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Environmental Protection Agency

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

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Title:

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

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Preface

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to diethylene glycol 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 ₄ H ₁₀ O ₃
Structural formula:	HO-CH ₂ -CH ₂ -O-CH ₂ -CH ₂ -OH
Molecular weight:	106.12
CAS-no.:	111-46-6
Synonyms:	Bis(2-hydroxyethyl)ether DEG Diglycol Glycoether Glycoethylether 2-(2-Hydroxyethoxy)ethanol 2-Hydroxyethylether 3-Oxapentane-1,5-diol 2,2'-Oxybisethanol 2,2'-Oxydiethanol

1.2 Physical / chemical properties

Description:	Diethylene glycol (DEG) is a colourless, practically odourless, viscous and hygroscopic liquid with a sharply sweetish taste but a bitter aftertaste.
Purity:	-
Melting point:	-8 – -10 °C
Boiling point:	245 °C (at 760 mmHg)
Density:	1.117 g/ml (at 20°C)
Vapour pressure:	< 0.01 mmHg (< 1.3 Pa) at 20°C 0.02 mmHg (2.7 Pa) at 20°C (MAK 1998)
Concentration of saturated vapours:	13 or 26 ppm (55 or 115 mg/m ³) (at 20°C and 760 mmHg) (calculated).
Vapour density:	-
Conversion factor:	1 ppm = 4.41 mg/m ³ 20°C 1 mg/m ³ = 0.227 ppm 1 atm
Flash point:	143 °C

Flammable limits:	1.6 -10.8 (v/v% in air)
Autoignition temp.:	230 °C (at 760 mmHg)
Solubility:	Water: Miscible. Miscible with lower aliphatic alcohols and ketones. Practically insoluble in aliphatic hydrocarbons and fatty oils.
logP _{octanol/water} :	Ca. -2 (at 20°C)
Henry's constant:	2 x 10 ⁻⁹ (atm x m ³)/mol at 20°C
pK _a -value:	-
Stability:	DEG is quite stable chemically and does not present a hazard due to flammability, except at high temperatures or where mist may be involved.
Incompatibilities:	-
Odour threshold, air:	-
References:	IUCLID (2000), HSDB (1999), A&H (1993), Cavender & Sowinski (1994), Merck Index (1996).

1.3 Production and use

DEG is produced commercially as a by-product of ethylene glycol production. It can also be produced directly by reaction between ethylene glycol and ethylene oxide. (Cavender & Sowinski 1994).

DEG is used in gas conditioning and in antifreeze formulations; as a constituent of brake fluids, lubricants, mould release agents, and inks; as a softening agent for textiles; as a plasticiser for cork, adhesives, paper, packaging materials, and coatings; as an intermediate in the production of the explosive diethylene glycol dinitrate; and as an intermediate in the production of certain resins, morpholine, and diethylene glycol ethers and esters. DEG was previously an ingredient in some medicines. (Cavender & Sowinski 1994, A&H 1993, Merck Index 1996).

1.4 Environmental occurrence

DEG is released to the environment during its production and use.

1.4.1 Air

No data have been found.

1.4.2 Water

DEG has been qualitatively detected in US drinking water supplies and in ground waters in the Netherlands at a maximum concentration of 3 µg/l (HSDB 1999).

1.4.3 Soil

No data have been found.

1.4.4 Foodstuffs

No data have been found.

1.5 Environmental fate

1.5.1 Air

If released to the atmosphere, vapour-phase DEG is degraded by reaction with photochemically produced hydroxyl radicals with an estimated half-life of about 13 hours. Particulate-phase DEG may be physically removed from the air by wet deposition. (HSDB 1999).

1.5.2 Water

If released to water, biodegradation is expected to be an important fate process. DEG is not expected to adsorb to suspended solids and sediment or to volatilise from water to the atmosphere. (HSDB 1999).

1.5.3 Soil

If released to soil, DEG is expected to biodegrade quickly. It is expected to have a very high mobility in soil. Volatilisation of DEG is not expected to be important from moist or dry soil surfaces. DEG will not be susceptible to direct photolysis on soil surfaces. (HSDB 1999).

1.5.4 Bioaccumulation

Based on the high water solubility and the low octanol/water partition coefficient, DEG is not expected to significantly bioconcentrate in aquatic organisms (HSDB 1999).

1.6 Human exposure

No data have been found.

2 Toxicokinetics

2.1 Absorption, distribution, and excretion

2.1.1 Inhalation

Because of the polar and hygroscopic characteristics of DEG, it can be assumed that most of the substance in vapour form is probably absorbed soon after it enters the upper respiratory passages. DEG in aerosol form is probably also absorbed in the upper respiratory passages. (A&H 1993).

