred to some extent. The rapid onset and greater intensity of effects after *i.p.* injection (Arco Chemical, 1996e) relative to that seen after ingestion also suggests that intestinal absorption is relatively slow. Dermal penetration has not been measured. The adaptive responses seen in liver following sub-chronic administration indicate that hepatic metabolism of DPGTBE occurs, with de-methylation or oxidation to a carboxylic acid favoured on structural grounds. Changes seen in male rat kidney, suggestive of $\alpha_{2\mu}$-globulin-nephropathy, also support formation of *tert*-butanol.

4.44.4.8 Neurotoxicity

No treatment-related neurobehavioural effects (FOB, motor activity test) were present in Crl:CD BR rats (5/sex/group) given by oral gavage 0 or 1,000 mg DPGTBE/kgbw/d for 13 weeks (as part of the subchronic study discussed in Section 4.44.4.3). Morphometric and micro-structural examination of the brain proved unremarkable: control and high dose animals of both sexes were indistinguishable. The NOAEL for neurobehavioural effects and neurotoxicology was 1,000 mg/kgbw/d (Arco Chemical, 1996c).

4.44.4.9 Immunotoxicity

No data are available.

4.44.5 Human effects data

No data are available.

Table 4.44.1: Systemic toxicity of DPGTBE in Crl:CD BR (SD) rats by oral gavage

Route / Species, strain, number and sex/group	**Dose (mg/kgbw/d)**	**Exposure regime**	**Result**	**Reference**
5 M, 5 F	0, 100, 300, 1,000	1 x/d for 14 d	No effects. ↑ salivation. ↑ relative liver and relative kidney weight (M, F), ↑ centrilobular hepatocyte, renal $\alpha_{2\mu}$-globulin droplets (M) ↓ relative adrenal weight, significant in F	Arco Chemical, 1996b
Crl:CD BR, 10 M, 10 F	0, 62.5, 250 1,000	1 x/d for 13 wk	No effects ↑ salivation, ↓ CNS depression. Slight ↓ total white cell and lymphocyte counts (M, F) at week 5 and in F at wk 13, but not dose-related and within historical control range; ↑ albumin, globulin and total protein in top dose animals (M, F) at wk 5 and 13; ↑ liver weights in high dose animals (M, F) with minimal centrilobular hepatocyte hypertrophy at 13 wk, but liver unremarkable at end of 4-wk recovery study; ↑ kidney weights in both sexes at 1,000 mg/kgbw/d, with increased degree and incidence of microscopic changes indicative of $\alpha_{2\mu}$-globulin-induced nephropathy in all M (F unaffected), only partially resolved at end of 4-wk recovery study. ↑ adrenal weights with minimal increase in width of zona fasiculata in high dose F only, fully resolved at end of 4-wk recovery	Arco Chemical, 1996c

Table 4.44.2: Genotoxicity of DPGTBE

Endpoint / Species	Strain or type / Target	Concentration	Result	Remark	Reference
In vitro					
Gene mutation		**(µg/plate)**			
Salmonella typhimurium	TA98, TA100, TA1535, TA1537	50 - 1,500	**–ve**	+/– S9, plate incorporation	Arco Chemical, 1996d
In vivo					
Micronucleus frequency		**(mg/kgbw)**			
Mouse	Swiss Bone marrow	M: 1 x 0, 200, 400 or 600 i.p. F: 1 x 0, 200, 400 or 800 i.p.	**–ve**	Bone marrow collected at 24, 48 and 72 h	Arco Chemical, 1996e

APPENDIX A: SPECIAL ABBREVIATIONS

ADH	Aldehyde dehydrogenase
ADME	Absorption distribution metabolism elimination
ALP or AP	Alkaline phosphatase
ALT	Alanine aminotransferase
ATP	Adenosine triphosphate
AUC	Area under the curve
BAA	2-Butoxy acetic acid
BAL	2-Butoxy acetaldehyde
BAT	Biologischer Arbeitsstoff-Toleranz-Wert [a]
BEAA	2-(2-butoxyethoxy)acetic acid
bw	Body weight
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
CNS	Central nervous system
Con A	Concanavalin A
CYP	Cytochrome P450
d	Day
D_4^{20}	Relative density
DCM	Dichloromethane
DNA	Deoxyribonucleic acid
EAA	Ethoxy acetic acid
EG	Ethylene glycol
EMH	Extramedullary haemopoiesis
EPA	Ethoxypropionic acid
F	Female
F344	Fischer 344
FSH	Follicle stimulating hormone
FOB	Functional observation battery
GC	Gas chromatography
g.d.	Gestation day
GI-tract	Gastrointestinal tract
GLP	Good laboratory practice
GSH	Glutathione
h	Hour
Hb	Haemoglobin
Hct	Haematocrit
HGPRT	Hypoxanthine-guanine-phosphoribosyl transferase

