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Evaluation of health hazards by exposure to

Ethylene glycol

and proposal of a health-based quality criterion for ambient air

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Evaluation of health hazards by exposure to Ethylene glycol and proposal of a health-based quality criterion for ambient air

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Content

Preface

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to N,N-Dimethyl-1,3-propanediamine and proposal of a health based quality criterion for ambient air. This resulted in 2006 in the present report, which was prepared by Elsa Nielsen and Ole Ladefoged, Department of Toxicology and Risk Assessment, Danish Institute for Food and Veterinary Research, i.e. the present Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark.

The report has been subjected to review and discussion and has been endorsed by a steering committee consisting of representatives from the following Danish authorities:

The Danish Working Environment Authority,
The Danish Ministry of Food, Agriculture and Fisheries (The Faculty of Agricultural Sciences),
The Danish Veterinary and Food Administration,
The National Board of Health, Denmark,
The Danish Environmental Protection Agency

The Danish Environmental Protection Agency
Copenhagen, September 2013.

1 General description

1.1 Identity

Molecular formula:	$C_2H_6O_2$
Structural formula:	HO-CH_2-CH_2-OH
Molecular weight:	62.07
CAS-no.:	107-21-1
Synonyms:	1,2-Dihydroxyethane EG 1,2-Ethanediol Ethane-1,2-diol 2-Hydroxyethanol Glycol MEG Monoethylene glycol

1.2 Physical / chemical properties

Description:	Ethylene glycol is a clear, colourless, slightly viscous, hygroscopic liquid with a sweet taste.
Purity:	The technical product contains > 99.3% of ethylene glycol.
Melting point:	-13 °C
Boiling point:	197.56 °C (at 760 mmHg)
Density:	1.113 g/ml (at 20 °C)
Vapour pressure:	0.06 mmHg (8 Pa) (at 20 °C)
Concentration of saturated vapours:	79 ppm (204 mg/m^3) (at 20 °C and 760 mmHg) (calculated)
Vapour density:	2.14 (air = 1)
Conversion factor:	1 ppm = 2.58 mg/m^3 (at 20 °C and 760 mmHg) 1 mg/m^3 = 0.388 ppm
Flash point:	115 ºC (open cup) 111.1 ºC (closed cup)

Flammable limits:	3.2 - 21.6%
Autoignition temp.:	398 - 412.93 °C
Solubility:	Water: Miscible. Miscible with lower aliphatic alcohols, glycerol, acetic acid, acetone and similar ketones, aldehydes and pyridine. Practically insoluble in benzene, chlorinated hydrocarbons, petroleum ether and oils.
$\log P_{octanol/water}$:	-1.93 to -1.36 (at 25 ºC)
Henry's constant:	2.34×10^{-10} (atm x m^3)/mole at 25ºC
pK_a-value	-
Stability:	Ethylene glycol is very hygroscopic.
Incompatibilities:	Ethylene glycol can react vigorously with oxidants.
Odour threshold, air:	62.5 mg/m^3 (ACGIH 2001) 25 ppm (64.5 mg/m^3) (AEA Technology 1994) 90 mg/m^3 (A&H 1980)
References:	IUCLID (2000), HSDB (2002), ATSDR (1997), BUA (1991), ACGIH (2001).

1.3 Production and use

Historically, ethylene glycol has been commercially produced on a large-scale basis by hydration of ethylene oxide. Currently, it is also produced by the oxidation of ethylene in the presence of acetic acid to form ethylene diacetate, which is then hydrolysed to the glycol, with the acetic acid being recycled in the process. (ATSDR 1997, ACGIH 2001).

On a worldwide base, approximately two-thirds of ethylene glycol is used as a chemical intermediate in the manufacture of polyesters for fibres, films, bottles, etc., with a further one-quarter used as an antifreeze in engine coolants. In Western Europe, the pattern is slightly different, with about half used in polyester manufacture and a quarter in coolants. Ethylene glycol is also used for runway de-icing, as a plasticiser for adhesives, as a softening agent for cellulose film, as an ingredient of electrolytic condensers, for various heat transfer applications, as a humectant in inks, and as antifreeze and plasticiser in paints. (CICAD 2000).

1.4 Environmental occurrence

Ethylene glycol in the environment is predominantly from its production and use with the major sources being from the disposal of used antifreeze and de-icing solutions (ATSDR 1997).

Ethylene glycol has been identified as the stable end metabolite of growth-regulating ethylene in a number of higher plants (Blomstrom & Beyer 1980 – quoted from BUA 1991).

Ethylene glycol has also been identified as one of the volatile aroma substances in the edible fungus *Tricholoma matsutake*. In the raw fungus, ethylene glycol constituted 0.17% of total aroma substances, while after cooking it was no longer detectable. (Ahn & Lee 1986 – quoted from BUA 1991).

1.4.1 Air

No relevant data have been found.

1.4.2 Water

Levels measured in surface waters have been generally low, at a few micrograms per litre. Concentrations in wastewater from production plants, prior to treatment, have averaged up to 1300 mg/litre. By far the highest reported concentrations relate to runoff water from airports, with levels up to 19000 mg/litre. (CICAD 2000).

1.4.3 Soil

No data have been found.

1.4.4 Foodstuffs

Ethylene glycol has been found to migrate into a number of foods from regenerated cellulose films containing triethylene glycol and polyethylene glycol as softening agents. Ethylene glycol has also been found to migrate into food simulants from polyethylene terephthalate (PET) bottles used in the packaging of carbonated beverages. (ATSDR 1997).

Ethylene glycol has also been detected in various wines (BUA 1991).

1.5 Environmental fate

1.5.1 Air

If released to the atmosphere, ethylene glycol will mainly exist in the vapour phase. Vapour-phase ethylene glycol is expected to undergo rapid photochemical oxidation via reaction with hydroxyl radicals. (CICAD 2000, ATSDR 1997, HSDB 2002, BUA 1991).
The half-life for the photochemical oxidation of ethylene glycol has been estimated to be 8 - 84 hours (ATSDR 1997, BUA 1991).

1.5.2 Water

If released to water, both aerobic and anaerobic biodegradation is expected to be major fate processes; aerobic degradation is essentially complete in 1 to 4 days. Ethylene glycol is not expected to volatilise from water surfaces to the atmosphere due to its relatively low vapour pressure, it is not expected to be hydrolysed in water, and is not expected to adsorb to suspended solids and sediment in the water. (HSDB 2002, ATSDR 1997, BUA 1991).

1.5.3 Soil

If released to soil, biodegradation is expected to be the major fate process; complete biodegradation has been shown in one soil within 2 days and 97% biodegradation in 12 days has been reported for a second soil. Ethylene glycol has little or no capacity to bind to particulates and will have a high mobility in soil. Volatilisation of ethylene glycol is not expected to be important from moist or dry soil surfaces, due to the relatively low vapour pressure. (HSDB 2002, CICAD 2000, ATSDR 1997, BUA 1991).

1.5.4 Bioaccumulation

The low octanol/water partition coefficient and measured bioconcentration factors indicate a low capacity for bioaccumulation (HSDB 2002, CICAD 2000, ATSDR 1997, BUA 1991).

1.6 Human exposure

The most important route of human exposure to ethylene glycol for members of the general population is dermal contact with fluids used in automobiles (e.g., antifreeze, coolants, brake fluids) (ATSDR 1997, HSDB 2002).

Persons living near airports where large amounts of ethylene glycol are used for de-icing of aircraft, near hazardous waste sites, or near production and/or processing sites can be exposed from consumption of contaminated groundwater (ATSDR 1997).

Intentional or accidental ingestion of antifreeze has also been reported (ATSDR 1997).

2 Toxicokinetics

2.1 Absorption, distribution, and excretion

2.1.1 Inhalation

In nose-only exposure experiments, rats were exposed to ^{14}C-ethylene glycol vapour (32 mg/m^3) for 30 minutes or to an ethylene glycol aerosol (184 mg/m^3) for 17 minutes. Estimates indicate that at least 60% of inhaled ethylene glycol from both exposures was deposited in the nasal cavity. Absorption and distribution from the site of deposition was rapid, since in animals, which were sacrificed immediately after exposure, 75-80% of the received dose had already been distributed in the animal's body. The half time for clearance of plasma ^{14}C-activity (ethylene glycol plus metabolites) was 39 hours for ethylene glycol vapour and 34 hours for the aerosol (Marshall & Cheng 1983 – quoted from BUA 1991, NTP 1993).

2.1.2 Oral intake

The approximate serum half-life of ethylene glycol has been reported to be 2.5 hours in children (Rothman et al. 1986 – quoted from ATSDR 1997), 2.7 hours for adults during haemodialysis (Cheng et al. 1987 – quoted from ATSDR 1997), and between 3.0 and 8.4 hours in untreated adults (Jacobsen et al. 1988, Peterson et al. 1981 – both quoted from ATSDR 1997).

Reif (1950 – quoted from Ware 1988), on three separate occasions, drank pure ethylene glycol in 100 ml of water. The amounts consumed were 5.5, 11.0 and 13.2 g (corresponding to 78.5, 157, and 188.6 mg/kg, respectively, assuming a body weight of 70 kg). Ethylene glycol was recovered in the urine at 24 to 31% of the administered dose within 24 to 48 hours. Oxalic acid concentrations in the urine were higher than normal for 8 to 12 days, with a peak on the fourth day.

In rats, ingested ethylene glycol is rapidly absorbed and evenly distributed throughout the body reaching peak blood levels at 1 to 4 hours after ingestion of doses of 7-29 mg/kg (Winek et al. 1978 – quoted from ATSDR 1997).

The kinetics of orally administered ethylene glycol and its major metabolites, glycolic acid and oxalic acid, in pregnant rats were compared across doses, and between pregnant (P) and non-pregnant (NP) rats. Groups of rats were administered ^{13}C-ethylene glycol by gavage at doses of 10 (P and NP), 150 (P), 500 (P), 1000 (P), or 2500 (P and NP) mg/kg b.w. (Pottenger et al. 2001).
Pregnancy status (gestation days 10-11) had no significant impact on the blood concentration-time profiles of ethylene glycol, glycolic acid, or oxalic acid. Thus, the kinetic parameters estimated (C_{max}, T_{max}, AUC, and $\beta t_{½}$) did not differ significantly between the pregnant and non-pregnant groups.
The T_{max} for ethylene glycol for all dose groups occurred at one hour after dosing; the blood concentration decreased in a linear fashion thereafter and was no longer detectable for the low dose group (10 mg/kg) by 12 hours post-dosing and by 24 hours for the 150 and 500 mg/kg dose groups. The estimated half time for elimination of ethylene glycol from blood was less than 2 hours for all dose levels. Blood levels of glycolic acid increased to a peak at 3 hours post-dosing, except at the

lowest dose level (10 mg/kg) where this metabolite could not be detected in the blood. The blood levels decreased by 24 hours post-dosing to undetectable levels. The concentrations of oxalic acid in the blood varied between undetectable and about 2 times the limit of quantification over the 24-hour collection period. There were no substantial differences in the urinary elimination profiles between the pregnant and non-pregnant dose groups, at comparable dose levels. Urinary elimination of ethylene glycol and its metabolites demonstrated dose-dependency, with the high dose groups (2500 mg/kg) eliminating almost 70% of the administered dose in urine, compared with about 16% in the low dose groups (10 mg/kg). The shift in urinary elimination was mainly due to increased urinary glycolic acid and ethylene glycol, and not to increased elimination of oxalic acid.

In rats, 10-20% of oral doses up to 1000 mg/kg of ethylene glycol were recovered from the body tissues and carcass 96 hours after dosing, whereas mice retained only a small percentage of the dose in their tissues. Total recovery of the oral doses in rats and mice was approximately 90-100%, indicating substantial absorption. The major excretory route of [^{14}C] was via exhalation of carbon dioxide (42%), while 24% of the dose was excreted via the urine and 3% via the faeces. (Frantz et al. 1989, 1991 – quoted from ATSDR 1997).
The elimination half-life in plasma has been estimated at 1.7 hours in rats given 2000 mg/kg and 1.4 to 2.5 hours following administration of 10 to 1000 mg/kg (Frantz et al. 1989 – quoted from ATSDR 1997).

Mice showed a similar profile, exhaling 55% of the dose, and excreting 24% in the urine and up to 12% in the faeces. The elimination half-life in plasma in mice has been estimated at 0.3 to 1.1 hours following administration of 10 to 1000 mg/kg. (Frantz et al. 1991 – quoted from ATSDR 1997).

In contrast, approximately 50% of an oral dose of ethylene glycol administered to dogs was excreted via the urine (Grauer et al. 1987 – quoted from ATSDR 1997).

2.1.3 Dermal contact

The *in vitro* permeability of human skin to ethylene glycol was determined by Loden (1986 – quoted from ATSDR 1997); the rate of resorption was 118 µg/cm^2/hour, with a steady state concentration of 0.97 mg/cm^2.

^{14}C-ethylene glycol was applied to the surface of three different fresh human skin samples at a dose of 8 µg/cm^2. After 24 hours of exposure, 18.3% of the applied dose was recovered from the receptor fluid (absorbed through the skin), 8.3% in the skin, and 12.5% in the skin surface, for a total of approximately 39% recovery of the applied dose. Individual differences existed for the three samples; average absorption was 26.6%. This represented an absorption rate of approximately 0.09 µg/cm^2/hour for ethylene glycol. (Driver et al. 1993 – quoted from ATSDR 1997).

In dermal applications using an occlusion bandage, approximately 30% of a dose of ethylene glycol up to 1000 mg/kg was absorbed through rat skin; 14% of the absorbed dose was expired, while 7% was excreted in the urine, and 1% was recovered from the faeces (Frantz et al. 1989 – quoted from ATSDR 1997).

Following administration of 100 mg/kg undiluted radiolabeled ethylene glycol to mice using an occlusive bandage, 99.5% of the dose was recovered, with tissues and excreta accounting for 76.5%. Most was recovered as volatile organic radioactivity (25-39%) or as radioactive carbon dioxide (8-12%). Urine and faeces each accounted for another 4.9% of the dose. Tissue recoveries were less than 1% of the

dose, while the residual carcass contained about 10-18% of the dose. Following application of 1000 mg/kg undiluted ethylene glycol or as an 50% aqueous solution, the total recovery was 89% of the dose with 84% in tissues and excreta, and approximately 7% in faeces, cage wash water, and carcass. According to the authors, mice absorbed 85-100% of the administered dermal dose. (Frantz et al. 1991 – quoted from ATSDR 1997).

2.2 Metabolism

The metabolic pathway for ethylene glycol is shown in Figure 2.2. Solid arrows represent the steps that are quantitatively most important, while the broken arrows indicate minor metabolic conversions in humans. (ATSDR 1997).

Ethylene glycol is oxidised to glycolaldehyde by NAD-dependent alcohol dehydrogenase in the liver and kidney. Glycolaldehyde is further oxidised to glycolic acid by mitochondrial aldehyde dehydrogenase and cytosolic aldehyde oxidase. There is no evidence for an accumulation of glycolaldehyde and it appears to be rapidly metabolised to glycolic acid (Jacobsen & McMartin 1997).
A small amount of glycolaldehyde is converted to glyoxal, which, in presence of lactate dehydrogenase and/or aldehyde oxidase, is further converted to glycolic acid or is directly metabolised via an oxidative mechanism to glyoxylic acid.
Further degradation of glycolic acid is oxidation to glyoxylic acid by glycolic acid oxidase or lactic dehydrogenase. This step occurs apparently at a slow rate since glycolate has been shown to accumulate in large amounts (Jacobsen & McMartin 1997).
Glyoxylic acid is further metabolised via a number of intermediate metabolic pathways, for example, to oxalic acid by the enzyme glycolic acid oxidase and, via formic acid, to carbon dioxide and water, or to glycine. The major metabolic route in terms of toxicological importance is the conversion to oxalate and the most important alternate pathway appears to be formation of glycine (Jacobsen & McMartin 1997). Glyoxylate can induce lactic acid formation via oxalomalate production and its inhibitory effects on the citric acid cycle.
(ATSDR 1997, Jacobsen & McMartin 1997, BUA 1991, Cavender & Sowinski 1994).