2.1.2 Oral intake

Oral doses of 1, 5, or 10 ml/kg ^{14}C -DEG (purity >99%) were given to male Sprague-Dawley rats (4, 4, and 2 animals per group, respectively) by gavage. The absorption was rapid with an initial steep increase of ^{14}C activity in plasma being observed for 10-14, 4, or 2 minutes, respectively, and maximal concentrations in plasma being observed after 30-120, 30-100, or 150-240 minutes, respectively. ^{14}C -DEG was rapidly distributed from the blood into organs and tissues in the order kidneys > brain > spleen > liver > muscle > fat; 64, 87, or 91% of ^{14}C activity in blood disappeared in 12-16 hours with a half-life of 3.4 hours. A total of 73-96% of ^{14}C activity in blood was excreted with the urine and 0.7-2.2% with the faeces. (Heilmair et al. 1993).

Rats given an oral dose of 1, 5, or 10 ml/kg of ^{14}C -DEG excreted 61 to 68% as DEG and 16 to 31% as 2-HEAA in the urine; elimination half-lives were 6, 6, or 10 hours, respectively (Lenk et al. 1989 - quoted from A&H 1993 and from Toxline).

2.1.3 Dermal contact

Dermal uptake by animals is indicated by the occurrence of toxic effects.

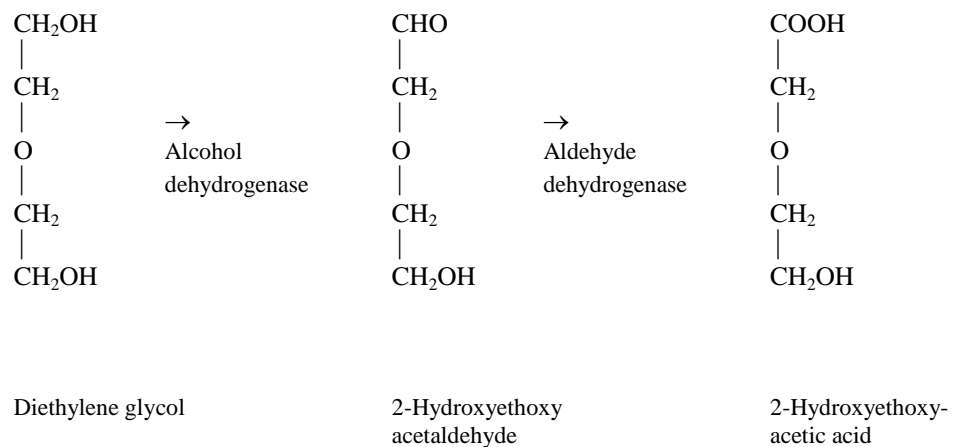
2.2 Metabolism

To determine the metabolic pathway for the oxidation of DEG, male Wistar rats (2 animals per group) were given 1.1 g/kg [1,2- ^{14}C]DEG (free of ethylene glycol) by gavage (3 groups) or by intravenous injection (1 group). In the urine, the parent compound (accounting for about 80% of the radioactivity present in the urine) and a single radiolabelled compound identified as 2-hydroxyethoxyacetic acid (2-HEAA, accounting for about 20% of the radioactivity) were detected. The route of administration did not alter the metabolic profiles. The suggested pathway for the oxidation of DEG is presented in Figure 1. DEG is first oxidised by alcohol dehydrogenase to 2-hydroxyethoxy acetaldehyde, which is rapidly oxidised by aldehyde dehydrogenase to HEAA. Following oral administration, about 43% of the administered radioactivity was recovered in the urine during the first 6 hours. The oxidation of DEG was inhibited by pyrazole, an alcohol dehydrogenase inhibitor, and by diethyldithiocarbamate, an aldehyde dehydrogenase inhibitor. Further oxidation of HEAA to diglycolic acid was not observed. No radioactivity was detected as eth-

ylene glycol or its metabolites, glycolaldehyde, glycolate, glyoxylate, oxalate or carbon dioxide indicating that the ether bond of DEG is not cleaved. (Wiener & Richardson 1989).

Older studies have suggested a metabolic cleavage of the ether bond in DEG with the formation of ethylene glycol and its metabolites, e.g., oxalic acid. More recent studies did not confirm this suggestion as no metabolites of ethylene glycol could be found in the urine of rats given single doses of pure DEG. (A&H 1993).

Figure 1. Proposed metabolic pathway for the oxidation of DEG. From Wiener & Richardson (1989).



2.3 Toxicological mechanisms

No data have been found.