[a] Biological tolerance value at the workplace

APPENDIX A: SPECIAL ABBREVIATIONS (CONT'D)

^{3}H -TdR	^{3}H -Thymidine reduction
IgG	Immunoglobulin class G
IgM	Immunoglobulin class M
i.p.	Intraperitoneal
i.v.	Intravenous
LC_{50}	Lethal concentration for 50% of the exposed animals
LD_{50}	Lethal dose for 50% of the exposed animals
LDH	Lactate dehydrogenase
LH	Luteinising hormone
LO(A)EL	Lowest observed (adverse) effect level
LP	Lymphoproliferative
M	Male
MAALD	2-Methoxyacetaldehyde
MAK	Maximale Arbeitsplatzkonzentration [a]
MCHb	Mean cell haemoglobin
MCV	Mean corpuscular volume
MEAA	(2-Methoxy-ethoxy) acetic acid
MEG	Monoethylene glycol
MEL	Maximum exposure limit
MFO	Mixed function oxidase
mg	Milligramme
mmol	Millimole
ml	Millilitre
MAA	Methoxy acetic acid
MPA	Methoxy propionic acid
NA	Not available
NCE	Normochromatic erthyrocytes
ND	Not detected
n	Number of samples or subjects
NK	Natural killer
NKA	Natural killer (cell) activity
NO(A)EL	No-observed (adverse) effect level
NS	Not specified, not stated
NZW	New Zealand white
OEL	Occupational exposure limit (value)
PAS	Periodic acid Schiff
PBPK	Physiologically-based pharmacokinetic

[a] Maximum workplace concentration

APPENDIX A: SPECIAL ABBREVIATIONS (CONT'D)

PCE	Polychromatic erthyrocytes
PCT	Proximal convoluted tubule
PD	Protective dose
PFC	Plaque-forming cell
PHA	Phytohaemagglutinine
PHAA	Phenoxy acetic acid
PNPH	p-Nitrophenol
ppm	Parts per million
PROD	Pentoxyresorufin O-dealkylase
PWN	Poak wead nitrogen
RBC	Red blood cell(s)
S9	Supernatant of centrifuged 9,000 x g liver homogenate
s.c.	Subcutaneous
sd	Standard deviation
SD	Sprague-Dawley
SCE	Sister chromatid exchange
SGPT	Serum glutamic pyruvic transaminase
SHE	Syrian hamster embryo
SRBC	Sheep red blood cell(s)
ST	Sulfphotransferase
STEL	Short-term exposure limit
TCA	Tricarboxylic acid
TK	Thymidine kinase
TLV	Threshold limit value
TNP-LPS	Trinitrophenyl-lipopolysaccharide
TPG	Tripropylene glycol
TWA	Time-weighted average
UGT	UDP- glucuronosyl transferase
WBC	White blood cell(s)
wk	Week
+/–	With or without, in the presence or absence of
–ve	Negative: no effects
+ve	Positive: effects (on organ or system)
±ve	Equivocal
↓	Decrease
↑	Increase
µmol	Micromole
µg	Microgramme

APPENDIX B: CONVERSION FACTORS FOR VAPOUR CONCENTRATIONS IN AIR

Conversion factors for vapour concentrations in air can be calculated from the molar volume of an ideal gas at 0°C: 22.4136 litre.

$$1\ mg/m^3 = 22.4136/Mw \times 1{,}013.25/P \times (273+T)/273\ ppm \quad \text{.....(Eq. B.1)}$$

$$1\ ppm = Mw/22.4136 \times P/1{,}013.25 \times 273/(273+T)\ mg/m^3 \quad \text{.....(Eq. B.2)}$$

where Mw = molecular weight, T = temperature (°C) and P = pressure (hPa).