2.3 Mode of action

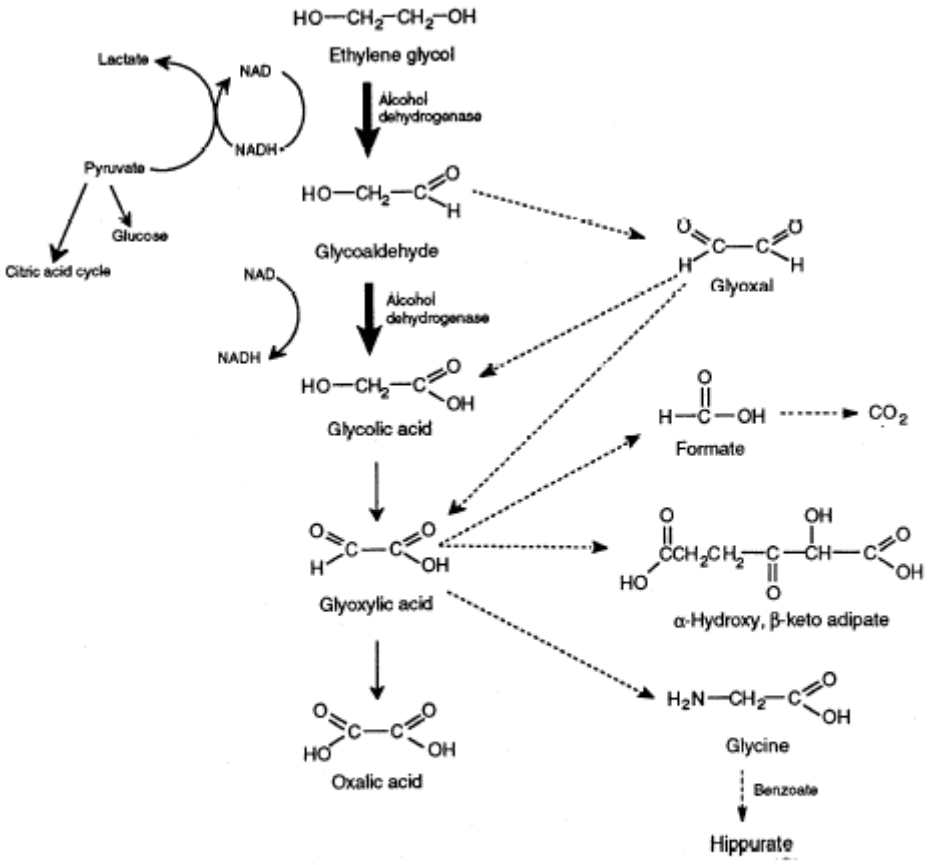
The toxicity of ethylene glycol is mainly a result of the effects of its metabolites such as glycolaldehyde, glycolic acid, glyoxylic acid, and oxalic acid. (ATSDR 1997, Jacobsen & McMartin 1997, LaKind et al. 1999, BUA 1991, NTP 1993).

The clinical symptoms of acute ethylene glycol poisoning in humans can be divided into three (and occasionally four) fairly distinct stages (see also part 3.1.2) (LaKind et al. 1999, Cavender & Sowinski 1994, BUA 1991, ACGIH 2001).

The first stage, which usually begins within 30 minutes to 12 hours after ingestion, consists primarily of central nervous system (CNS) toxicity and is usually attributed both to unmetabolised ethylene glycol and to the formation of aldehydes that peak 6 to 12 hours after ingestion (LaKind et al. 1999). The appearance of cerebral symptoms coincides with the highest concentration of the metabolite, glycolaldehyde (Balazs et al. 1982 – quoted from BUA 1991).

The second stage has been described as the cardio-pulmonary toxicity stage that occurs 12 to 72 hours after ingestion. This stage may also be characterised by severe metabolic acidosis. (LaKind et al. 1999).
Aldehyde intermediates are held responsible for the cytotoxic effects and glycolaldehyde seems to be responsible for the observed cardio-pulmonary symptoms (Balazs et al. 1982 – quoted from BUA 1991).

Figure 2.2. Metabolic pathway of oxidation of ethylene glycol. From ATSDR (1997).



Adapted from Gabow et al. 1986; Jacobsen et al. 1988; Robinson and McCoy 1989; Vale 1979; Wiener and Richardson 1988.

Remark: glycolaldehyde is oxidised to glycolic acid by aldehyde dehydrogenase, not by alcohol dehydrogenase as stated in the figure.

The third stage is known as the “renal failure” stage and occurs 24 to 72 hours after ingestion. This stage is characterised by profound acidosis caused, according to LaKind et al. (1999), primarily by the accumulation of the metabolites glycolic acid and glyoxylic acid.
Recent studies of cases of human ethylene glycol poisoning have demonstrated that the major determinant of the metabolic acidosis is the degree of glycolic acid accumulation and glycolate accumulation correlates with the increase in anion gap or

decrease in arterial bicarbonate concentrations in humans, as well as in animals. Several studies have suggested accumulation of glyoxylate, although at much lower levels (60 µM) than that of glycolate (15 mM). Glycolate is less toxic *in vitro* than either glycolaldehyde or glyoxylate. Whether the levels of glyoxylate that have been measured in human cases can contribute to the clinical features cannot yet be determined. (Jacobsen & McMartin 1997).
Calcium oxalate precipitation within the renal tubules has long been accepted as an important pathogenic factor for the development of renal toxicity; however, the mechanism of the renal toxicity is not yet known and there is no evidence directly linking oxalate precipitation with development of renal tubular necrosis. Furthermore, the renal damage can occur at levels of exposure where no or few crystals of oxalate are detected. Therefore, the renal toxicity has also been suggested to occur from a metabolite-induced cytotoxicity. (Jacobsen & McMartin 1997, LaKind et al. 1999).
Glycolic acid and glyoxylic acid react with bicarbonate causing a decrease in the pH in body fluids, particularly in blood. An elevated anion gap develops, and the serum osmolal gap across cells increases, resulting in renal oedema that compromises intrarenal blood flow and promotes renal failure. Blood phosphorous (inorganic) levels are increased due to uncoupling of oxidative phosphorylation, and, as a result, blood calcium levels are lowered. Acidosis results in oliguric or anuric renal failure with renal changes. (LaKind et al. 1999).

Generally, the role of oxalate accumulation in the toxicity of ethylene glycol has not been clarified. Urinary calcium oxalate crystals are an important hallmark of ethylene glycol poisoning. However, plasma oxalate determinations in human cases have showed low levels (< 0.4 mM) possibly because the oxalate in plasma rapidly precipitates as calcium oxalate, whose crystals have been noted in the kidney, brain and other tissues. Such precipitation probably leads to the hypocalcaemia that is characteristic of ethylene glycol poisonings. (Jacobsen & McMartin 1997).

Other neurological symptoms, effects on cranial nerves, have been identified 6 or more days after ingestion and constitute a possible fourth clinical stage of toxicity. The mechanism(s) of these delayed neurological effects is unknown, but is distinctly different from the pathological events of the first three stages. The cause may be related to oxalate crystal deposition or, perhaps, ethylene glycol may induce pyridoxine deficiency, resulting in peripheral neuropathy. (LaKind et al. 1999).

The mechanism(s) of ethylene glycol toxicity as related to developmental effects warrants further exploration. A link between maternal metabolic acidosis and developmental toxicity has been suggested. (LaKind et al. 1999).
Pottenger et al. (2001) has suggested that glycolic acid is the proximate developmental toxicant.

3 Human toxicity

3.1 Single and repeated dose toxicity

3.1.1 Inhalation

Twenty male volunteers (prisoners, age not reported) were exposed for 30 days, 20-22 hours a day, to ethylene glycol atmospheres (aerosol, diameters of droplets 1-5 µm) containing mean concentrations of 17 to 49 mg/m^3 (lowest concentration: 0.8 mg/m^3; highest concentration: 67 mg/m^3). Fourteen other volunteers, as a control group, were treated as similarly as possible to the exposed group. Follow-up observations were made on both groups two weeks after the end of the exposure period. No subject experienced any serious signs of toxicity, but there were complaints of irritation of the throat and slight headache and low backache were also reported occasionally.
During the last two weeks of the experiment, the concentration of ethylene glycol was intentionally increased up to 308 mg/m^3 during the absence of the volunteers and observation was made of their responses to these elevated concentrations of the aerosol when they re-entered the exposure chamber. When the volunteers returned to a concentration of ethylene glycol in the chamber air of 188 mg/m^3, this concentration was irritating but could be tolerated for 15 minutes. A concentration of 244 mg/m^3 could not be tolerated for more than a minute or two and a concentration of 308 mg/m^3 could not be tolerated at all. Other similar trials revealed that concentrations of ethylene glycol in the air greater than about 200 mg/m^3 were intolerable due to strong irritation of the upper respiratory tract, with a burning sensation along the trachea and a burning cough. The irritation became common when the concentration of ethylene glycol in the chamber air was raised to about 140 mg/m^3.
Exposure to ethylene glycol under the conditions of this study produced no significant alterations of the haematological, clinically chemical, or clinically pathological parameters studied, including the concentrations of urea nitrogen and creatinine in the blood of the exposed volunteers.
(Wills et al 1974).

Troisi (1950 – quoted from LaKind et al. 1999 and from BUA 1991) examined 38 female workers at a condenser factory who were exposed for 1½ to 5 years to vapour from a mixture of ethylene glycol (40%), boric acid (55%), and ammonia (5%) kept at a temperature of 105 °C. Nine workers reportedly suffered from nystagmus and frequently lost consciousness; five other workers exhibited nystagmus only. Following changes to the production process, which precluded further exposure, the reported symptoms ceased to occur.

3.1.2 Oral intake

There are numerous case reports in the literature of poisoning in humans due to accidental or intentional ingestion of ethylene glycol. In the United States, 6 to 60 deaths/year have been attributed to ethylene glycol ingestion. (LaKind et al. 1999, Jacobsen & McMartin 1997, ACGIH 2001, BUA 1991, ATSDR 1997).

The clinical symptoms of acute ethylene glycol poisoning can be divided into three (and occasionally four) fairly distinct stages. The severity of these stages and the

advance from one stage to the next depends greatly on the amount of ethylene glycol entering the body. (LaKind et al. 1999, Jacobsen & McMartin 1997, Cavender & Sowinski 1994, BUA 1991, ACGIH 2001).
The first stage consists primarily of "central nervous system (CNS) effects". This stage usually occurs shortly after ingestion of ethylene glycol, within 30 minutes to 12 hours. The CNS effects are characterised by signs of drunkenness (but without the characteristic breath odour of alcohol), nausea, vomiting, and at large doses coma followed by convulsions and death in some cases. Mild hypotension, tachycardia, low-grade fever, depressed reflexes, generalised or focal seizures, myoclonic jerks, and titanic contractions can occur. Ocular signs (nystagmus, ophthalmoplegia, papilledema, and subsequent optic atrophy) have also been reported.
The second stage has been described as the "cardiopulmonary effects" stage that occurs 12 to 72 hours after ingestion. This stage may also be characterised by severe metabolic acidosis. Symptoms that occur include tachypnoea, tachycardia, mild hypotension, and cyanosis. In severe cases, pulmonary oedema, bronchopneumonia, cardiac enlargement, and congestive failure are present. Hypocalcaemia may occur secondary to precipitation of metabolically formed oxalate and calcium deposits. Death in this stage usually occurs between 24 and 72 hours after exposure and is attributed to pulmonary oedema, cardiac dilation, and bronchopneumonia.
The third stage is known as the "renal failure" stage and occurs 24 to 72 hours after ingestion. This stage is characterised by profound acidosis. The renal damage may vary from mild increase in blood urea nitrogen and creatinine followed by recovery, to complete anuria with acute tubular necrosis that can lead to death. Histological changes include tubular epithelial degeneration and necrosis and oxalate crystal deposition in the kidney, lower urinary tract, and other organs (e.g., the brain).
The three clinical stages of acute oral ethylene glycol toxicity may overlap with the different latency periods before each stage, depending on the amount of ethylene glycol ingested. Often, Stage II never develops, yet the patient shows symptoms of the third stage of intoxication. (LaKind et al. 1999).
Other neurological symptoms, effects on cranial nerves, have been identified six or more days after ingestion and constitute a possible fourth clinical stage. These delayed neurological effects have resulted in facial paralysis, facial palsy, hearing loss, elevated protein levels in the cerebrospinal fluid, and bilateral cranial nerve dysfunction. These symptoms are uncommon and the mechanism of injury is unknown, but is distinctly different from the pathological events of the first three stages. (LaKind et al. 1999).

The oral dose of ethylene glycol required to cause death in humans is not well defined in the literature (ATSDR 1997).
The minimal lethal dose has been estimated, based on poisonings from accidental or intentional ingestion, at about 100 ml (about 111 g corresponding to about 1.6 g/kg b.w. for an adult) (LaKind et al. 1999, ACGIH 2001, BUA 1991, Cavender & Sowinski 1994, ATSDR 1997, NTP 1993).
However, early diagnosis and appropriate therapeutic measures can significantly reduce mortality, so that even doses of 970 ml (about 1080 g corresponding to 15 g/kg b.w. for an adult) have been survived (Gaultier et al. 1976 – quoted from BUA 1991).

3.1.3 Dermal contact

The potential for subchronic human exposure to ethylene glycol (as well as other substances) to induce effects in motor-servicing workers who performed various tasks that brought them into contact with motor vehicle antifreeze has been studied by Laitinen et al. (1995 – quoted from LaKind et al. 1999). Individual exposures

were intermittent, but the workers had been performing their jobs for up to 23 years. Exposures resulted in enhanced urinary excretion of ethylene glycol by workers compared with office worker controls, as well as enhanced ammonia excretion (typical of chronic acidosis associated with ethylene glycol exposure). Because measured airborne levels of ethylene glycol were below the detection limits, the authors concluded that exposure occurred primarily via dermal contact with ethylene glycol during extended contact with the antifreeze.

3.2 Irritation

3.2.1 Skin irritation

In order to study the irritation and sensitisation potential, ethylene glycol was applied to infrascapular skin of 401 human volunteers nine times over a 3-week period, in 24-hour occluded and semioccluded patch tests. Sites were evaluated after 24 hours for local irritancy. Initial and challenge applications resulted in marginal erythematous reactions in 9.3 and 12.2% of the individuals, respectively. Definite erythema, seen in a smaller group during the induction period, suggested potential cumulative irritation. (Union Carbide 1990 – quoted from LaKind et al. 1999).

One-inch-square gauze pads completely wetted with 0.11, 1.1, or 10% (v/v) ethylene glycol were applied to the backs of 13 volunteers with no known previous exposure to ethylene glycol. No skin reactions were reported from any of the patches after 1, 2, 4, or 8 hours. (Shupack et al. 1981 – quoted from LaKind et al. 1999 and from ACGIH 2001).

Out of 1556 dermatitis patients subjected to 20/24-hour closed patch tests, 3.9% (61) experienced an irritant response (of unspecified severity) to neat ethylene glycol (Hannuksela et al. 1975 – quoted from IUCLID 2000).

After treatment with a concentration of 5% ethylene glycol in water, one woman showed strong skin reactions while 10 other subjects showed no reactions (Hindson & Ratcliffe 1975 – quoted from IUCLID 2000).

The application of 3% ethylene glycol in ethanol (patch test) resulted in a positive reaction in 1 out of 9 persons (Dawson 1976 – quoted from IUCLID 2000).