3 Human toxicity

3.1 Single and repeated dose toxicity

DEG poisoning in man is characterised by nausea, dizziness and pain in the region of the kidneys, initial polyuria followed by oliguria, anuria and death in a uraemic coma. Depression of the central nervous system is the main effect after exposure to single high doses. Severe pathological effects on the kidneys and liver occur. The kidneys become pale and swollen. Histopathological examination reveals hydropic degeneration of the kidney tubules with desquamation of the tubule epithelium and obstruction of the tubules. In general, there are not effects on the glomeruli. Centri-lobular hydropic degeneration is seen in the liver. Oxaluria has also been reported in some cases following high oral doses. (Anon 1995).

3.1.1 Inhalation

No data have been found.

3.1.2 Oral intake

There have been several deaths due to the use of DEG in medicinals and from accidents. In virtually all of these cases the ultimate cause of death was kidney failure. (A&H 1993, Cavender & Sowinski 1994).

In general, pathology observed in human victims resembles closely that which has been observed in laboratory animals and consists primarily of degeneration of the kidney with fewer lesions in the liver (Cavender & Sowinski 1994).

In 1937, 105 persons died after repeated doses of a sulpha elixir that contained 72% DEG. The symptoms began with nausea, followed in order by vomiting, greatly increased urine production, which then declined and ceased entirely, pains in the stomach and back, grogginess, coma, trembling, spasms, and death. Based upon this episode, the acute lethal dose for humans has been estimated to be about 1 g/kg b.w. (Calvery & Klump 1939 - quoted from Cavender & Sowinski 1994, A&H 1993 and from IUCLID 2000).

More recent cases include three epidemics among children in Nigeria, Bangladesh, and in Haiti:

In 1990, 47 Nigerian children died, within 2 weeks from admission, from ingestion of paracetamol syrup adulterated with diethylene glycol. Most of the children presented with anuria, fever, vomiting, diarrhoea, and convulsions. Signs on admission were tachycardia, acidotic breathing, pallor, oedema, and hepatomegaly. Laboratory findings included hyperkalaemia, acidosis, elevated creatinine level and hypoglycaemia. The clinical course was rapid and the average time from admission to death was 4 days, most died within 1-2 days of admission. (Okuonghae et al. 1992 - quoted from Toxline and from IUCLID 2000).

In Bangladesh, 339 children with initially unexplained renal failure were significantly more likely, when compared to a control group of children with cause of renal failure identified, to have hepatomegaly, oedema and hypertension, to have a higher serum creatinine concentration and lower serum bicarbonate concentration,

to have been given a drug for fever, to have ingested a brand of paracetamol shown to contain 20% DEG, and to have died in hospital. All children were admitted to hospital during 35 months after January 1990. Based upon these observations, it was concluded that paracetamol elixirs with DEG as a diluent were responsible for a large outbreak of fatal renal failure. (Hanif et al. 1995 - quoted from Toxline). In Haiti, an epidemic of severe systemic toxicity and deaths from DEG-contaminated acetaminophen (paracetamol) syrup occurred in 1996 with 109 identified cases of acute renal failure among children. The clinical syndrome included renal failure, hepatitis, pancreatitis, central nervous system impairment, coma, and death. The median estimated toxic dose was 1.34 ml/kg b.w. (1.57 g/kg b.w.) (range 0.22-4.42 ml/kg). (O'Brien et al. 1998 - quoted from Toxline).

In 1985, wine produced in Austria that was contaminated with DEG appeared on the European market. The concentration of DEG ranged mostly from 1 to 10 g/l wine with the highest concentration being 48 g/l. The assessment of potential health risks was difficult, since there was a clear lack of observations on the possible toxicological effects of small DEG doses. (Freundt & Weis 1989).

3.1.3 Dermal contact

Five patients with third-degree burns died of kidney failure following treatment of the damaged skin, which covered 7 to 63% of the body surface, with 500 to 1400 ml of a solution that contained 6 to 7 mg/ml of DEG. The calculated amount of DEG applied to the skin was 0.05 to 0.5 g/kg b.w./day. (Cantarell et al. 1987 - quoted from A&H 1993).

3.2 Irritation

3.2.1 Skin irritation

DEG is not particularly irritating to skin; prolonged exposure can result in skin maceration (A&H 1993, Cavender & Sowinski 1994).

DEG (112 mg) for 3 days resulted in a mild reaction to human skin (Drill & Lazar 1976 – quoted from IUCLID 2000).

When undiluted DEG was applied two times daily for 2 hours to the forearm under occlusion, no irritation was observed. No further details are available in IUCLID. (Loeser et al. 1954 – quoted from IUCLID 2000).

3.2.2 Eye irritation

DEG is not particularly irritating to eyes (A&H 1993, Cavender & Sowinski 1994).

3.3 Sensitisation

DEG was not sensitising in a Patch-Test in humans (Meneghini et al. 1971 – quoted from IUCLID 2000).

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 adequate data have been found.