For European standard conditions, 20°C and 1,013.25 hPa (=1 atm = 760 mm Hg), the formulae become

$$1\ mg/m^3 = 24.0556/Mw\ ppm \quad \text{.....(Eq. B.3)}$$

$$1\ ppm = Mw/24.0556\ mg/m^3 \quad \text{.....(Eq. B.4)}$$

In the USA and other countries 25°C is used, and the formulae are:

$$1\ mg/m^3 = 24.4661/Mw\ ppm \quad \text{.....(Eq. B.5)}$$

$$1\ ppm = Mw/24.4661\ mg/m^3 \quad \text{.....(Eq. B.6)}$$

APPENDIX C: OCCUPATIONAL EXPOSURE LIMIT VALUES

Several countries have adopted OEL values (Table C.1). The justification of some OELs (also) takes account of critical effects on the reproductive system.

Table C.1: OEL values [a,b]

Country [c]	Concentration, 8-h [d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
1. Ethylene glycol methyl ether (EGME), CAS No. 109-86-4, structural formula: $CH_3–O–CH_2–CH_2–OH$						
Austria	5	15	10	30	Yes	
Belgium	5	16	-	-	Yes	
Denmark	5	16	-	-	Yes	
EU	-	-	-	-	-	May impair fertility (R60), May cause harm to the unborn child (R61), Toxic for reproduction (Category 2)
Finland	0.5	1.6	-	-	Yes	
France	5	16	-	-	Yes [f]	
Germany	5	16	20	64	Yes	Toxic for reproductive purposes
Ireland	5	16	-	-	Yes	Category 2 reproductive toxins: toxic for reproduction for humans
Italy	5	16	-	-	Yes	
Netherlands	0.3	1	-	-	Yes	Toxic to reproduction
Norway	5	16	-	-	Yes	Reproduction damaging substance
Portugal	-	-	-	-	-	
Spain	5	16	-	-	Yes	Substance that can and should be considered harmful for the fertility of human beings or should be considered toxic for their development.
Sweden	0.1	-	-	-	Yes	Reproduction-disturbing substances. Observation list: properties impairing reproduction.

Table C.1: OEL values[a,b] (cont'd)

Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
Switzerland	5	15	10	30	Yes	Harm to the foetus is possible even when the MAK value is complied with.
UK	5	16	-	-	Yes	
US-ACGIH	5	-	-	-	Yes	Critical effect: reproductive
US-NIOSH	0.1	0.3	-	-	-	Reproductive and developmental effects
US-OSHA	25	80	-	-	Yes	
Japan	5	16	-	-	Yes	
2. Ethylene glycol methyl ether acetate (EGMEA), CAS No. 110-49-6, structural formula $CH_3–O–CH_2–CH_2–O–CO–CH_3$						
Austria	5	25	10	50	Yes	
Belgium	5	24	-	-	Yes	
Denmark	5	24	-	-	Yes	
EU	-	-	-	-	-	May impair fertility (R60), May cause harm to the unborn child (R61), Toxic for reproduction (Category 2)
Finland	0.5	2.5	-	-	Yes	
France	5	24	-	-	Yes [f]	
Germany	5	25	20	100	Yes	Toxic for reproductive purposes
Ireland	5	24	-	-	Yes	Category 2 reproductive toxins: toxic for reproduction for humans
Italy	5	24	-	-	Yes	

Table C.1: OEL values[a,b] (cont'd)

Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
Netherlands	0.3	1.5	-	-	Yes	Toxic to reproduction
Norway	5	22	-	-	Yes	Reproduction damaging substance
Spain	5	24	-	-	Yes	Teratogen Category TR2: Substance that can and should be considered harmful for the fertility of human beings or should be considered toxic for their development.
Sweden	0.1	-	-	-	Yes	Group B: reproduction-disturbing substances. Observation list: properties impairing reproduction.
Switzerland	5	25	10	50	Yes	Harm to the foetus is possible even when the MAK value is complied with.
UK	5	25	-	-	Yes	
US-ACGIH	5	-	-	-	Yes	Critical effect: reproductive
US-NIOSH	0.1	0.5	-	-	Yes	Reproductive and developmental effects
US-OSHA	25	120	-	-	Yes	
Japan	5	24	-	-	Yes	
3. Ethylene glycol dimethyl ether (EGDME), CAS No. 110-71-4, structural formula: $CH_3–O–CH_2–CH_2–O–CH_3$						
	-	-	-	-	-	
4. Diethylene glycol methyl ether (DEGME), CAS No. 111-77-3, structural formula: $CH_3–(O–CH_2–CH_2)_2–OH$						
Denmark	25	-	-	-	-	
EU	-	-	-	-	-	Possible risk of harm to the unborn child (R63), Toxic for reproduction (Category 3)