Ethylene glycol produces no significant irritant effect on the skin. A slight maceration of the skin may result from prolonged exposures. (Rowe & Wolf 1982 – quoted from Cavender & Sowinski 1994 and from IUCLID 2000).

3.2.2 Eye irritation

Exposure of human eyes to vapour or spray of ethylene glycol for 4 weeks at 17 mg/m^3 resulted in no effects (Grant 1986 – quoted from Cavender & Sowinski 1994 and from ACGIH 2001).

A splash of neat ethylene glycol into the eye of a worker produced marked inflammation, but no permanent damage (BIBRA 1993 – quoted from IUCLID 2000).

3.3 Sensitisation

Ethylene glycol was applied to infrascapular skin of 401 human volunteers nine times over a 3-week period, in 24-hour occluded and semioccluded patch tests. Sensitisation was assessed after a 3-week rest, by 24-hour patch test challenges at distal sites. Sensitisation was suggested in fewer than 1% of the volunteers, but this response was not confirmed after rechallenge. (Union Carbide 1990 – quoted from LaKind et al. 1999).

In 15 (1%) out of 1556 dermatitis patients subjected to 20/24 hour closed patch tests, the skin reaction was described as allergic in character (Hannuksela et al. 1975 – quoted from IUCLID 2000).

Allergic dermatitis have been reported in two workers handling a fluid containing ethylene glycol (25 or 33%) in the preparation of contact lenses for periods of 3 to 4 months. The sensitisation was confirmed by their positive reactions to 48-hour closed patch tests. The application of 3% ethylene glycol in ethanol or 5% in water to the skin of the two workers induced a positive reaction. (Dawson 1975, Hindson & Ratcliffe 1975 – both quoted from IUCLID 2000 and from BUA 1991).

3.4 Toxicity to reproduction

No data have been found.

3.5 Mutagenic and genotoxic effects

No data have been found.

3.6 Carcinogenic effects

No data have been found.

4 Animal toxicity

4.1 Single dose toxicity

The acute toxicity of ethylene glycol in experimental animals closely mirrors the acute effects seen in humans (see part 3). One exception is the fourth clinical stage of delayed cranial neurological consequences observed in humans; no studies have been found that reported this stage in animals (LaKind et al 1999).

Susceptibility to ethylene glycol intoxication varies with species, sex, and individual (NTP 1993). Cats appear to share the susceptibility of humans; however, because cats are unusually sensitive to ethylene glycol due to their high baseline production of oxalic acid, they are not representative of experimental animal species predictive of human responses to ethylene glycol exposure (LaKind et al 1999).

4.1.1 Inhalation

According to Cavender & Sowinski (1994), the one-hour LC_{50}-value in rats is 10.9 g/m^3.

In a study of rats, all animals survived an 8-hour exposure to saturated atmosphere (20 ºC: ca. 200 mg/m^3 (calculated)) of ethylene glycol (BASF 1961 – quoted from IUCLID 2000).

4.1.2 Oral intake

The reported oral LD_{50}-values for ethylene glycol in rats ranged from >2.0 to 11.3 g/kg b.w. (IUCLID 2000, LaKind et al. 1999, NTP 1993, BUA 1991, Cavender & Sowinski 1994, A&H 1993).

In other experimental animals, the reported oral LD_{50}-values ranged from 5.89 to 15.4 g/kg b.w. in mice, from 7.0 to 9.3 g/kg b.w. in rabbits, and from 4.0 to 8.2 g/kg b.w. in guinea pigs (IUCLID 2000, LaKind et al. 1999, NTP 1993, BUA 1991, Cavender & Sowinski 1994, A&H 1993).

For the cat, oral LD_{50}-values of 1670 and 4700 mg/kg b.w. have been reported and for the dog, from 4000 to 8200 mg/kg b.w. (IUCLID 2000, A&H 1993).

A minimal lethal dose of 3800 mg/kg b.w. has been reported for rats, of 1000 mg/kg b.w. for cats, and of 6700 and 7300 mg/kg b.w. for dogs (LaKind et al. 1999).

Monkeys (Macaca fascicularis) received ethylene glycol orally at doses of 1000, 2000, or 4000 mg/kg b.w. and were observed 14 days after dosing. At the highest dose level, the two animals were found comatose 22 hours after dosing and died 26 and 28 hours after treatment. No further details are given. (ICI 1979 – quoted from IUCLID 2000).

4.1.3 Dermal contact

Dermal LD_{50}-values for ethylene glycol of 9.53 g/kg b.w. (Cavender & Sowinski 1994, A&H 1993) and of 10.6 g/kg b.w. have been reported for the rabbit (IUCLID 2000, BUA 1991).

4.2 Irritation

4.2.1 Skin irritation

Guillot et al. (1982) have evaluated skin irritation in rabbits by determination of the primary cutaneous irritation index under patch-test and by determination of the cumulative irritation index after repeated exposure (6 weeks) according to official French methods but with some complements or modifications.
In the primary cutaneous irritation test (the experimental procedure is quoted from BUA 1991), 0.5 ml of neat ethylene glycol was applied to undamaged and scarified skin of 6 rabbits and covered with an occlusive dressing. The duration of exposure was 23 hours and assessments of the effects were made one hour and 48 hours after removal of the dressing. Ethylene glycol showed a primary irritation index of 0.08 (an index of up to 0.5 is considered non-irritant and an index of 0.5 to 2.0 as slightly irritant).
In the cumulative cutaneous irritation test (the experimental procedure is quoted from IUCLID (2000), three rabbits were treated daily with 2 ml of neat ethylene glycol or with a 10% aqueous solution for 6 weeks. Ethylene glycol undiluted showed a mean maximum irritation index of 0.47 interpreted as 'well tolerated' and the 10% solution an index of 0 interpreted as 'very well tolerated (the maximum index that could be scored is not stated; the highest index scored was 1.83 for polypropylene glycol and interpreted as 'relatively well tolerated').

Ethylene glycol was tested for skin irritative properties according to the Draize test in female New Zealand rabbits. Skin response was evaluated 24 and 72 hours after treatment. Ethylene glycol had a low potential for skin irritation (score 0.4, with a score of <2.0 meaning mild or no irritation). (Clark et al. 1979).

4.2.2 Eye irritation

Moderate to severe eye irritation was observed in rats and rabbits exposed continuously for 90 days to ethylene glycol (vapour) at a concentration of 12 mg/m^3. Two rats developed corneal opacity after 8 days and appeared to be blind for the remainder of the exposure. Erythema, oedema, and discharge began in rabbits after 3 days of exposure, the oedema being severe enough to result in virtual closure of the eyes. Guinea pigs, dogs, and monkeys exposed similarly showed no effects on the eyes. See also part 4.2.1. (Coon et al. 1970).

Rats, guinea pigs, rabbits, dogs, and monkeys were exposed to a concentration of 57 mg/m^3 of ethylene glycol (vapour) 8 hours a day, 5 days per week for 6 weeks; none of the animals showed any signs of ocular irritation. In rabbits exposed similarly to 10 mg/m^3, mild conjunctivitis was noted in one eye of each of 2 rabbits during the 4th and 5th weeks, which persisted until the end of the exposure; each of these rabbits also developed a small lesion over the irritated eye. These signs were, according to the authors, probably brought on by accidental trauma, which may have been aggravated by the exposure. See also part 4.2.1. (Coon et al. 1970).

Diluted ethylene glycol (10, 20, or 50 % solution in water) caused only slight oedema and erythema under occlusive conditions in the eyes of rabbits. Instillation of neat ethylene glycol produced moderate to severe oedema and erythema. No effects were observed 48 hours post-treatment. According to IUCLID, ethylene glycol is evaluated as being moderately irritating to the eyes. (Star 1980 – quoted from IUCLID 2000 and from BUA 1991).

Toxicity and irritation of ethylene glycol were assessed in rabbit eyes following multiple topical or multiple intraocular (anterior chamber) administrations. The concentration of the test solutions was 0.04, 0.4, 4.0, and 40% (topical administration only) in balanced salt solution. The balanced salt solution and saline were used as negative controls. Only sterile test solutions were used. (McDonald et al. 1972).
Regarding multiple topical administration, one drop (approximately 0.05 ml) of the test solution was instilled into the cul-de-sac of the test eye (6 eyes per concentration) at 10-minute intervals for a total of 36 applications in a 6-hour period. All eyes were examined with a biomicroscope at 6 hours after the first application and the eyes were graded (iris, flare, cornea) according to an arbitrary numerical score. Eyes were also scored for palpebral and bulbar conjunctival irritation by the method of Draize at 2, 4, and 6 hours after treatment and daily thereafter, and scored. Based on the Draize scores, 0.4% was the highest concentration to be non-irritating. Irritation at higher concentrations consisted of chemosis, swelling, and conjunctival redness. No test concentration elicited significant toxic findings on biomicroscopic examination. All eyes were normal by 7 days.
Regarding multiple intraocular administration, 0.5 ml of the test solution was instilled once per day for 5 days into the anterior chamber. All eyes were examined with a biomicroscope before test and on days 2, 4, 7, and 14 after the first injection and the eyes were graded (iris, flare, cornea) according to an arbitrary numerical score. Based on the biomicroscopic scores, 0.4% was the highest concentration to be non-toxic.
No evidence of systemic toxicity was, according to the authors, observed following extraocular and intraocular administration, based on behaviour, appearance, and body weight.

Twenty-four hours after the application of 0.5 ml 80% ethylene glycol into the eye of rabbits, no eye irritation could be observed. The lowest non-irritating concentration of ethylene glycol was 20% when applied as 0.1 ml solution 5 times a day for 21 consecutive days. (McDonald et al. 1977 – quoted from IUCLID 2000 and from BUA 1991).

Guillot et al. (1982) have evaluated eye irritation in rabbits by determination of the ocular irritation index according to official French methods but with some complements or modifications. Ethylene glycol (0.1 ml) was instilled into the eyes of six rabbits. It is not stated whether the test substance was washed out or not. Assessments of the irritative effect were made 1, 24, 48, 72, 96, and 168 hours after treatment. Ethylene glycol showed an ocular irritation index of 11.33; a compound was not considered to provoke any significant injury to the eye mucous membrane when no opacity of the cornea occurred and when the ocular irritation index was less than 15. The experimental procedure is quoted from BUA (1991).

Ethylene glycol was tested for eye irritative properties according to the Draize test in female New Zealand rabbits. Eyes were inspected 1, 24, 48, 72 and 96 hours after instillation of 0.1 ml of the fluid (>99% ethylene glycol). A slight irritation was observed one hour after treatment (score: 3.0), but continually diminished with time and could not be noticed after 96 hours. The maximum score possible for a single evaluation was 110. (Clark et al. 1979).

4.2.3 Sensitisation

No data have been found.

4.3 Repeated dose toxicity

4.3.1 Inhalation

4.3.1.1 Rats

Rats (15 Sprague-Dawley and Long-Evans male and female animals per group, no information about distribution between species and sexes) were exposed to ethylene glycol (vapour) continuously at a concentration of 12 ± 2 mg/m^3 for 90 days (continuous study), or at concentrations of 10 ± 1 or 57 ± 14 mg/m^3 for 8 hours a day, 5 days per week for 6 weeks (repeated study). The control group consisted of 123 animals. (Coon et al. 1970).
In the continuous study, 1 rat died during exposure and 4 rats in the control group. All haematological data were within normal limits. Observations during necropsy revealed normal organs and tissues. Histopathological examination showed inflammatory changes in the lungs of exposed animals and to a lesser degree in controls. Moderate to severe eye irritation was observed and 2 animals developed corneal opacity after 8 days and appeared to be blind for the remainder of the exposure.
In the repeated study, there were no deaths at both exposure levels and all haematological values were within normal limits. At 10 mg/m^3, histopathological examination revealed fatty changes and focal necrosis in the liver in 1 of 8 rats. At 57 mg/m^3, histopathological examinations revealed non-specific inflammatory changes in the lungs and occasionally the hearts of exposed animals. None of the animals showed any signs of ocular or nasal irritation.

Rats (10 animals) were exposed to ethylene glycol at concentrations of 350 to 400 mg/m^3 8 hours a day, 5 days per week for 16 weeks. One rat died. The histological examination of brain, lung, heart, liver, pancreas, spleen, lymph nodes, kidneys, adrenals, testes, stomach, and caecum did not show any substance-related pathological changes. (Wiley et al. 1936 – quoted from BUA 1991, ACGIH 2001, Cavender & Sowinski 1994 and from IUCLID 2000).

4.3.1.2 Mice

Mice (20 animals) were exposed to ethylene glycol at concentrations of 350 to 400 mg/m^3 8 hours a day, 5 days per week for 16 weeks. Three mice died. The histological examination of brain, lung, heart, liver, pancreas, spleen, lymph nodes, kidneys, adrenals, testes, stomach, and caecum did not show any substance-related pathological changes. (Wiley et al. 1936 – quoted from BUA 1991, ACGIH 2001, Cavender & Sowinski 1994 and from IUCLID 2000).

4.3.1.3 Guinea pigs

Guinea pigs (15 male and female animals per group, no information about distribution between sexes) were exposed to ethylene glycol (vapour) continuously at a concentration of 12 ± 2 mg/m^3 for 90 days (continuous study), or at concentrations

of 10 ± 1 or 57 ± 14 mg/m^3 for 8 hours a day, 5 days per week for 6 weeks (repeated study). The control group consisted of 73 animals. (Coon et al. 1970).
In the continuous study, 3 animals died during exposure. All haematological data were within normal limits. Observations during necropsy revealed normal organs and tissues. Histopathological examination showed inflammatory changes in the lungs of exposed animals and to a lesser degree in controls. Occasional foci of inflammatory cells were seen in kidneys from several animals, but this was not interpreted, by the authors, as being specific chemically induced changes.
In the repeated study, there were no deaths at both exposure levels and all haematological values were within normal limits. At 10 mg/m^3, histopathological examination revealed hepatic fatty changes in 2 of 8 animals and focal necrosis in the liver in 1 of 8 animals; focal necrosis of the liver was also seen in 1 of 3 control guinea pigs. At 57 mg/m^3, histopathological examinations revealed non-specific inflammatory changes in the lungs and occasionally the hearts of exposed animals. The livers of 1 of 8 animals revealed areas of focal necrosis; this was not considered, by the authors, to be chemically induced. None of the animals showed any signs of ocular or nasal irritation.

4.3.1.4 Rabbits

Male New Zealand albino rabbits (3 animals per group) were exposed to ethylene glycol (vapour) continuously at a concentration of 12 ± 2 mg/m^3 for 90 days (continuous study), or at concentrations of 10 ± 1 or 57 ± 14 mg/m^3 for 8 hours a day, 5 days per week for 6 weeks (repeated study). The control group consisted of 12 animals. (Coon et al. 1970).
In the continuous study, 1 rabbit died during exposure. All haematological data were within normal limits. Observations during necropsy revealed normal organs and tissues. Histopathological examination showed inflammatory changes in the lungs of exposed animals and to a lesser degree in controls. One rabbit had hamartomatosis (a benign tumour-like nodule) in liver bile ducts, but this was not interpreted, by the authors, as being a specific chemically induced change. Moderate to severe eye irritation was observed and erythema, oedema, and discharge began after 3 days of exposure, the oedema being severe enough to result in virtual closure of the eyes.
In the repeated study, there were no deaths at both exposure levels and all haematological values were within normal limits. At 10 mg/m^3, mild conjunctivitis was noted in one eye of each of two rabbits during the 4th and 5th weeks, which persisted to the end of the exposure; each of these rabbits also developed a small lesion over the irritated eye. These signs were, according to the authors, probably brought on by accidental trauma, which may have been aggravated by the exposure. At 57 mg/m^3, histopathological examinations revealed non-specific inflammatory changes in the lungs and occasionally the hearts of exposed animals. None of the animals showed any signs of ocular or nasal irritation.