Table C.1: OEL values[a,b] (cont'd)

Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
Netherlands	9	45	-	-		Toxic to reproduction
5. Diethylene glycol dimethyl ether (DEGDME), CAS No. 111-96-6, structural formula: $CH_3–(O–CH_2–CH_2)_2–O–CH_3$						
Austria	5	27	20	108	-	
Germany	5	28	20	112	Skin	
Netherlands	5	27	-	-		
Norway	-	-	-	-	-	Reproduction damaging substance
Switzerland	5	27	10	54	-	
6. Triethylene glycol methyl ether (TEGME), CAS No. 112-35-6, structural formula: $CH_3–(O–CH_2–CH_2)_3–OH$						
	-	-	-	-	-	
7. Triethylene glycol dimethyl ether (TEGDME), CAS No. 112-49-2, structural formula: $CH_3–(O–CH_2–CH_2)_3–O–CH_3$						
	-	-	-	-	-	
8. Methoxy-acetic acid (MAA), CAS No. 625-45-6, structural formula: $CH_3–O–CH_2–COOH$						
Netherlands	5	19	-	-	-	
EU	-	-	-	-	-	May impair fertility (R60), May cause harm to the unborn child (R61), Toxic for reproduction (Category 2)
Switzerland	5	19	10	38	-	Harm to the foetus is possible even when the MAK value is complied with.

Table C.1: OEL values[a,b] (cont'd)

Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
9. Ethylene glycol ethyl ether (EGEE), CAS No. 110-80-5, structural formula: $C_2H_5–O–CH_2–CH_2–OH$						
Austria	5	19	20	76	Yes	
Belgium	5	18	-	-	Yes	
Denmark	5	18.5	-	-	Yes	
EU	-	-	-	-	-	May impair fertility (R60), May cause harm to the unborn child (R61), Toxic for reproduction (Category 2)
France	5	19	-	-	Yes [f]	
Finland	2	7.5	-	-	Yes	
Germany	5	19	20	76	Yes	Substances which are Carcinogenic, Mutagenic or Toxic for Reproductive Purposes
Ireland	5	18	-	-	Yes	Substance should be regarded as if it is toxic for reproduction for humans (Category 2 reproductive toxins).
Italy	5	18	-	-	Yes	
Netherlands	5	19	-	-	Yes	Substances toxic to reproduction
Norway	5	18	-	-	Yes	Reproduction Damaging Substance
Spain	5	18	-	-	Yes	Substance that can and should be considered harmful for the fertility of human beings or should be considered toxic for their development)
Sweden	5	19	10	40	Yes	Substance has reproduction-disturbing effects. Properties impairing reproduction.

Table C.1: OEL values[a,b] (cont'd)

Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
Switzerland	5	19	10	38	Yes	Harm to the foetus is possible even when the MAK value is complied with.
UK	10	37	-	-	Yes	
US-ACGIH	5	-	-	-	Yes	Critical Effect(s): Reproductive
US-NIOSH	0.5	1.8	-	-	Yes	Reproductive and developmental effects
US-OSHA	200	740	-	-	Yes	
Japan	5	18	-	-	Yes	
10. Ethylene glycol ethyl ether acetate (EGEEA), CAS No. 111-15-9, structural formula: $C_2H_5–O–CH_2–CH_2–O–CO–CH_3$						
Austria	5	27	20	108	Yes	
Belgium	5	27	-	-	Yes	
Denmark	5	27.0	-	-	Yes	
EU	-	-	-	-	-	May impair fertility (R60), May cause harm to the unborn child (R61), Toxic for reproduction (Category 2)
France	5	27	-	-	Yes [f]	
Finland	2	11	-	-	Yes	
Germany	5	27	20	108	Yes	Substances which are carcinogenic, mutagenic or toxic for reproductive purposes
Ireland	10	54	-	-	Yes	Substance should be regarded as if it is toxic for reproduction for humans (Category 2 reproductive toxins).
Italy	5	27	-	-	Yes	

Table C.1: OEL values[a,b] (cont'd)

Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
Norway	5	27	-	-	Yes	Reproduction damaging substance
Spain	5	27	-	-	Yes	Substance that can and should be considered harmful for the fertility of human beings or should be considered toxic for their development
Sweden	5	30	10	50	Yes	Substance has reproduction-disturbing effects. Properties impairing reproduction
Switzerland	5	27	10	54	Yes	Harm to the foetus is possible even when the MAK value is complied with
Netherlands	5	27	-	-	Yes	Substances toxic to reproduction
UK	10	55	-	-	Yes	
US-ACGIH	5	-	-	-	Yes	Critical Effect(s): Reproductive
US-NIOSH	0.5	2.7	-	-	Yes	Reproductive and developmental effects
US-OSHA	100	540	-	-	Yes	
Japan	5	27	-	-	Yes	
11. Ethylene glycol diethyl ether (EGDEE), CAS No. 629-14-1, structural formula: C_2H_5–O–CH_2–CH_2–O–C_2H_5						
	-	-	-	-	-	
12. Diethylene glycol ethyl ether (DEGEE), CAS No. 111-90-0, structural formula: C_2H_5–(O–CH_2–CH_2)$_2$–OH						
Netherlands	32	180	-	-	Yes	
Sweden	15	80	30	170	Yes	
US-AIHA	25	140	-	-	-	

Table C.1: OEL values[a,b] (cont'd)

Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
13. Diethylene glycol ethyl ether acetate (DEGEEA), CAS No. 112-15-2, structural formula: $C_2H_5–(O–CH_2–CH_2)_2–O–CO–CH_3$						
Sweden	15	110	30	220	Yes	
14. Diethylene glycol diethyl ether (DEGDEE), CAS No. 112-36-7, structural formula: $C_2H_5–(O–CH_2–CH_2)_2–O–C_2H_5$						
	-	-	-	-	-	
15. Triethylene glycol(mono) ethyl ether (TEGEE), CAS No. 112-50-5, structural formula: $C_2H_5–(O–CH_2–CH_2)_3–OH$						
	-	-	-	-	-	
16. Ethylene glycol isopropyl ether (EgiPE), CAS No. 109-59-1, structural formula: $(CH_3)_2CH–O–CH_2–CH_2–OH$						
Austria	5	22	10	44	Yes	
Belgium	25	108	-	-	Yes	
Denmark	5	22	-	-	Yes	
France	25	105	-	-	Yes [f]	
Germany	5	22	20	88	Yes	If the MAK and BAT values are complied with, there should be no risk for the foetus
Ireland	25	106	-	-	Yes	
Italy	25	106	-	-	Yes	
Netherlands	10	44	-	-	Yes	
Norway	20	80	-	-	-	
Sweden	10	45	20	90	Yes	

Table C.1: OEL values[a,b] (cont'd)

Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
Switzerland	5	22	10	44	Yes	The foetus will not be harmed if the MAK value is complied with.
US-ACGIH	25	-	-	-	Yes	
17. Ethylene glycol isopropyl ether acetate (EGiPEA), CAS No. 91598-97-9, structural formula: $(CH_3)_2CH–O–CH_2–CH_2–O–CO–CH_3$						
	-	-	-	-	-	
18. Ethylene glycol *n*-propyl ether (EgnPE), CAS No. 2807-30-9, structural formula: $C_3H_7–O–CH_2–CH_2–OH$						
Denmark	25	110	-	-	-	
Germany	20	86	20	86	Yes	If the MAK and BAT values are complied with, there should be no risk for the foetus
Netherlands	10	44	-	-	Yes	
Sweden	10	45	20	90	Yes	
Switzerland	20	85	40	170	Yes	The foetus will not be harmed if the MAK value is complied with.
19. Ethylene glycol *n*-propyl ether acetate (EGnPEA), CAS No. 20706-25-6, structural formula: $C_3H_7–O–CH_2–CH_2–O–CO–CH_3$						
Germany	20	120	20	120	Yes	If the MAK and BAT values are complied with, there should be no risk for the foetus
Netherlands	10	60	-	-	Yes	
Switzerland	20	120	40	240	Yes	The foetus will not be harmed if the MAK value is complied with.