4.3.1.5 Dogs

Male Beagle dogs (2 animals per group) were exposed to ethylene glycol (vapour) continuously at a concentration of 12 ± 2 mg/m^3 for 90 days (continuous study), or at concentrations of 10 ± 1 or 57 ± 14 mg/m^3 for 8 hours a day, 5 days per week for 6 weeks (repeated study). The control group consisted of 12 animals. (Coon et al. 1970).
In the continuous study, all haematological data were within normal limits. Observations during necropsy revealed normal organs and tissues. Histopathological ex-

amination showed inflammatory changes in the lungs of exposed animals and to a lesser degree in controls.
In the repeated study, there were no deaths at both exposure levels and all haematological values were within normal limits. At 10 mg/m^3, histopathological examination revealed mild congestion in the spleens of both exposed animals. At 57 mg/m^3, histopathological examinations revealed non-specific inflammatory changes in the lungs and occasionally the hearts of exposed animals. None of the animals showed any signs of ocular or nasal irritation.

4.3.1.6 Monkeys

Male squirrel monkeys (2/3 animals per group) were exposed to ethylene glycol (vapour) continuously at a concentration of 12 ± 2 mg/m^3 for 90 days (continuous study), or at concentrations of 10 ± 1 or 57 ± 14 mg/m^3 for 8 hours a day, 5 days per week for 6 weeks (repeated study). The control group consisted of 8 animals. (Coon et al. 1970).
In the continuous study, all haematological data were within normal limits. Observations during necropsy revealed normal organs and tissues. Histopathological examination showed inflammatory changes in the lungs of exposed animals and to a lesser degree in controls.
In the repeated study, there were no deaths at both exposure levels and all haematological values were within normal limits. At 57 mg/m^3, histopathological examinations revealed non-specific inflammatory changes in the lungs and occasionally the hearts of exposed animals. The livers of 2 of 3 animals revealed areas of focal necrosis; this was not considered, by the authors, to be chemically induced. None of the animals showed any signs of ocular or nasal irritation.

Two chimpanzees were exposed by inhalation to air saturated with ethylene glycol (stated to be 256 mg/m^3) for 28 days. A biopsy revealed that one animal had oxalate crystals in the kidney. Both animals experienced an unexplained rise in haemoglobin concentration and mean red cell volume, as well as a decreased ability to concentrate urine. (Felts 1969 (abstract) – quoted from LaKind et al. 1999, Cavender & Sowinski 1994).

When monkeys were exposed to an ethylene glycol aerosol (500 mg/m^3) for up to 30 weeks, oxalate crystals were found in the kidneys (Harris 1969 (abstract) – quoted from LaKind et al. 1999).

4.3.2 Oral intake

4.3.2.1 Rats

Sprague-Dawley rats (10 males and females per group) received ethylene glycol in their drinking water for 10 or 90 days. In the 10-day study, the concentrations were 0, 0.5, 1.0, 2.0, or 4.0% (according to IUCLID corresponding to 0, 554, 1108, 2216, or 4432 mg/kg b.w./day assuming a water consumption of 100 ml/kg b.w. per day. In the 90-day study, females received the same concentrations in the drinking water whereas males received 0, 0.25 (227 mg/kg b.w./day), 0.5, 1.0, or 2%. (Robinson et al. 1990 – quoted from BUA 1991 and from IUCLID 2000).
In the 10-day study, high-dose (4%) male rats showed a strong reduction in body weight and in heart, liver, spleen and thymus weights. High-dose female rats had changes in blood parameters (decreased haemoglobin and haematocrit and markedly reduced numbers of erythrocytes and leucocytes) and decreased thymus weights. In male animals at the 2 and 4% dose levels, histopathological examination of the

kidneys revealed a dose-dependent increase in the incidence and severity of kidney damage, which involved dilation, degeneration and necrosis of the renal tubules, acute inflammation and intratubular deposits of proteinaceous material and calcium oxalate crystals. In females, the effects on kidneys were limited to tubular dilation and intratubular deposition of proteinaceous material at the highest dose level. A NOAEL of 1% ethylene glycol in the drinking water (1108 mg/kg b.w./day) for 10 days of exposure can be considered.
In the 90-day study, 8 female and 2 male animals from the high-dose group (4% and 2%, respectively) died. Body weight gain was markedly reduced in high-dose (2%) males. In females, the leucocyte count was significantly reduced at 0.5, 2. and 4%. In male animals at the 1 and 2% dose levels and in female animals at the 2 and 4% dose levels, histopathological examination of the kidneys showed a dose-dependent increase in the incidence and severity of kidney damage, which involved dilation, degeneration and inflammation of the renal tubules, and renal pelvis. A NOAEL of 0.5% ethylene glycol in the drinking water (554 mg/kg b.w./day) for 90 days of exposure can be considered based on the renal effects in male animals.

In a 13-week study, ethylene glycol was administered in the feed to Fischer 344/N rats (10 males and 10 females) at dose levels of 0, 0.32, 0.63, 1.25, 2.5, or 5.0% (equivalent to 0, 160, 315, 625, 1250, or 2500 mg/kg b.w./day assuming than an adult rat consumes 50 g feed/kg b.w./day). Four high-dose male rats died. The body weight gain for male rats in the 2.5 and 5% dose groups was depressed by more than 10% when compared to the control group. The relative kidney weight was significantly increased in both male and female rats in the two highest dose groups (2.5 and 5%) and the relative thymus weight was significantly decreased in high-dose (5%) male rats. Serum urea nitrogen and serum creatinine levels were significantly elevated in male animals at the two highest dose levels (2.5 and 5%) and kidney lesions were observed in all male rats at these dose levels. Damaged kidneys contained calcium oxalate crystals mainly located within tubular lumens in the renal cortex, but were also occasionally found in tubules in the medulla. Crystals were also observed in the urinary bladder, the urethral lumen, and in the brain of some high-dose male rats (5%). Severe toxic nephrosis (distension and dilation of renal tubules, necrosis and regeneration of tubule epithelium, thickening of basement membranes, and fibrosis) was diagnosed in the 5% male dose group and moderate toxic nephrosis in the 2.5% male dose group. Toxic lesions in the kidneys of female rats, which were multifocal and tended to be subcapsular, were only observed in the high-dose group (5%); no crystals were observed. Based upon the renal effects observed in male rats, a NOAEL of 1.25% (equivalent to 625 mg/kg b.w./day) can be considered. According to the author, this dose level corresponds to 0.6 to 1.0 g/kg b.w./day. (Melnick 1984).

Wistar rats (15 animals of each sex per group) were administered dietary doses of 0, 0.05, 0.1, 0.25, or 1% (according to IUCLID corresponding to 0, 35/38, 71/85, 180/185, or 715/1128 mg/kg b.w. per day for males and females, respectively) for 16 weeks. In male rats at the two highest dose levels, increased levels of oxalic acid and oxalate crystals were found in the urine and histopathological examination revealed damage to the kidneys. In addition, in males at the highest dose level, increased kidney weight and impairment of renal function was observed. In female animals, the same effects were less severe and only occurred at the highest dose level. Based on the effects observed in male animals, a NOAEL of 71 mg/kg b.w. per day is considered. (Gaunt et al. 1974 – quoted from BUA 1991 and from IUCLID 2000).

Fischer 344 rats (130 males and females per group) were fed diets (0.1, 0.5, or 2.5%) yielding approximate dosages of 40, 200, or 1000 mg/kg b.w. per day of

ethylene glycol for 24 months. Two untreated control groups were included. (De-Pass et al. 1986a).
High-dose (2.5%) male rats had a significant increase in mortality rate from the 9th through the 16th month of the study and the last high-dose male rat died after 474 days of the study. Other significant findings in high-dose male rats only included: increased water intake; decreased body weight gain; decreased red blood cell count, haematocrit, and haemoglobin concentration; increased neutrophilic leukocyte count; increased serum levels of creatinine and urea; increased urine volume; increased absolute and relative kidney weights after 6 and 12 months of treatment; and decreased absolute and relative liver weights after 12 months of treatment. In female rats of the highest dosage group, kidney weights were increased after 6 and 18 months of treatment, but not after 12 months. Calcium oxalate crystals were found in urine samples from all but one male of the high-dose animals after 12 months of treatment; at 18 and 24 months, all but one of the high-dose female rats had calcium oxalate crystals.
At the 6-month sacrifice, the incidence of renal lesions (tubular hyperplasia, tubular dilation, peritubular nephritis, and calcium oxalate crystalluria) was significantly increased in the high-dose males (2.5%) and calcium oxalate crystals were present in the urinary bladder in two high-dose male rats; these conditions were absent in the other male dose groups and in females.
At the 12-month sacrifice, all high-dose males (2.5%) had chronic nephritis (multiple severe histopathological changes including tubular dilation and proteinosis, glomerular shrinkage, tubular cell hyperplasia, and chronic interstitial nephritis) with calcium oxalate crystalluria and 50% had oxalate crystals in the urinary bladder; these findings were not present in males at the lower dose levels or in females.
By the time of the 18-month sacrifice, all of the high-dose males (2.5%) had died or were sacrificed moribund. In most of these animals, oxalate nephrosis was the primary cause of death. Kidneys from these animals had tubular obstruction by large crystals with secondary tubular dilation and degeneration. Calculi were sometimes found within the renal pelvic space, urethras, and urinary bladder, often with an associated hydronephrosis. Extrarenal lesions included cellular hyperplasia of the parathyroid glands and a significant increase in the incidence of splenic haemosiderosis.
Among high-dose females sacrificed at 2 years, an increased incidence of mild fatty metamorphosis of the liver (dose-related, but only significant at the high-dose level) and of haemosiderosis of the mesenteric lymph node (not significant) was observed. No biologically significant lesions were observed in the male rats sacrificed at 2 years.
Based on the effects observed in the kidneys, a NOAEL of 200 mg/kg b.w. per day is considered. For females, a NOAEL of 40 mg/kg b.w. per day can be considered based on the effects observed in the liver of high-dose females.

Sprague-Dawley rats (16 male and female animals per group) received ethylene glycol in their diet at concentrations of 0, 0.1, 0.2, 0.5, 1.0, or 4.0% (equivalent to 0, 50, 100, 250, 500 or 2000 mg/kg b.w./day assuming than an adult rat consumes 50 g feed/kg b.w./day). An increased mortality rate was observed in males of the two highest dose groups (1 and 4%) and in females of the highest dose group (4%). A significant decrease in growth was observed in male rats from week 16 at 4% and after week 70 at 1%, and in female rats after about one year at 4%. A significantly increased water consumption was noted in male rats at 1 and 4% and in female rats at 4%, and in these dose groups protein was found in the urine. The mean terminal kidney, lung and liver weights were lower (whether the reduction is significant is not stated in the publication) in male animals at 0.1% and above compared to controls. Calculi and crystal deposition in the kidneys were observed in male rats from 0.5% (only 1/16 animals at 0.5%) and in female rats at 4% and from 1%, respectively. Morphological changes in the kidneys also included degeneration

of the tubular epithelium, manifested mainly as cytoplasmic vacuolisation; however, no details are provided at which dose levels these morphological changes were observed. According to the author, it appears probable that the NOEL is no higher than 0.2% (equivalent to 100 mg/kg b.w./day) ethylene glycol in the diet but may be less. (Blood 1965).

4.3.2.2 Mice

In a 13-week study, ethylene glycol was administered in the feed to B6C3F1 mice (10 males and 10 females) at dose levels of 0, 0.32, 0.63, 1.25, 2.5, or 5.0% (equivalent to 0, 480, 945, 1875, 3750, or 7500 mg/kg b.w./day assuming than an adult mouse consumes 150 g feed/kg b.w./day). There were no deaths, the relative weight gain data did not show any clear dose-related effects, and there were no differences in organ weights. Renal lesions diagnosed as mild toxic nephrosis (tubular dilation, cytoplasmic vacuolisation, and regenerative hyperplasia with piling up of nuclei) were observed in about half of the male high-dose mice (5%) and in one male mouse in the 2.5% dose group. There was no evidence of crystal formation in the affected tubules. A degenerative change (accumulation of an eosinophilic hyaline material in the cytoplasm of hepatocytes adjacent to or close to central veins) was present in the livers of all the male mice in the 2.5 and 5% dose groups. There were no adverse effects observed in female mice at any of the dose levels. Based upon the effects observed in kidneys and livers in male mice, a NOAEL of 1.25% (equivalent to 1875 mg/kg b.w./day) can be considered. (Melnick 1984).

B6C3F1 mice (60 animals of each sex per group) were fed diets containing ethylene glycol for 103 weeks. Male mice received 0, 6250, 12500, or 25000 ppm (equal to average daily levels of approximately 0, 1500, 3000, or 6000 mg/kg b.w./day) and female mice 0, 12500, 25000, or 50000 ppm (equal to average daily levels of approximately 0, 3000, 6000, or 12000 mg/kg b.w./day). There were no significant differences in survival between dosed and control groups. Mean body weights of exposed and control animals were similar and no treatment-related clinical findings or gross lesions were noted. Hepatocellular hyaline degeneration was seen in mid- and high-dose male and high-dose female mice. Pulmonary arterial medial hyperplasia was observed at an increased incidence in exposed females but not in exposed males. Incidence and severity of nephropathy were not affected by treatment in either sex. Small numbers of oxalate-like crystals, calculi, or both were noted in renal tubules, urethras, and/or urinary bladders in a few high-dose male mice. Based upon the effects observed in livers of female mice, a NOAEL of 12500 ppm (equal to approximately 3000 mg/kg b.w./day) can be considered. (NTP 1993).

CD-1 mice (80 males and females per group) were fed diets (0.1-0.05, 0.7-0.24, or 0.35-1.27%) yielding approximate dosages of 40, 200, or 1000 mg/kg b.w. per day of ethylene glycol for 24 months. Two untreated control groups were included. Renal tubular degeneration occurred slightly more frequently in high-dose females than in controls at the 18-month sacrifice, but was absent in the 15 high-dose females sacrificed at 24 month. A NOAEL of 1000 mg/kg b.w. per day can be considered. (DePass et al. 1986a).

4.3.2.3 Monkey

Rhesus monkeys (2 males and 1 female) were given ethylene glycol in their diet at 0.2% (males) or 0.5% (female) for 3 years. No calculi or abnormal calcium depos-

its were demonstrated by X-ray examination and no other effects on organs and tissues were observed at the histopathological examination. (Blood et al. 1962).

4.3.3 Dermal contact

No data have been found.