Table C.1: OEL values[a,b] (cont'd)

Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
20. Ethylene glycol phenyl ether (EGPhE), CAS No. 122-99-6, structural formula: C_6H_5–O–CH_2–CH_2–OH						
Germany	20	110	20	110	-	If the MAK and BAT values are complied with, there should be no risk for the foetus
Netherlands	20	110	-	-	-	
Switzerland	20	110	40	220	Yes	The foetus will not be harmed if the MAK value is complied with.
21. Ethylene glycol *n*-butyl ether (EGBE), CAS No. 111-76-2, structural formula: C_4H_9–O–CH_2–CH_2–OH						
Austria	20	100	40	200	Yes	
Belgium	25	123	-	-	Yes	
Denmark	20	98	-	-	Yes	
EU	20	98	50	246	Yes	
Finland	20	98	50	250	Yes	
France	25	120	-	-	Yes [f]	
Germany	20	98	80	392	Yes	If the MAK and BAT values are complied with, there should be no risk for the foetus
Ireland	25	120	-	-	Yes	
Italy	20	97	-	-	Yes	
Netherlands	20	100	50	246	Yes	
Norway	10	50	-	-	Yes	
Spain	20	98	-	-	Yes	

Table C.1: OEL values[a,b] (cont'd)

Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
Sweden	10	50	20	100	Yes	
Switzerland	20	100	40	200	Yes	
UK	25	123	-	-	Yes	
US-ACGIH	20	-	-	-	Yes	
US-NIOSH	5	24	-	-	Yes	
US-OSHA	25	120	-	-	Yes	
22. Ethylene glycol *n*-butyl ether acetate (EGBEA), CAS No. 112-07-2, structural formula: $C_4H_9–O–CH_2–CH_2–O–CO–CH_3$						
Austria	20	135	40	270	Yes	
Denmark	20	130	-	-	Yes	
EU	20	133	50	333	Yes	
Finland	20	130	50	330	Yes	
Germany	20	130	80	520	Yes	If the MAK and BAT values are complied with, there should be no risk for the foetus
Netherlands	20	135	50	333	Yes	
Norway	10	65	-	-	Yes	
Spain	20	133	50	333	Yes	
Sweden	10	70	20	140	Yes	
Switzerland	20	135	40	270	Yes	
US-ACGIH	20	-	-	-	-	
US-NIOSH	5 [g]	33	-	-	-	

Table C.1: OEL values[a,b] (cont'd)

Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
23. Diethylene glycol butyl ether (DEGBE), CAS No. 112-34-5, structural formula: C_4H_9–(O–CH_2–CH_2)$_2$–OH						
Austria	15	100	15	100	-	
Denmark	-	100	-	-	-	
Germany	-	100	-	100	-	If the MAK and BAT values are complied with, there should be no risk for the foetus
Netherlands	9	50	-	-	Yes	
Sweden	15	100	30	200	-	
Switzerland	-	100	-	100	-	The foetus will not be harmed if the MAK value is complied with.
24. Diethylene glycol butyl ether acetate (DEGBEA), CAS No. 124-17-4, structural formula: C_4H_9–(O–CH_2–CH_2)$_2$–O–CO–CH_3						
Netherlands	15	130	30	250	-	
Sweden	15	130	30	250	-	
25. Triethylene glycol *n*-butyl ether (TEGBE), CAS No. 143-22-6, structural formula: C_4H_9–(O–CH_2–CH_2)$_3$–OH						
	-	-	-	-	-	
26. Ethylene glycol (mono) *n*-hexyl ether (EGHE), CAS No. 112-25-4, structural formula: C_6H_{13}–O–CH_2–CH_2–OH						
	-	-	-	-	-	
27. Diethylene glycol (mono) hexyl ether (DEGHE), CAS No. 112-59-4, structural formula: C_6H_{13}–(O–CH_2–CH_2)$_2$–OH						
	-	-	-	-	-	

Table C.1: OEL values[a,b] (cont'd)

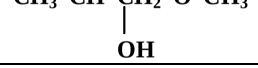
Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
28. 2-Propylene glycol 1-methyl ether (2PG1ME), CAS No. 107-98-2, structural formula: 						
Austria	50	187	50	187	Yes	
Belgium	100	374	150	561	-	
Denmark	50	185	-	-	-	
EU	100	375	150	568	Yes	
France	100	360	-	-	- [f]	
Finland	100	370	150	560	-	
Germany	100	370	100	370	-	If the MAK and BAT values are complied with, there should be no risk for the foetus
Ireland	100	360	300	1,080	Yes	
Italy	100	369	150	553	-	
Netherlands	100	375				
Norway	50	180	-	-	Yes	
Spain	100	374	200	748	Yes	
Sweden	50	190	75	300	Yes	
Switzerland	100	360	200	720	-	The foetus will not be harmed if the MAK value is complied with.
UK	100	375	300	1,120	Yes	
US-ACGIH	100	-	150	-	-	
US-NIOSH	100	360	150	540	-	

Table C.1: OEL values[a,b] (cont'd)

Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
29. 2-Propylene glycol 1-methyl ether 2-acetate (2PG1MEA), CAS No. 108-65-6, structural formula:					$CH_3–CH(O–CO–CH_3)–CH_2–O–CH_3$	
Austria	50	275	100	550	-	
Belgium	50	275	100	550	Yes	
Denmark	50	270	-	-	-	
EU	50	275	100	550	Yes	
Finland	50	270	100	550	Yes	
Germany	50	270	50	270	-	If the MAK and BAT values are complied with, there should be no risk for the foetus
Ireland	50	275	100	550	Yes	
Italy	50	275	100	550	-	
Netherlands	100	550	-	-	-	
Norway	50	270	-	-	Yes	
Spain	50	275	100	550	Yes	
Sweden	50	250	75	400	Yes	
Switzerland	50	275	50	275	-	The foetus will not be harmed if the MAK value is complied with.
UK	50	274	150	822	-	
Canada	50	75	-	-	Yes	

Table C.1: OEL values[a,b] (cont'd)

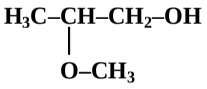
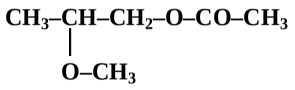
Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
US-AIHA	100	541	-	-	-	
US-California	100	541	150	811	-	
30. 1-Propylene glycol 2-methyl ether (1PG2ME), CAS No. 1589-47-5, structural formula:						
Austria	20	75	40	150	-	
Denmark	20	75	-	-	-	
EU						May cause harm to the unborn child (R60), Toxic for reproduction (Category 2)
Germany	20	75	80	300	-	
Netherlands	-	-	-	-	-	Substances toxic to reproduction
Norway	20	75	-	-	Yes	Reproduction damaging substance
Spain	20	75	-	-	-	
Sweden	50	190	75	300	Yes	
Switzerland	20	75	40	150	Yes	Harm to the foetus is possible even when the MAK value is complied with
Canada	20	-	40	-	-	Possible reproductive toxin
31. 1-Propylene glycol 2-methyl ether 1-acetate (1PG2MEA), CAS No. 70657-70-4, structural formula:						
Austria	20	110	40	220	-	

Table C.1: OEL values[a,b] (cont'd)

Country [c]	**Concentration, 8-h[d] TWA**		**STEL, 15-min**		**Skin notation**	**Pregnancy group**
	(ppm)	**(mg/m³) [e]**	**(ppm)**	**(mg/m³) [e]**		
Denmark	20	110	-	-	-	
EU	-	-	-	-	-	May cause harm to the unborn child (R61), Toxic for reproduction (Category 2)
Germany	20	110	80	440	-	
Netherlands	-	-	-	-	-	Substances toxic to reproduction
Norway	20	110	-	-	Yes	Reproduction damaging substance
Switzerland	20	110	40	220	Yes	Harm to the foetus is possible even when the MAK value is complied with
Canada	20	-	40	-	-	Possible reproductive toxin
32. Dipropylene glycol methyl ether (DPGME), CAS No. 34590-94-8, structural formula:					**$CH_3–(O–CH_2–CH(CH_3))_2–OH$**	
Austria	50	307	100	614	-	
Belgium	50	308	-	-	Yes	
Denmark	50	300	-	-	Yes	
EU	50	308	-	-	Yes	
Finland	50	310	-	-	Yes	
France	100	600	-	-	- [f]	
Germany	50	310	50	310	-	
Ireland	100	606	150	909	Yes	
Italy	50	308	-	-	Yes	

Table C.1: OEL values[a,b] (cont'd)

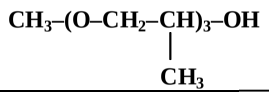
Country [c]	Concentration, 8-h[d] TWA		STEL, 15-min		Skin notation	Pregnancy group
	(ppm)	(mg/m^3) [e]	(ppm)	(mg/m^3) [e]		
Netherlands	50	300				
Norway	50	300	-	-	Yes	
Spain	50	308	-	-	Yes	
Sweden	50	300	75	450	Yes	
Switzerland	50	300	50	300	Yes	
UK	50	308	-	-	Yes	
Canada-Alberta	100	606	150	909	Yes	
Canada, British Columbia	100	-	150	-	Yes	
Canada, Ontario	100	605	150	910	Yes	
Mexico	100	600	150	900	Yes	
US-ACGIH	100	-	150	-	Yes	
US-NIOSH	100	600	-	-	Yes	
US-OSHA	100	600	-	-	Yes	
US-California	100	600	150	900	Yes	
US-North Carolina	100	600	150	900	Yes	
33. Tripropylene glycol methyl ether (TPGME), CAS No. 25498-49-1, structural formula: 						
-	-	-	-	-		