4.4 Toxicity to reproduction

4.4.1 Inhalation

CD rats and CD-1 mice (25 animals per group) were exposed to a respirable ethylene glycol aerosol (mass median aerodynamic diameter (MMAD) 2.3 µm) on gestational days (GD) 6 to 15, 6 hours a day, by whole-body exposures (target concentrations: 0, 150, 1000, or 2500 mg/m^3). Rats were sacrificed on gestation day 21, and mice on gestation day 18. (Tyl et al. 1995a).
The total concentrations of ethylene glycol (aerosol plus vapour) were 79, 89, or 84% of target concentrations, respectively (119 ± 13, 888 ± 149, or 2090 ± 244 mg/m^3). The vapour phase was 82% of the total concentration for the 150 mg/m^3 group; for the higher aerosol concentrations, the vapour phase was 19-20% of the total concentration.
All rat dams survived to scheduled termination. Food and water consumption, maternal body weights and weight gain, and maternal organ weights (other than liver) were unaffected by exposure. A significant increase in absolute and relative liver weight was observed at 2500 mg/m^3. Gestational parameters (pre- and post-implantation loss, live foetuses/litter, sex ratio, and foetal body weight/litter) were unaffected by exposure. There was no significant increase in the incidence of any individual malformation, in the incidence of pooled external, visceral, or skeletal malformations, or in the incidence of total malformations by foetus or by litter. There were no significant increases in the incidence in any foetal external or visceral variations. There was some evidence of treatment-related reductions in ossification of the foetal skeleton, including an increase in the incidence of poorly ossified humerus (forelimb) and zygomatic arch (face) at the highest exposure level (2500 mg/m^3) and an increase in the incidence of poorly ossified metatarsals and proximal phalanges of the hindlimb at 1000 mg/m^3 but not at 2500 mg/m^3. According to the authors, the NOAEL was 1000 mg/m^3 for maternal and 150 mg/m^3 for developmental toxicity.
All mouse dams survived to scheduled termination. One female at 2500 mg/m^3 had a totally resorbed litter at termination; all other pregnant animals had one or more live foetuses at sacrifice. Clinical signs included only wet fur for all ethylene glycol exposed mice. Reduced body weight and body weight gain were observed at 1000 and 2500 mg/m^3 both during and after the exposure period. Gravid uterine weight was also reduced at the two highest exposure levels so that body weight corrected for gravid uterine weight was unaffected by treatment. Liver and kidney weights were unaffected by treatment. The following gestational parameters were affected: the number of viable implantations per litter was reduced at 2500 mg/m^3, the number of nonviable implantations per litter was increased at 1000 and 2500 mg/m^3, the number of early resorptions was increased (not significantly) at 2500 mg/m^3; the sex ratio was reduced at 1000 mg/m^3 but not at 2500 mg/m^3, and the foetal body weights per litter (male, female, and total) were reduced at 1000 and 2500 mg/m^3. There was a significant increase in the incidence of a number of external, visceral, and skeletal malformations at 1000 and 2500 mg/m^3, as well as in the incidence of pooled external, visceral, and skeletal malformations, and in the incidence of total malformations. Malformations were found in the head (exenceph-

aly), face (cleft palate, foreshortened and abnormal face, and abnormal facial bones), and skeleton (vertebral fusions, and fused, forked, and missing ribs). The incidences of many foetal variations were also increased at the two highest dose levels, but only a few at the lowest dose level (150 mg/m^3). According to the authors, the NOAEL was 150 mg/m^3 for maternal and at or below 150 mg/m^3 for developmental toxicity.

Ethylene glycol was teratogenic to mice by whole-body exposure to aerosol (1000 and 2500 mg/m^3), see the study described above. According to the authors, the results were confounded by possible exposure from ingestion after grooming and/or from percutaneous absorption. Therefore, CD-1 mice (30 animals per group) were exposed to ethylene glycol aerosol (MMAD 2.6 ± 1.7 µm) on gestational days 6 to 15, 6 hours a day, by nose-only (target concentrations: 0, 500, 1000, or 2500 mg/m^3) or whole-body exposures (target concentrations: 0 or 2100 mg/m^3). Control environments were water aerosol (4200 mg/m^3 for nose-only and 2700 mg/m^3 for whole-body). On gestation day 18, the dams were sacrificed. (Tyl et al. 1995b).
In either exposure regimen, body weights, body weight gain (absolute or corrected for the weight of the gravid uterus), and liver weights (absolute or relative) were unaffected by treatment. Microscopic examination of maternal kidneys indicated no treatment-related incidence or severity of renal lesions. There were no treatment-related differences among groups in the number of corpora lutea per dam, the number of total or viable implantations per litter, or on foetal sex ratio. There was no significant increase in the incidence of any individual external or visceral malformation or variations, or of all external or visceral malformations or in total variations.
In the nose-only experiment, maternal clinical signs associated with the animals struggling while in restraint were observed. Maternal kidney weights were increased at concentrations of 1000 (absolute) and 2500 (absolute and relative) mg/m^3 and there was a trend toward reduced gravid uterine weight at these concentrations as well. The percentage of live foetuses was reduced slightly (but not significantly) at the two highest concentrations. At the highest concentration (2500 mg/m^3), the foetal body weights per litter were significantly reduced and the incidences of one skeletal malformation (fused ribs) and 18 skeletal variations were increased. According to the authors, exposure of CD-1 mice to a respirable ethylene glycol aerosol during organogenesis by nose-only inhalation resulted in minimal maternal toxicity at 1000 and 2500 mg/m^3 and developmental toxicity at 2500 mg/m^3; the NOAEL was 500 mg/m^3 for maternal and 1000 mg/m^3 for developmental toxicity.
In the whole-body dose group (2100 mg/m^3), the gravid uterine weight, the percentage of live foetuses (due to an increase in late resorptions), and the foetal body weights per litter were significantly reduced. There was an increase in the incidence of a number of skeletal malformations, including fused thoracic arches, extra thoracic arches, fused lumbar arches and centra, fused ribs, and extra ribs between existing ribs, as well as in the incidence of pooled skeletal malformations and a total of 63 skeletal variations exhibited significantly increased incidences.

4.4.2 Oral intake

4.4.2.1 Rats

To assess the possible effects of ethylene glycol on reproductive performance, a three-generation reproduction study was performed in Fischer 344 rats. Ethylene glycol was administered in the diet at approximate doses of 40, 200, or 1000 mg/kg per day (weekly calculated doses ranged from 40-50, 200-300, or 1000-1300 mg/kg/day for males and from 40-60, 200-300, or 900-1200 for females). Two un-

treated diet control groups were included. At approximately 100 days of age, 10 males were mated to 20 females in each dose group. Necropsies and microscopic examinations of several organs and tissues were performed (5 animals of each sex from each dose level) on the F_2 parents and on the F_3 weanlings. There was no treatment-related effect on body weight gain or diet consumption, nor was there any mortality among parental rats. No treatment-related effect was observed for any of the reproductive indices (fertility index, gestation index, gestation survival index, survival indices, and days from first mating to litter) for all three generations, or on neonatal body weight at days 4, 14, or 21 postpartum. The histopathological examinations revealed no treatment-related findings in the F_2 parents and in the F_3 weanlings, including kidney damage. The NOAEL for reproductive toxicity was 1000 mg/kg b.w./day. (DePass et al. 1986b).

Fischer 344 rats were fed ethylene glycol in their diet from gestation day 6 to 15 so that the animals received doses of 0, 40, 200, or 1000 mg/kg b.w./day. Animals were sacrificed on gestation day 21 and the foetuses were examined. The only effects found were a non-significant increase in pre-implantation losses at the highest dose level and a delay in ossification of the foetal skeleton. The incidence of malformations was not significantly different to the negative controls. No maternal effects were observed at any of the dose levels; however, only body weight of the dams was examined. (Maronpot et al. 1983 – quoted from BUA 1991 and from IUCLID 2000).

In order to determine a NOAEL for developmental toxicity of ethylene glycol administered orally, CD rats received 0, 150, 500, 1000, or 2500 mg/kg b.w. per day by gavage on gestation days 6 to 15. At the highest dose level, water consumption was increased during treatment and body weights were reduced throughout gestation; liver and kidney weights were increased at sacrifice (gestation day 21). Relative liver weights were also increased at 1000 mg/kg/day. Effects observed in foetuses at 2500 mg/kg/day included hydrocephaly; gastroschisis; umbilical hernia; fused, duplicated, or missing arches, centra, and ribs; poor ossification in thoracic and lumbar regions; and reduced body weights. At 1000 mg/kg/day, reduced body weights; duplicated or missing ribs, centra, and arches; and poor ossification were observed. According to the authors, the NOAEL for developmental toxicity was 500 mg/kg b.w./day. (Neeper-Bradley et al. 1995 – abstract quoted from TOXLINE 1995-1998).

Timed-pregnant CD rats (at least 20 animals per group) were dosed by gavage with ethylene glycol in distilled water on gestational days 6 through 15 at doses of 0, 1250, 2500, or 5000 mg/kg b.w. No maternal deaths or distinctive clinical signs were noted, except for piloerection, which was seen in all treated groups but not in controls. Maternal body weight gain during treatment was significantly reduced in all dose groups. Gravid uterine weight was reduced at the mid and high doses, and corrected maternal gestational weight gain showed a significant decreasing trend. Absolute liver weight was significantly decreased at the high dose and relative kidney weight was increased in the mid- and high-dose groups. Dose-related increases in post-implantation loss per litter were observed with the high dose significantly above controls. The number of live foetuses per litter and foetal body weight per litter was significantly reduced at the mid and high doses. The percentage of malformed live foetuses per litter and/or the percentage of litters with malformed foetuses were significantly elevated in all dose groups and more than 95% of litters were affected at the high dose. A wide variety of malformations (external, visceral and skeletal) were observed with the most common being craniofacial and neural tube closure defects and axial skeletal dysplasia. (Price et al. 1985).

Price et al. (1988 – quoted from BUA 1991 and from IUCLID 2000) also have administered ethylene glycol by gavage to CD rats from gestation day 6 to 20 at doses of 0, 250, 1250, or 2250 mg/kg b.w./day. The offspring were reared by untreated dams and examined in respect to postnatal growth and survival rate, bodily development, the onset of sexual maturity, locomotive activity, and performance in a complex test of learning ability. At the two highest dose levels, there was a significant increase in the duration of gestation; microscopic examination revealed an increase in kidney damage. At the highest dose level, maternal body weight gain was reduced. In offspring from dams exposed at the highest dose level, foetal mortality was increased, there was a reduction in live litter size and in the weight of the neonates, and there was a significant increase in the number of malformations (particularly in the form of hydrocephaly and abnormalities of the axial skeleton). Prenatal exposure to ethylene glycol had no adverse effect on postnatal learning behaviour.

Ethylene glycol was administered to pregnant Wistar rats from gestation day 6 to 15 orally by a stomach tube at dose levels of 253, 638, 858, 1073, or 1595 mg/kg b.w. The foetal body weight and crown-rump length were significantly reduced from 858 mg/kg and 1.8 to 43.6% of foetuses among these groups presented gastroschisis, exencephaly, meningoencephalocele, harelip, and rib malformation; malformation frequencies showed a dose-response relationship. (Longzhan et al. 1989).

4.4.2.2 Mice

Examination of testicular weight in mice, which were administered ethylene glycol by gavage at doses of 0, 500, 1000, 2000, or 4000 mg/kg b.w. per day, 5 days a week for 5 weeks, gave no indication of any testicular damage (Nagano et al. 1984 – quoted from BUA 1991).

In a continuous breeding study, CD-1 mice (20 animals of each sex in the dose groups; 40 animals of each sex in the control group) were given ethylene glycol in their drinking water at concentrations of 0, 0.25, 0.5, or 1% for 14 weeks. As a rough estimate, the dose (on a mg/kg body weight basis) was calculated using the average daily water consumption multiplied by the concentration of the chemical in that dose group and divided by the body weight. Between days 98 to 105, the average doses were 0, 410, 840, and 1640 mg/kg b.w., respectively. (Lamb et al. 1985). No treatment-related effects were observed on body weight or water consumption, or in clinical signs of toxicity. At the highest dose level (1%), significant decreases in the number of litters per fertile pair, the mean number of live pups per litter, and the mean live pup weight were observed as compared to control F_0 mice. In the F_1 generation, the number of live pups per litter and the live pup weight were lower in the dosed group (1%), but differences were not significant. Facial anomalies were noted in a number of offspring of high-dose mice (1%) and an examination for skeletal defects demonstrated a pattern including reduction in the size of bones in the skull, fused ribs, and abnormally shaped sternebrae and vertebrae; examination by light microscopy of bones from treated mice did not reveal histological alterations. At least six pups from three different litters had cleft lip when observed grossly at birth. The NOAEL for reproductive effects was 0.5% (corresponding to an average dose of 840 mg/kg b.w./day).

In order to determine a NOAEL for developmental toxicity of ethylene glycol administered orally, CD-1 mice received 0, 50, 150, 500, or 1500 mg/kg b.w. per day by gavage on gestation days 6 to 15. There were no apparent treatment-related effects in dams. Effects observed in foetuses at 1500 mg/kg/day included reduced body weights; fused ribs and arches; poor ossification in thoracic and lumbar cen-

tra; and increased occurrence of an extra 14th rib. At 500 mg/kg/day, slight reductions in foetal body weight and increased incidences of extra ribs were observed. According to the authors, the NOAEL for developmental toxicity was 150 mg/kg b.w./day. (Neeper-Bradley et al. 1995 – abstract quoted from TOXLINE 1995-1998).

Timed-pregnant CD-1 mice (at least 20 animals per group) were dosed by gavage with ethylene glycol in distilled water on gestational days 6 through 15 at doses of 0, 750, 1500, or 3000 mg/kg b.w. No maternal deaths or distinctive clinical signs were noted, except for piloerection, which was seen in all treated groups but not in controls. Maternal body weight gain during treatment was significantly reduced in mid- and high-dose groups. Gravid uterine weight was reduced at the mid and high doses, and corrected maternal gestational weight gain showed a significant decreasing trend. Absolute liver weight was significantly decreased at the mid and high dose. Dose-related increases in postimplantation loss per litter were observed. The number of live foetuses per litter was significantly reduced at the high dose and foetal body weight per litter was significantly reduced at the mid and high doses. The percentage of malformed live foetuses per litter and/or the percentage of litters with malformed foetuses were significantly elevated in all dose groups and more than 95% of litters were affected at the high dose. A wide variety of malformations (external, visceral and skeletal) were observed with the most common being craniofacial and neural tube closure defects and axial skeletal dysplasia. (Price et al. 1985).

4.4.2.3 Rabbits

New Zealand White (NZW) rabbits (23-24 inseminated animals per group) were administered ethylene glycol by gavage on gestational day 6 through 19 at doses of 0, 100, 500, 1000, or 2000 mg/kg/day. Dams were sacrificed at gestation day 30. Profound maternal toxicity (42% mortality, three early deliveries, and one spontaneous abortion) was observed at the highest dose (2000 mg/kg b.w./day). At necropsy, there were no significant effects on gravid uterine weight, and liver or kidney weights. Kidney damage observed at 2000 mg/kg b.w./day was limited to the cortical renal tubules and included intraluminal oxalate crystals, epithelial necrosis and tubular dilatation and renal tubular degeneration. There were no effects on pre- or postimplantation loss, the number of foetuses per litter, foetal body weight per litter, or sex ratio (percent male foetuses per litter), and no evidence of teratogenicity based on evaluation of external, visceral including craniofacial, skeletal or total malformations, or variations at any dose level. The NOAEL for maternal toxicity was 1000 mg/kg b.w./day and the NOAEL for developmental toxicity was 2000 mg/kg b.w./day. (NTP 1991, Tyl et al. 1993 – abstract quoted from Toxline 1990-1994).

4.4.3 Dermal contact

CD-1 mice (30 animals per group) were exposed to ethylene glycol on gestation days 6 to 15, 6 hours per day by occluded cutaneous application at 0, 12.5, 50, or 100% ethylene glycol (0.1 ml/animal, equivalent to approximately 0, 404, 1677, or 3549 mg/kg b.w./day). There were no treatment-related maternal effects, and no differences in pre- or postimplantation loss or in foetal body weights/litter, and no increased incidences of any foetal malformations. Two skeletal variations were observed at the highest exposure level. According to the authors, the NOAEL for maternal and developmental toxicity was the highest exposure level (approximately

3549 mg/kg b.w./day). (Tyl et al. 1995 – abstract quoted from TOXLINE 1995-1998).

4.5 Mutagenic and genotoxic effects

4.5.1 *In vitro* studies

Ethylene glycol was negative when tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 at concentrations from 1 to 10000 µg/plate with and without metabolic activation (Various studies quoted in IUCLID 2000, BUA 1991, and ATSDR 1997).

A negative result has been reported for ethylene glycol in the SOS chromotest in *Escherichia coli* PQ37 when tested at concentrations of up to 60000 µg/plate with and without metabolic activation (von der Hude et al. 1988).