Table C.1: OEL values[a,b] (cont'd)

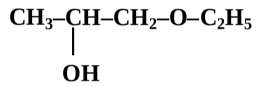
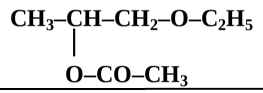
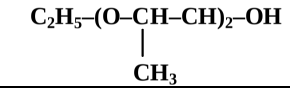
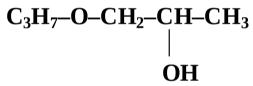
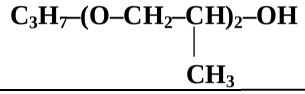
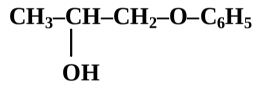
Country [c]	Concentration, 8-h[d] TWA (ppm)	(mg/m^3) [e]	STEL, 15-min (ppm)	(mg/m^3) [e]	Skin notation	Pregnancy group
34. 2-Propylene glycol 1-ethyl ether (2PG1EE), CAS No. 1569-02-4, structural formula: 						
Denmark	100	-	-	-	-	
35. 2-Propylene glycol 1-ethyl ether 2-acetate (2PG1EEA), CAS No. 54839-24-6, structural formula: 						
	-	-	-	-	-	
36. Dipropylene glycol ethyl ether (DPGEE), CAS No. 30025-38-8, structural formula: 						
	-	-	-	-	-	
37. Propylene glycol *n*-propyl ether (PGPE), CAS No. 1569-01-3, structural formula: 						
Denmark	100	-	-	-	-	
38. Dipropylene glycol *n*-propyl ether (DPGPE), CAS No. 29911-27-1, structural formula: 						
	-	-	-	-	-	
39. 2-Propylene glycol 1-phenyl ether (2PG1PhE), CAS No. 770-35-4, structural formula: 						
	-	-	-	-	-	

Table C.1: OEL values[a,b] (cont'd)

Country [c]	Concentration, 8-h[d] TWA (ppm)	(mg/m^3) [e]	STEL, 15-min (ppm)	(mg/m^3) [e]	Skin notation	Pregnancy group
40. 2-Propylene glycol 1-n-butyl ether (2PG1BE), CAS No. 5131-66-8, structural formula:					$C_4H_9–O–CH_2–CH(CH_3)–OH$	
Denmark	100	-	-	-	-	
41. Dipropylene glycol 1-butyl ether (DPGBE), CAS No. 29911-28-2, structural formula:					$C_4H_9–(O–CH_2–CH(CH_3))_2–OH$	
	-	-	-	-	-	
42. Tripropylene glycol 1-butyl ether (TPGBE), CAS No. 55934-93-5, structural formula:					$C_4H_9–(O–CH_2–CH(CH_3))_3–OH$	
	-	-	-	-	-	
43. Propylene glycol *tert*-butyl ether (PGTBE), CAS No. 57018-52-7, structural formula:					$CH_3–CH(OH)–CH_2–O–C(CH_3)_3$	
	-	-	-	-	-	
44. Dipropylene glycol *tert*-butyl ether (DPGTBE), CAS No. 132739-31-2, structural formula:					$C_6H_{13}–O–CH_2–CH_2–OH$	
	-	-	-	-	-	

[a] The value may be advisory or official (tentative or legally binding)

[b] Ariel Research, 2002

[c] For additional EU national information, see: http://europe.osha.eu.int/good_practice/risks/ds/oel/
For EU OEL activities, see: http://europe.eu.int/comm/employment_social/hands/areas/oels_en.htm
For Japan, see: http://joh.med.uoeh-u.ac.jp/oel/index.html.

[d] NIOSH 10-h TWA

[e] Some agencies use (slightly) different conversion factors based on variations in temperature, pressure and/or normal gas volume, cf. Appendix C.

[f] Affections engendrées par les solvants organiques liquides à usage professionnel: …, glycols et leurs éthers … (France, 1985)

[g] Recommended value (EGBEA)

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No. 3 Workshop on the Use of Human Data in Risk Assessment
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No. 5 Workshop on Alternative Testing Approaches in Environmental Risk Assessment
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