When tested in the DNA damage and repair assay in *Escherichia coli* (WP2, WP2uvrA, WP67, CM611, WP100, W3100polA+, p3478polA-) at concentrations up to 60000 µg/plate with and without metabolic activation, negative results were obtained (McCarroll et al. 1991 – quoted from IUCLID 2000, BUA 1991 and from ATSDR 1997).

Ethylene glycol was negative when tested in the gene conversion assay in *Saccharomyces cerevisiae* with and without metabolic activation. According to IUCLID, genetic changes induced by 11% ethylene glycol appeared to be due to elevated osmotic pressure; no further details are given. (BIBRA 1988 – quoted from IUCLID 2000).

A negative result has also been obtained for aneuploidy induction in the fungus *Neurospora crassa* when tested with and without metabolic activation (Griffiths 1979, 1981 – quoted from ATSDR 1997).

A negative result has been reported for gene mutation in the HGPRT assay in Chinese Hamster Ovary cells with and without metabolic activation (Ballantyne 1985, Slesinski et al. 1986 – both quoted from IUCLID 2000 and BUA from 1991). In another HGPRT assay in Chinese Hamster Ovary cells, there was no dose-related increase in the incidence of mutagenic events either in the presence or absence of metabolic activations; according to IUCLID, the result of the assay was ambiguous (Union Carbide Corporation 1981 – quoted from IUCLID 2000).

Ethylene glycol has been reported to yield a negative result when tested for point mutations in the mouse lymphoma assay in L5178Y with and without metabolic activation (Brown et al. 1980 – quoted from IUCLID 2000 and from BUA 1991, McGregor et al. 1991 – quoted from IUCLID 2000 and NTP from 1993).

Negative results were obtained for sister chromatid exchanges when ethylene glycol was tested in Chinese Hamster Ovary cells with and without metabolic activation (Ballantyne 1985, Slesinski et al. 1986 – both quoted from IUCLID 2000 and from BUA 1991, Galloway et al. 1985,1987 – quoted from NTP 1993) or without metabolic activation at concentrations for 2 to 20% (v/v) (Union Carbide Corporation 1981 – quoted from IUCLID 2000).

A negative result was also obtained for chromosome aberrations when ethylene glycol was tested in Chinese Hamster Ovary cells with and without metabolic activation (Galloway et al. 1985,1987 – quoted from NTP 1993, Ballantyne 1985,

Slesinski et al. 1986 – both quoted from IUCLID 2000 and from BUA 1991). When tested in another assay at concentrations from 10 to 100 mg/ml, ethylene glycol did not produce an increase in the incidence of chromosome aberrations; according to IUCLID, the result of the assay was ambiguous (Union Carbide Corporation 1985 – quoted from IUCLID 2000).

Negative results were also obtained for unscheduled DNA synthesis when ethylene glycol was tested in rat hepatocytes with and without metabolic activation (Slesinski et al. 1986, Union Carbide Corporation 1981 – quoted from IUCLID 2000 and from BUA 1991).

4.5.2 *In vivo* studies

Ethylene glycol has been tested for mutagenic effects in the micronucleus assay in Swiss mice by oral administration of 2.5, 3.125, 6.25, or 12.5 ml/kg b.w. (corresponding to 2.8, 3.5, 7.0, or 13.9 g/kg b.w.; 4, 4, 4, or 2 animals in the dose groups, respectively) and by intraperitoneal injection of 1.25, 2.5, or 6.25 ml/kg b.w. (corresponding to 1.4, 2.8, or 7.0 g/kg b.w.; 5, 5, or 1 animal in the dose groups, respectively). A control group of 19 animals was included. Animals in the high-dose oral group (13.9 g/kg b.w.) showed toxic effects. At all, but the lowest oral dose level (2.8 g/kg b.w.), there was an increase in the numbers of micronuclei in polychromatic erythrocytes when compared to the control. According to IUCLID, a weak positive but not dose dependent result was obtained. (Conan et al. 1979).

When tested in mice (5 animals) for chromosome aberrations following an intraperitoneal injection of 2.5 ml/kg b.w., there was no significant difference in the occurrence of chromosome aberrations in bone marrow cells (Conan et al. 1979 – quoted from BUA 1991).

In a combined 3-generation reproduction and dominant lethal study, ethylene glycol was administered to Fischer 344 rats (20 females and 10 males) in their diet at approximate dose levels of 40, 200, or 1000 mg/kg b.w. per day. Two control groups received the same diet without ethylene glycol. Males from each dosage group of the F_2 generation, which had received an ethylene glycol containing diet for 155 days, were bred with 15 untreated females at weekly intervals for 3 weeks. On day 12 of gestation, the females were sacrificed, and uteri and ovaries were examined for the numbers of living and dead foetuses. No significant changes were observed in any of the test groups. (DePass et al. 1986b).

4.6 Carcinogenic effects

4.6.1.1 Rats

Fischer 344 rats (130 males and females per group) were fed diets (0.1, 0.5, or 2.5%) yielding approximate dosages of 40, 200, or 1000 mg/kg b.w. per day of ethylene glycol for 24 months. Two untreated control groups were included. The only tumour type for which there was a significant difference was fibroadenoma of the mammary gland in females at the lowest dose level (0.1%). According to the authors, this finding was most probably unrelated to ethylene glycol treatment because of the absence of an effect at the two higher dose levels. Non-neoplastic findings are described in 4.4.2.1. (DePass et al. 1986a).

In Sprague-Dawley rats (16 male and female animals per group), which received ethylene glycol in their diet at concentrations of 0, 0.1, 0.2, 0.5, 1.0, or 4.0%

(equivalent to 0, 50, 100, 250, 500 or 2000 mg/kg b.w./day assuming than an adult rat consumes 50 g feed/kg b.w./day) for 2 years, mammary tumours developed in many female rats towards the end of the experiment. According to the author, the distribution of the tumours in both males and females was such that it was impossible to correlate incidence with treatment. Few tumours were identified in organs within the body and none was associated with the occurrence of calculi. Non-neoplastic findings are described in 4.4.2.1. (Blood 1965).

4.6.1.2 Mice

In B6C3F1 mice (60 animals of each sex per group), which were fed diets containing ethylene glycol for 103 weeks (male mice: approximately 0, 1500, 3000, or 6000 mg/kg b.w./day; female mice: approximately 0, 3000, 6000, or 12000 mg/kg b.w./day), no treatment-related neoplasms were observed at the 15-month interim evaluations or at the end of the 2-year studies. Non-neoplastic findings are described in 4.4.2.2. (NTP 1993).

CD-1 mice (80 males and females per group) were fed diets (0.1-0.05, 0.7-0.24, or 0.35-1.27%) yielding approximate dosages of 40, 200, or 1000 mg/kg b.w. per day of ethylene glycol for 24 months. Two untreated control groups were included. The only tumour type for which there was any evidence of a possible increased incidence was lymphosarcoma in females. The time-adjusted incidence was significantly increased according to one of three trend tests. Comparisons among groups for differences in tumour proportions indicated that the observed results could have occurred by chance alone. Non-neoplastic findings are described in 4.4.2.2. (DePass et al. 1986).

5 Regulations

5.1 Ambient air

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5.2 Drinking water

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5.3 Soil

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5.4 Occupational Exposure Limits

Denmark:	10 ppm (26 mg/m^3), notation H (At 2005). Atomised 10 mg/m^3 (At 2005).
ACGIH:	39 ppm (100 mg/m^3) (ACGIH 2001).
Germany:	-
EU:	10 ppm (26 mg/m^3) (8 hour TWA), skin notation 20 ppm (52 mg/m^3) (STEL, 15 minutes) (SEG 1993).

5.5 Classification

Ethylene glycol is classified for acute toxic effects (Xn;R22 – harmful if swallowed) (MM 2002).

5.6 IARC

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5.7 US-EPA

Oral reference dose (RfD): 2 mg/kg b.w. per day. The oral RfD is based on the NOAEL of 200 mg/kg b.w. per day for renal toxicity in a 2 year feeding study in rats (DePass et al. 1986 – quoted in IRIS 2002) and the application of a uncertainty factor of 100 (10 for interspecies extrapolation and 10 for differences in individual human sensitivity) (IRIS 2002).

6 Summary and evaluation

6.1 Description

Ethylene glycol (EG) is a clear, colourless, slightly viscous, hygroscopic liquid with a sweet taste. It is miscible with water and has a low vapour pressure (0.06 mmHg at 20 °C). Odour thresholds of around 62-65 mg/m^3 and 90 mg/m^3 have been reported.

6.2 Environment

EG in the environment is predominantly due to its production and use with the major sources being from the disposal of used antifreeze and de-icing solutions. It has been identified naturally in some higher plants and in one fungus.

In the atmosphere, EG will mainly exist in the vapour phase and is degraded by reaction with photochemically produced hydroxyl radicals with an estimated half-life of 8 to 84 hours. No information about atmospheric levels has been found.

If released to soil and water, EG is expected to biodegrade (both aerobic and anaerobic) rapidly. EG is not expected to adsorb to sediment or suspended organic matter in water and soil or to volatilise from either soil or water surfaces. It is expected to display a very high mobility in soil and potentially to leach into the ground water. It is not expected to bioaccumulate.

6.3 Human exposure

The most important route of human exposure to EG for the general population is dermal contact with fluids used in automobiles. Persons living near airports (where large amounts of EG are used for de-icing of aircraft), near hazardous waste sites, or near production and/or processing sites can be exposed from consumption of contaminated groundwater. Intentional or accidental ingestion of antifreeze has also been reported.

6.4 Toxicokinetics

EG is rapidly absorbed and distributed following inhalation, oral and dermal administration. In one inhalation study in rats, 75-80% of inhaled EG (vapour or aerosol) was distributed immediately after exposure. Total recovery of oral doses in rats and mice is approximately 90-100%, indicating substantial absorption. After dermal application, approximately 30% of a dose was absorbed through rat skin, whereas mice absorbed 85-100% of the administered dose. An average absorption of approximately 25% was observed when EG was applied to the surface of three different human skin samples.

The metabolism of EG occurs primarily in the liver and kidney. The initial step is a conversion of the parent compound to glycolaldehyde, which is further oxidised to glycolic acid. Glycolic acid is then converted to glyoxylic acid, which is converted

either to carbon dioxide or to oxalic acid. Generally, metabolism begins immediately after administration of EG, and excretion of most of the parent compound and metabolites is complete 12 to 48 hours after dosing. The major excretory end products are carbon dioxide in exhaled air, and glycolate and unchanged EG in the urine.

6.5 Mode of action

The toxicity of EG is primarily a result of the effects of its metabolites although the effects on the central nervous system observed shortly after ingestion is partly attributed to unmetabolised EG as well as to formation of aldehydes, primarily glycolaldehyde.

The metabolic acidosis is usually attributed to the metabolites glycolic acid and glyoxylic acid, which react with bicarbonate causing a decrease in the pH in body fluids, particularly in blood. Recent studies have demonstrated that the major determinant of the metabolic acidosis is the degree of glycolic acid accumulation.

Calcium oxalate precipitation within the renal tubules has long been accepted as an important pathogenic factor for the development of renal toxicity; however, the mechanism of the renal toxicity is not yet known and there is no evidence directly linking oxalate precipitation with development of renal tubular necrosis. The renal toxicity has also been suggested to occur from a metabolite-induced cytotoxicity or from metabolic acidosis.

The mechanism(s) causing the delayed neurological effects from acute ingestion is unknown, but is distinctly different from the pathological events of the first three stages; it has been suggested that these effects could be related to oxalate crystal deposition in the brain.

The mechanism of EG toxicity as related to developmental effects warrants further exploration. A link between maternal metabolic acidosis and developmental toxicity has been suggested with glycolic acid being the toxic metabolite.

6.6 Human toxicity

Twenty male volunteers exposed for 30 days, 20-22 hours a day, to EG atmospheres (aerosol, diameters of droplets 1-5 µm) containing mean concentrations of 17 to 49 mg/m^3 did not experience any serious signs of toxicity, but there were complaints of irritation of the throat. The irritation became common when the volunteers re-entered the chamber in which the concentration of EG (during the absence of volunteers) was raised to about 140 mg/m^3 and concentrations greater than about 200 mg/m^3 were intolerable due to strong irritation of the upper respiratory tract. No significant alterations of the haematological, clinically chemical, or clinically pathological parameters studied, including the concentrations of urea nitrogen and creatinine in the blood of the exposed volunteers were observed.

Several deaths due to accidental or intentional ingestion of EG have been reported; the minimal lethal oral dose for humans has been estimated to be about 1.6 g/kg b.w. (adults). The clinical signs which follow acute poisoning after ingestion of EG can be divided into three (possibly four) stages: 1) effects on the central nervous system, which occur 30 minutes to 12 hours after ingestion; 2) effects on the cardiopulmonary system occurring 12 to 72 hours after ingestion, this stage may also be characterised by severe metabolic acidosis; 3) effects on the kidneys, which occur

24 to 72 hours after ingestion, this stage is characterised by profound metabolic acidosis; and 4) degenerative effects on the central nervous system occurring 6 or more days after ingestion, symptoms which are uncommon.

EG has not shown a particularly irritating potential to eyes or skin and was not shown to have a strong sensitising potential although some case reports are available. Prolonged dermal exposure can result in skin maceration.

No data on toxicity to reproduction, mutagenic and genotoxic effects, or carcinogenic effects of EG in humans have been found.

6.7 Animal toxicity

6.7.1 Single dose toxicity

An LC_{50}-value (one hour) of 10.9 g/m^3 has been reported in rats. In a study of rats, all animals survived an 8-hour exposure to a saturated atmosphere (ca. 200 mg/m^3 (calculated)).
The reported oral LD_{50}-values ranged from >2.0 to 11.3 g/kg b.w. in rats, from 5.89 to 15.4 g/kg b.w. in mice, from 7.0 to 9.3 g/kg b.w. in rabbits, from 4.0 to 8.2 g/kg b.w. in guinea pigs, from 4 to 8.2 g/kg b.w. in dogs, and of 1.67 and 4.7 g/kg b.w. in cats. A minimal lethal dose of 3.8 g/kg b.w. has been reported for rats, of 1 g/kg b.w. for cats, and of 6.7/7.3 g/kg b.w. for dogs.
Dermal LD_{50}-values of 9.53 and 10.6 g/kg b.w. have been reported for the rabbit.

6.7.2 Irritation

EG did not show irritating properties when applied to the skin of rabbits.

Moderate to severe eye irritation has been observed in rats and rabbits exposed continuously for 90 days to EG (vapour) at a concentration of 12 mg/m^3; guinea pigs, dogs, and monkeys exposed similarly showed no effects on the eyes. Following exposure to a concentration of 57 mg/m^3 of EG (vapour, 8 hours a day, 5 days per week for 6 weeks), rats, guinea pigs, rabbits, dogs, and monkeys did not show any signs of ocular irritation.
EG (10, 20, or 50 % solution in water) caused slight oedema and erythema under occlusive conditions in the eyes of rabbits whereas instillation of neat EG produced moderate to severe oedema and erythema. Irritation consisting of chemosis, swelling, and conjunctival redness has also been observed in rabbit eyes following instillation of 4 and 40% EG in balanced salt solutions. The lowest non-irritating concentration of EG has been reported to be 20% when applied as 0.1 ml solution 5 times a day for 21 consecutive days. In other studies, neat EG has been reported to be only slightly irritating one hour after instillation of 0.1 ml of the fluid or to show an extremely low potential for eye irritation.

6.7.3 Sensitisation

No data on sensitisation in experimental animals have been found.

6.7.4 Repeated dose toxicity

Rats, guinea pigs, rabbits, dogs and monkeys were exposed to EG (vapour) either continuously at a concentration of 12 mg/m^3 for 90 days (continuous study), or at concentrations of 10 or 57 mg/m^3 for 8 hours a day, 5 days per week for 6 weeks (repeated study) (Coon et al. 1970). In the continuous study, one rat, one rabbit and 3 guinea pigs died during exposure as well as four control rats; moderate to severe eye irritation was observed in rats and rabbits; and histopathological examination showed inflammatory changes in the lungs of exposed animals and to a lesser degree in controls. In the repeated study, at 10 mg/m^3, histopathological examination revealed mild congestion in the spleens of both dogs, hepatic fatty changes in 2/8 guinea pigs and in 1/8 rats, and focal necrosis in the liver of 1/8 guinea pigs and of 1/8 rats; focal necrosis of the liver was also seen in 1 of 3 control guinea pigs; at 57 mg/m^3, histopathological examinations revealed non-specific inflammatory changes in the lungs and occasionally the hearts of exposed animals.
No substance-related pathological changes were observed in a number of organs and tissues of rats and mice exposed to EG at concentrations of 350 to 400 mg/m^3 8 hours a day, 5 days per week for 16 weeks.

In a 90-day drinking water study (Robinson et al. 1990 – quoted from BUA 1991 and from IUCLID 2000), a dose-dependent increase in the incidence and severity of kidney damage (dilation, degeneration, and inflammation of the renal tubules, and renal pelvis) were observed in male Sprague-Dawley rats at concentrations from 1% EG in the drinking water (corresponding to about 1100 mg/kg b.w./day) and in female rats from 2% (corresponding to about 2200 mg/kg b.w./day); the NOAEL for renal effects (in males) in this study was 0.5% EG in the drinking water (corresponding to about 550 mg/kg b.w./day).
In a 13-week feeding study (Melnick 1984) in Fischer 344/N rats, kidney lesions were observed at dose levels from 2.5% (equivalent to 1250 mg/kg b.w./day) in male rats (toxic nephrosis) and at 5% (equivalent to 2500 mg/kg b.w./day) in female rats; the relative kidney weight was significantly increased in both male and female rats from 2.5% and serum urea nitrogen and serum creatinine levels were significantly elevated in male animals from 2.5%. The NOAEL for renal effects (in males) in this study was 1.25% (equivalent to 625 mg/kg b.w./day, or according to the author: 600-1000 mg/kg b.w./day).
In another dietary study (Gaunt et al. 1974 – quoted from BUA 1991 and from IUCLID 2000) in Wistar rats of similar duration (16 weeks), damage to the kidneys were observed from 0.25% (corresponding to 180 mg/kg b.w./day) in male rats and at 1% (corresponding to 1130 mg/kg b.w./day) in female rats; the NOAEL for renal effects (in males) in this study was 0.1% (corresponding to about 70 mg/kg b.w./day).
In a 2-year feeding study (DePass et al. 1986a) in Fischer 344/N rats, kidney lesions were observed at a dose level of 2.5% (corresponding to 1000 mg/kg b.w./day) in male rats and all male rats at this dose level had died after 16 months of exposure due to oxalate nephrosis; kidney lesions were not observed in female rats at dose levels up to 2.5%. Kidney weights were increased in both male and female rats at 2.5%. The NOAEL for renal effects (in males) in this study was 0.5% (corresponding to 200 mg/kg b.w./day). Mild fatty metamorphosis of the liver was observed in female rats at 2.5% (corresponding to 1000 mg/kg b.w./day); a NOAEL of 0.5% (corresponding to 200 mg/kg b.w./day) can be considered for liver effects in female rats.
In another 2-year dietary study (Blood 1965) in Sprague-Dawley rats, kidney damage was observed from 0.5% (equivalent to 250 mg/kg b.w./day) in male rats (crystal deposition in the kidney, degeneration of the tubular epithelium in 1/11 animals) and in female rats at 4% (equivalent to 2000 mg/kg b.w./day); the NOAEL for renal effects (in males) in this study was 0.2% (equivalent to 100 mg/kg b.w./day).

In B6C3F1 mice, mild toxic nephrosis (only one animal) and a degenerative change in the livers were observed in male mice from a dietary level of 2.5% EG (equivalent to 3750 mg/kg b.w./day) for 13 weeks (Melnick 1984) ; no effects were seen in female mice. The NOAEL in this study was 1.25% (equivalent to 1875 mg/kg b.w./day).
In a 2-year feeding study (NTP 1993) in B6C3F1 mice, hepatocellular hyaline degeneration was seen in female mice at dietary levels from 2.5% (equal to 6000 mg/kg b.w./day). The incidence and severity of nephropathy was not affected in either sex at dietary levels of up to 2.5% (equal to 6000 mg/kg b.w./day) in males and up to 5% (equal to 12000 mg/kg b.w./day) in females. The NOAEL in this study was 1.25% (equal to 3000 mg/kg b.w./day).
In another 2-year feeding study (DePass et al. 1986a) in CD-1 mice, no adverse effects on the kidneys were observed at dietary levels up to 1000 mg/kg b.w./day (the highest dose level in the study).

6.7.5 Toxicity to reproduction

When CD rats were exposed to a respirable EG aerosol (target concentrations: 0, 150, 1000, or 2500 mg/m^3) by whole-body exposures (6 hours a day) on gestational days 6 to 15 (Tyl et al. 1995a), the only maternal effect observed was a significant increase in liver weight (absolute and relative) at the highest exposure level. Gestational parameters were unaffected by exposure and there was no significant increase in the incidence of any malformations or in any external or visceral variations. There was some evidence of treatment-related reductions in ossification of the foetal skeleton at the highest exposure level and an increase in the incidence of poorly ossified metatarsals and proximal phalanges of the hindlimb at 1000 mg/m^3. The NOAEL was 1000 mg/m^3 for maternal and 150 mg/m^3 for developmental toxicity.
In CD-1 mice exposed similarly as the rats, reduced body weight and body weight gain and reduced gravid uterine weight were observed at the two highest exposure levels. Several gestational parameters were affected at these exposure levels and there was a significant increase in the incidence of a number of external, visceral, and skeletal malformations. The incidences of many foetal variations were also increased at the two highest dose levels, but only a few at the lowest dose level. The NOAEL was 150 mg/m^3 for maternal and at or below 150 mg/m^3 for developmental toxicity.
In a subsequent study (Tyl et al. 1995b) in CD-1 mice, dams were exposed (6 hours a day) to EG aerosol by nose-only (target concentrations: 0, 500, 1000, or 2500 mg/m^3) or whole-body exposures (target concentrations: 0 or 2100 mg/m^3) on gestational days 6 to 15. In the nose-only experiment, maternal kidney weights were increased at the two highest exposure levels. At the highest exposure level, the foetal body weights per litter were significantly reduced and the incidences of one skeletal malformation (fused ribs) and 18 skeletal variations were increased. The NOAEL was 500 mg/m^3 for maternal and 1000 mg/m^3 for developmental toxicity. In the whole-body dose group, the gravid uterine weight, the percentage of live foetuses, and the foetal body weights per litter were significantly reduced. There was an increase in the incidence of a number of skeletal malformations and a total of 63 skeletal variations exhibited significantly increased incidences.

In a three-generation dietary reproduction study (DePass et al. 1986b) in Fischer 344 rats, no treatment-related effects were observed in the F_2 parents and in the F_3 weanlings, including kidney damage; the NOAEL for reproductive toxicity was 1000 mg/kg b.w./day (the highest dose level in the study). In a continuous breeding study (Lamb et al. 1985) in CD-1 mice, EG was administered in the drinking water for 14 weeks; the NOAEL for reproductive effects was 0.5% (corresponding to an

average dose of 840 mg/kg b.w./day) with foetotoxic effects, including malformations, being observed at the higher concentration of 1% (corresponding to an average dose of 1640 mg/kg b.w./day).

In Fischer 344 rats, the NOAEL for developmental toxicity as well as for maternal toxicity (only body weights of the dams were examined) was 1000 mg/kg b.w./day (the highest dose level in the study) when EG was administered in the diet from gestation day 6 to 15 (Maronpot et al. 1983 – quoted from BUA 1991 and from IUCLID 2000). When EG was administered by gavage (from gestation day 6 to 15) to CD rats (Neeper-Bradley et al. 1995 – quoted from Toxline), the NOAEL for developmental toxicity was 500 mg/kg b.w./day with effects (reduced body weights; duplicated or missing ribs, centra, and arches; and poor ossification) being observed at 1000 mg/kg b.w./day; maternal effects (increased relative liver weight) was observed as well at this dose level. Similarly, another gavage study (Price et al. 1985) in CD rats showed that administration of EG (from 1250 mg/kg b.w./day, the lowest dose level in the study) during organogenesis (from gestation day 6 to 15) produced severe dose-related developmental toxicity, including malformations, at dose levels where no serious maternal effects (reduced maternal body weight gain at 1250 mg/kg b.w./day) were observed. However, in a third gavage study (Price et al. 1988 – quoted from BUA 1991 and from IUCLID 2000), where EG was administered to CD-rats from gestation day 6 to 20, no symptoms of substance-related toxicity were observed in offspring at dose levels up to 1250 mg/kg b.w./day; maternal effects at this dose level included kidney damage. In Wistar rats, a NOAEL of 638 mg/kg b.w./day for developmental toxicity (foetotoxicity as well as malformations) was observed following administration of EG by a stomach tube from gestation day 6 to 15 (Longzhan et al. 1989); no information was given with regard to maternal effects.
When EG was administered by gavage (from gestation day 6 to 15) to CD-1 mice, the NOAEL for developmental toxicity was 150 mg/kg b.w./day with effects (slight reductions in foetal body weight and increased incidences of extra ribs) being observed at 500 mg/kg b.w./day (Neeper-Bradley et al. 1995 – quoted from Toxline)); no maternal effects were observed at any dose level (up to 1500 mg/kg b.w./day). Another gavage study (Price et al. 1985) in CD-1 mice showed that administration of EG (from 750 mg/kg b.w./day, the lowest dose level in the study) during organogenesis (from gestation day 6 to 15) produced severe dose-related developmental toxicity, including malformations, a dose level (750 mg/kg b.w./day) where no maternal effects were observed.
In New Zealand White rabbits, the NOAEL for maternal toxicity was 1000 mg/kg b.w./day and the NOAEL for developmental toxicity was 2000 mg/kg b.w./day (the highest dose level in the study), when EG was administered by gavage on gestational day 6 through 19 (NTP 1991).

Following occluded cutaneous application of EG to CD-1 mice on gestation days 6 to 15 (6 hours per day), the NOAEL for maternal and developmental toxicity was the highest exposure level (approximately 3550 mg/kg b.w./day) (Tyl et al. 1995 – quoted from Toxline).

6.7.6 Mutagenic and genotoxic effects

EG has shown negative results in the following *in vitro* test systems: in the Ames test (several tests in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; TA1538), in the SOS chromotest in *Escherichia coli* PQ37, in the DNA damage and repair assay in *Escherichia coli* (WP2, WP2uvrA, WP67, CM611, WP100, W3100polA+, p3478polA-), for gene conversion in *Saccharomyces cerevisiae*, for aneuploidy induction in the fungus *Neurospora crassa*, in a cytogenetic

assay in Chinese Hamster Ovary (CHO) cells, for gene mutation in the HGPRT assay in CHO cells, in the mouse lymphoma assay, for sister chromatid exchanges and chromosome aberrations in CHO cells, and for unscheduled DNA synthesis in rat hepatocytes. In one HGPRT assay and in one test for chromosome aberration, both in CHO cells, the result of the assay was reported as being ambiguous. Most of the assays were performed both with and without metabolic activation.

Negative results have also been reported in *in vivo* studies: in the dominant lethal assay following oral administration in the feed to Fischer 344 rats and for chromosome aberrations in mice following intraperitoneal injection. In an micronucleus assay in mice, an increase in the numbers of micronuclei was observed following oral administration of very high doses (2.8 to 13.9 g/kg b.w.), except at the lowest dose level, and following intraperitoneal injection (doses of 1.4 to 7.0 g/kg b.w.).

6.7.7 Carcinogenic effects

No evidence of a carcinogenic effect of EG was observed in Fischer 344 rats administered diets yielding dosages of up to approximately 1000 mg/kg b.w./day for 24 months, in Sprague-Dawley rats receiving dietary concentrations of up to 4% (equivalent to 2000 mg/kg b.w./day) for 2 years, in B6C3F1 mice fed diets containing EG for 103 weeks (male mice: up to 2.5% equivalent to approximately 6000 mg/kg b.w./day; female mice: up to 5% equivalent to approximately 12000 mg/kg b.w./day), or in CD-1 mice administered diets yielding dosages of up to approximately 1000 mg/kg b.w./day for 24 months.

6.8 Evaluation

Ethylene glycol (EG) is rapidly and almost completely absorbed (inhalation, rats: 75-80% of inhaled EG (vapour or aerosol); oral, rats and mice: 90-100%; dermal, rats: 30%; dermal, mice: 85-100%), distributed, metabolised and cleared (almost completely 12 to 48 hours after dosing) following exposure.
EG is metabolised by oxidation via glycolaldehyde and glycolic acid to glyoxylic acid, which is converted either to carbon dioxide or to oxalic acid. The kinetic study published by Pottenger et al. (2001) indicated that the metabolic conversion of EG to glycolic acid was approaching saturation at EG blood levels obtained following oral administration (gavage) of 2500 mg/kg b.w., and that glycolic acid was being formed at a maximum rate between 1000 and 2500 mg/kg b.w.
The major excretory end products are carbon dioxide in exhaled air, and glycolate and unchanged EG in the urine. The study published by Pottenger et al. (2001) showed that urinary elimination demonstrated dose-dependency, with the high dose groups (2500 mg/kg b.w.) eliminating almost 70% of the administered dose in urine, compared with about 16% in the low dose groups (10 mg/kg b.w.); the shift in urinary elimination was mainly due to increased urinary glycolic acid and EG, and not to increased elimination of oxalic acid. The study also showed that oxalate was a very minor metabolite in both blood and urine at all dose levels (up to 2500 mg/kg b.w.).

There are numerous case reports in the literature of poisoning in humans due to accidental or intentional ingestion of EG; the minimal lethal oral dose for humans has been estimated to be about 1600 mg/kg b.w. for adults. The clinical symptoms of acute EG poisoning in humans can be divided into three (possibly four) fairly distinct stages (effects on the CNS, cardiopulmonary effects, renal effects, and delayed neurological effects); toxicity in stage 2 and 3 is characterised by severe met-

abolic acidosis. The severity of these stages and the advance from one stage to the next depends greatly on the amount of EG absorbed.
The acute toxicity of EG in experimental animals closely mirrors the acute effects seen in humans; one exception is the fourth clinical stage as no studies have been found that reported this stage in animals. EG is of low acute toxicity in experimental animals, except the cat, with reported oral LD_{50}-values ranging from >2000 to 15400 mg/kg b.w.; a minimal lethal dose of 3800 mg/kg b.w. has been reported for rats, of around 7000 mg/kg b.w. for dogs and, of 1000 mg/kg b.w. for cats. Comparing the lethal oral dose in humans to the minimum lethal dose in experimental animals, EG appears to be two to five times more acutely toxic to humans and cats, on a body weight basis, than to rats and dogs.
The very limited data on acute inhalation and dermal toxicity in experimental animals also indicate a low acute toxicity by these routes with a reported LC_{50}-value (one hour) of 10.9 g/m^3 in rats and dermal LD_{50}-values of around 10600 mg/kg b.w. for the rabbit.

EG has not shown a particularly irritating potential to eyes or skin in humans and did not show irritating properties when applied to the skin of rabbits. Prolonged dermal exposure to humans can result in skin maceration.
The data on eye irritation in experimental animals are conflicting but overall, the data indicate an eye irritating potential following instillation of either neat EG or solutions of EG to rabbit eyes. Moderate to severe eye irritation has been observed in rats and rabbits exposed continuously for 90 days to EG (vapour, 12 mg/m^3) but not following exposure to a concentration of 57 mg/m^3 (vapour, 8 hours a day, 5 days per week for 6 weeks); guinea pigs, dogs, and monkeys exposed similarly showed no effects on the eyes.
Male volunteers complained of irritation of the throat following exposure to 17 to 49 mg/m^3 (aerosol, 20-22 hours a day for 30 days); the irritation became common at about 140 mg/m^3 when the volunteers re-entered the exposure chamber in which the concentration of EG (during the absence of volunteers) was raised and concentrations greater than about 200 mg/m^3 were intolerable due to strong irritation of the upper respiratory tract.

EG is not considered to have a sensitising potential in humans although some case reports are available. No data on sensitisation in experimental animals have been found.

Male volunteers (exposed for 30 days, 20-22 hours a day, aerosol, mean concentrations of 17 to 49 mg/m^3) did not experience any serious signs of toxicity and no indications of renal toxicity (alterations in urea nitrogen and creatinine in the blood) were observed. Similarly, no indications of renal toxicity were observed in rats, guinea pigs, rabbits, dogs and monkeys exposed to EG (vapour) either continuously (12 mg/m^3 for 90 days), or repeatedly (10 or 57 mg/m^3 for 8 hours a day, 5 days per week for 6 weeks). Inflammatory changes were observed in the lungs of exposed animals and to a lesser degree in controls as well as some effects in the liver in a few animals; most of the observed effects were interpreted, by the authors, as being unrelated to the exposure to EG, however, these interpretations cannot be evaluated from the data provided in the publication (Coon et al. 1970).

Repeated oral administration of EG to rats results primarily in toxic effects in the kidneys. Male rats are far more sensitive to the renal effects of EG than are female rats as renal effects occur in male rats at dose levels from about 200-250 mg/kg b.w./day (16-week and 2-year feeding studies) but in female rats at dose levels above 1000 mg/kg b.w./day. Mice appear to be relatively resistant to EG induced kidney damage when compared to rat as incidence and severity of nephropathy

were not affected in either sex at dietary levels (2-year feeding study) of up to 6000 mg/kg b.w./day in males and of up to 12000 mg/kg b.w./day in females.
The incidence and severity of the renal effects appear to depend on the dose level as well as on the exposure duration. A NOAEL for renal effects (in male rats) of about 550 mg/kg b.w./day can be considered from a 90-day drinking water study (Robinson et al. 1990 – quoted from BUA 1991 and from IUCLID 2000) and of about 625 mg/kg b.w./day from a 13-week feeding study (Melnick 1984) whereas 2-year feeding studies have revealed NOAELs of 100 (Blood 1965) and 200 mg/kg b.w./day (DePass et al. 1986a), respectively. This difference in the NOAELs could be a result of different sensitivity in the rat strains used in the two studies (Blood: Sprague-Dawley; DePass et al.: Fischer 344/N), but no data are available to further elucidate this aspect. However, the validity of the study by DePass et al. is considered to be better than that of the study by Blood, e.g., 130 animals of each sex per group compared to 16 animals of each sex per group; more detailed descriptions of results including incidence and severity of renal effects in the various dose groups.
The lowest NOAEL (about 70 mg/kg b.w./day) for damage to the kidneys in male Wistar rats has been observed in a 16-week dietary study (Gaunt et al. 1974 – quoted from BUA 1991 and IUCLID 2000); however, BUA and IUCLID do not give any details about the kidney damage (type, incidence, severity) and the study report is not public available. Overall, a NOAEL for renal effects in male rats, the most sensitive species, of 200 mg/kg b.w./day is considered taken into account the reliability of the various studies as discussed above. According to NTP (1993), the study by DePass et al. (1986a) was considered adequate to evaluate the chronic toxicity of EG in F344 rats; therefore, NTP only has conducted a 2-year study in mice.
According to DePass et al. (1986), the greater susceptibility of male rats to EG induced renal toxicity may be the result of more efficient conversion of EG to toxic metabolites including oxalate in male rats, as well as to the more rapid progression of spontaneous nephropathy in the male. High-dose females had significant amounts of urinary oxalate crystals at 12, 18, and 24 months, so conversion of EG to oxalate clearly occurred in females also. The critical factor responsible for the more severe nephrotoxicity in male rats was probably the greater incidence and severity of spontaneous nephropathy in the male.
In female rats, mild fatty metamorphosis of the liver has been observed in a 2-year feeding study (DePass et al. 1986a) (at 2.5% in the feed corresponding to 1000 mg/kg b.w./day); a NOAEL of 0.5% (corresponding to 200 mg/kg b.w./day) is considered for liver effects in female rats.

No data on toxicity to reproduction in humans have been found.
Dietary exposure of male and female F344 rats to EG at dose levels up to 1000 mg/kg b.w./day (the highest dose level in the study) for three generations produced no effects on fertility, fecundity, or reproductive performance. When EG was administered to CD-1 mice in the drinking water for 14 weeks (continuous breeding study), reduced fertility and fecundity, and foetotoxic effects, including malformations were observed at about 1640 mg/kg b.w./day; the NOAEL was about 840 mg/kg b.w./day.
Administration of EG via the gastrointestinal route (gavage) at high concentrations has resulted in developmental toxicity, including teratogenicity in rats and mice. Developmental toxicity was observed in CD rats in two gavage studies at dose levels from about 1000 mg/kg b.w./day (gavage) and in Wistar rats from about 860 mg/kg b.w./day whereas one gavage study did not show any developmental toxicity in CD rats at 1250 mg/kg b.w./day. When EG was administered to F344 rats in the diet, no developmental effects were observed at dose levels up to 1000 mg/kg b.w./day (the highest dose level in the study). No serious maternal effects were noted at the dose levels resulting in developmental toxicity. Mice appear to be far more sensitive to the developmental effect exerted by EG with severe developmen-

tal toxicity, including malformations, being observed in a gavage study at dose levels from 750 mg/kg b.w./day (the lowest dose level in the study), a dose level where no maternal effects were observed. In another gavage study, lower dose levels were administered and a NOAEL for developmental toxicity of 150 mg/kg b.w./day can be considered from this study. One gavage study in rabbits indicates that this species is fairly resistant to the developmental effects exerted by EG as no developmental effects were observed at dose levels up to 2000 mg/kg b.w./day (the highest dose level in the study).
Following dermal application of EG to CD-1 mice, no developmental effects were observed at dose levels up to about 3550 mg/kg b.w./day (the highest dose level in the study).
Developmental toxicity, including teratogenicity, has been observed in CD-1 mice following whole-body exposures to EG respirable aerosol at concentrations from 1000 mg/m^3 (6 hours a day); CD rats exposed similarly exhibited developmental toxicity, but no teratogenicity at the same exposure levels. This is consistent with the results observed in the oral studies discussed above, in which mice appeared to be far more sensitive to the developmental effect exerted by EG than rats. The NOAEL for developmental effects in the whole-body study was 150 mg/m^3 for rats and at or below 150 mg/m^3 for mice with NOAELs for maternal effects of 1000 and 150 mg/m^3, respectively (no concentrations between 150 and 1000 mg/m^3). Analysis of EG on the fur of rats and mice during and after the exposure period (2500 mg/m^3) showed significant amounts of EG on the fur, which, according to the authors (Tyl et al. 1995a,b), alone could have produced the effects seen in mice if it were ingested by grooming and/or percutaneously absorbed. Therefore, a nose-only study was performed in CD-1 mice in order to evaluate the toxicity of EG aerosol from inhalation exposure alone (500, 1000, and 2500 mg/m^3). In this study, the NOAEL for maternal and developmental effects, including teratogenicity, was 500 and 1000 mg/m^3, respectively. Assuming (for pregnant mice) an inhalation rate of 25 ml/minute (corresponding to 0.036 m^3/day), a body weight of 0.035 kg, and 100% absorption of EG by inhalation, the NOAELs correspond to about 500 and 1000 mg/kg b.w./day, respectively.

Most of the mutagenicity and genotoxicity tests available indicate that EG is not a mutagenic or genotoxic substance although some positive results have been reported. In the micronucleus assay in mice, the increased numbers of micronuclei was observed following administration (oral, intraperitoneal injection) of very high doses (2.8 to 13.9 g/kg b.w.) and thus, the result is not considered as being reliable. overall, EG is considered not to be a mutagenic or genotoxic substance.

No evidence of a carcinogenic effect of EG was observed at dietary concentrations of up to approximately to 2000 mg/kg b.w./day for 2 years in rats or of up to approximately 12000 mg/kg b.w./day for 2 years in mice.
No data on mutagenic and genotoxic effects, or carcinogenic effects of EG in humans have been found.

The toxicity of EG is primarily a result of the effects of its metabolites although the effects on the central nervous system observed shortly after acute ingestion is partly attributed to unmetabolised EG.
The metabolic acidosis is usually attributed to the acidic metabolites of EG and recent studies of cases of human EG poisoning (discussed in Jacobsen & McMartin 1997) have demonstrated that the major determinant of the metabolic acidosis is the degree of glycolic acid accumulation as glycolate accumulation correlates with the increase in anion gap or decrease in arterial bicarbonate concentrations observed in poisoned humans, as well as in animals.
The mechanism of the renal toxicity is not yet known. Calcium oxalate precipitation within the renal tubules has long been accepted as an important pathogenic

factor for the development of renal toxicity; however, there is no evidence directly linking oxalate precipitation with development of renal tubular necrosis and renal damage can occur at exposure levels where no or few oxalate crystals are detected. The renal toxicity has also been suggested to occur from a metabolite-induced cytotoxicity, such as from glycolaldehyde or glyoxylate, which are both highly toxic *in vitro*, or via the metabolic acidosis resulting from accumulation of glycolic acid. As a result of the decrease in the pH in body fluids, an elevated anion gap develops and the serum osmolal gap across cells increases, resulting in renal oedema that compromises intrarenal blood flow and promotes renal failure. These suggestions are in concordance with the data published by Pottenger et al. (2001) showing that oxalate was a very minor metabolite in rats in both blood and urine at oral (gavage) dose levels up to 2500 mg/kg b.w. and the data discussed in Jacobsen & McMartin (1997) showing that glycolate accumulation correlates with the increase in anion gap or decrease in arterial bicarbonate concentrations observed in poisoned humans, as well as in animals.
Although the mechanism(s) behind the developmental effects is not yet known, a link between maternal metabolic acidosis and developmental toxicity has been suggested with glycolic acid being the predominant toxic metabolite.

6.8.1 Critical effects and NOAELs

The critical effects following exposure to EG are the effects in the kidneys, which are observed in both humans and experimental animals; the developmental effects observed in experimental animals; and the irritative effects observed in humans and experimental animals following inhalation of EG.
In female rats, effects on the liver (mild fatty metamorphosis) has been observed with a NOAEL of about 200 mg/kg b.w./day; however, the liver effects observed are not considered to be as serious as the renal lesions observed in male rats although a NOAEL of about 200 mg/kg b.w./day for renal effects in male rats has been considered as well.

The mechanism(s) behind the nephrotoxic and developmental effects are not known but are probably due to the metabolic acidosis resulting from an accumulation of the EG metabolite glycolic acid. Glycolic acid is a major metabolite in both humans and experimental animals whereas oxalate appears to be a minor metabolite.

Based on the results reported in the available oral studies, male rats are far more sensitive to the renal effects than are female rats and mice (both sexes). No data are available in order to evaluate the sensitivity of humans to the renal effects as no long term studies in humans are available. Following acute ingestion of EG, the same type of renal effects are observed in humans as in experimental animals. Therefore, humans are considered to be as sensitive as male rats to the nephrotoxic effects of EG; a NOAEL of 200 mg/kg b.w./day has been considered for renal effects in male rats from a 2-year dietary study as discussed above. The exposure levels in the available inhalation studies (volunteers: 17-49 mg/m^3 for 30 days; experimental animals: 12 mg/m^3 continuously for 90 days or 10-57 mg/m^3 for 8 hours a day, 5 days per week for 6 weeks) are considered to be far too low to result in any renal effects.

Mice appear to be far more sensitive to the developmental toxicity exerted by EG than rats and rabbits. No data on reproductive toxicity in humans are available. Therefore, humans are considered to be as sensitive as mice to the developmental effects of EG. In a nose-only inhalation study, the NOAEC for developmental effects, including teratogenicity, in mice was 1000 mg/m^3 while the NOAEC was at

or below 150 mg/m^3 in a whole-body inhalation study (no concentrations between 150 and 1000 mg/m^3). Assuming (for pregnant mice) an inhalation rate of 25 ml/day (corresponding to 0.036 m^3/day), a body weight of 0.035 kg, and 100% absorption of EG by inhalation, these NOAECs correspond to about 1000 and 150 mg/kg b.w./day, respectively. A NOAEL for developmental toxicity in mice following gavage of 150 mg/kg b.w./day can be considered as discussed above.

Male volunteers complained of irritation of the throat following exposure to mean concentrations of 17 to 49 mg/m^3 (aerosol, 20-22 hours a day for 30 days). Moderate to severe eye irritation has been observed in rats and rabbits exposed continuously for 90 days to EG (vapour, 12 mg/m^3) but not following exposure to a concentration of 57 mg/m^3 (vapour, 8 hours a day, 5 days per week for 6 weeks). Based on these data, a LOAEC for irritative effects of 17 mg/m^3 is considered; furthermore, the data indicate an eye irritating potential of EG following instillation of either neat EG or solutions of EG to rabbit eyes. Assuming (for adults) an inhalation rate of 20 m^3/day, a body weight of 70 kg, and 100% absorption of EG by inhalation, this LOAEC corresponds to about 5 mg/kg b.w./day, which is far below the NOAELs considered for renal (200 mg/kg b.w./day) and developmental toxicity (150 mg/kg b.w./day).

For the purpose of estimating a quality criterion in air, 17 mg/m^3 is considered as a LOAEC for irritative effects of EG in the throat in humans observed in the study by Wills et al. (1974). The study by Wills et al. (1974) was considered by the EU Scientific Expert Group on Occupational Exposure Limits *"to be the best available basis for proposing occupational exposure limits"*. However, the Danish Environmental Protection Agency could not accept the study by Wills et al. (1974) as the basis for the quality criterion in air, primarily due to ethical reasons. Therefore, the LOAEC of 12 mg/m^3 for inflammatory changes observed in the lungs of exposed animals, and to a lesser degree in controls, in the study by Coon et al. (1970) has been selected as the point of departure acknowledging the limitations of this study (most of the observed effects were interpreted, by Coon et al., as being unrelated to the exposure to EG).

7 Quality criterion in air

The quality criterion in air QC_{air} is calculated based on a LOAEC of 12 mg/m^3 for inflammatory changes observed in the lungs of exposed animals, and to a lesser degree in controls, in the study by Coon et al. (1970):

$$QC_{air} = \frac{LOAEC}{UF_I * UF_{II} * UF_{III}} = \frac{12\ mg/m^3}{10 * 10 * 10}$$

$$= 0.012\ mg/m^3$$

The uncertainty factor UF_I accounting for interspecies variability is set to 10 assuming that humans are more sensitive than animals. The UF_{II} accounting for intraspecies variability is set to 10 reflecting the range in biological sensitivity within the human population. The UF_{III} is set to 10 because a LOAEC is used in stead of a NOAEC and because of the limitations in the study by Coon et al. (1970).

A quality criterion of 0.012 mg/m^3 has been calculated.

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Evaluation of health hazards by exposure to Ethylene glycol and proposal of a health-based quality criterion for ambient air

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to ethylene glycol. This resulted in 2006 in the present report which includes a health-based quality criterion for the substance in ambient air.

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