4 Animal toxicity

4.1 Single dose toxicity

The symptom of acute poisoning with DEG reported to data following oral, dermal and injection exposure are essentially similar for all species. The initial signs of toxicity are thirst, increased diuresis, loss of appetite and excitation, followed by a subsequent reduction in urine volume and even anuria, with proteinuria, metabolic acidosis, accelerated breathing, stupor, weakness, narcosis, disturbances in coordination, a reduction in body temperature, and coma. Death occurs in a uraemic coma due to cessation of breathing and cardiac arrest. Characteristic macroscopic and histopathological effects following the administration of lethal doses include hydropic degeneration of the kidney tubules and the centrilobular areas of the liver, with generalised oedema and haemorrhages in the lungs, intestinal tract and kidney.

The acute toxicity of DEG is based on a dose-dependent increase in urine excretion due to the hygroscopic properties of the substance, a narcotic effect, and the development of metabolic acidosis, which is correlated, with formation of the metabolite (2-hydroxyethoxyacetic acid - HEAA). (Anon 1995).

4.1.1 Inhalation

Rats were exposed to DEG (aerosol) at the maximum attainable concentration of 4400-4600 mg/m³ for 4 hours; the mass median aerodynamic diameter (MMAD) of particles ranged from 2.6 to 3.1 µm and greater than 96% were below 10 µm. No deaths were observed over a 14-day observation period. Observed signs consisted of decreased activity during exposure with rapid recovery on removal, a transient body weight loss, and nasal discharge or lachrymation, which persisted for several days. (Cascieri et al. 1991, abstract).

An essentially saturated atmosphere generated at approximately 170 °C and a fog generated at about 70 °C caused no deaths of rats exposed for 8 hours (Union Carbide Corporation unpublished data - quoted from Cavender & Sowinski 1994).

4.1.2 Oral intake

The reported oral LD₅₀-values for DEG ranged from 12.6 to 32.0 g/kg b.w. in rats, from 13.3 to 28.2 g/kg b.w. in mice, from 7.8 to 14.0 g/kg b.w. in guinea pigs, and of 4.4 or 26.9 g/kg b.w. in rabbits (IUCALID 2000, Cavender & Sowinski 1994, A&H 1993).

DEG produced metabolic acidosis, which was completely balanced after single doses of 1 or 5 ml/kg, but doses greater than 10 ml/kg produced non-compensated metabolic acidosis, hydropic degeneration of the tubuli, oliguria, anuria, accumulation of urea-nitrogen, and death in uraemic coma (Heilmair et al. 1993).

Rats given a single oral dose of 17.5 ml/kg b.w. died after 2 to 7 days from metabolic acidosis and uraemic poisoning (Lenk et al. 1989 - quoted from A&H 1993).

4.1.3 Dermal contact

Dermal LD₅₀-values for DEG of 11.9 and 13.1 g/kg have been reported for the rabbit (IUCLID 2000).

4.2 Irritation

4.2.1 Skin irritation

DEG produces no significant skin irritation; however, prolonged contact over an extended period of time may produce a macerating action comparable to that caused by glycerol (Cavender & Sowinski 1994).

DEG was reported as being not irritating (rabbit, guinea pig, and rat) or slightly irritating (rabbit) when applied to the skin (IUCLID 2000).

4.2.2 Eye irritation

DEG did not cause appreciable irritation when introduced into the eyes of rabbits (Cavender & Sowinski 1994, IUCLID 2000).

4.3 Sensitisation

DEG was not sensitising when tested in the guinea pig maximization test according to EEC Guideline B.6 (BASF 1989 – quoted from IUCLID 2000).

4.4 Repeated dose toxicity

On repeated administration of high doses, as on acute administration, the nephrotoxicity of the substance is particularly evident and in addition, effects on the liver were found. Increased diuresis with subsequent anuria, proteinuria and haemoglobinuria, unconsciousness, breathing difficulties and coma occurred with hydropic effects on the proximal kidney tubules and in the centrilobular areas of the liver. (Anon 1995).

4.4.1 Inhalation

No data have been found.

4.4.2 Oral intake

4.4.2.1 Rats

In rats given 1 to 20% of DEG in their drinking water (subchronic study according to Cavender & Sowinski 1994, no further details given), DEG had a CNS depressant effect and caused central paralysis of the respiratory and cardiac centres (Bornman 1954, 1955 - quoted from Cavender & Sowinski 1994).

In adult female Sprague-Dawley rats (8 animals in the dose group and in the control group) given 200 mg/kg b.w./day (the only dose level tested) of DEG in the drinking water for 90 days, no changes in renal function or in relative kidney weight were observed; histological examinations were not performed. The renal function was evaluated by analysis of a number of urinary parameters: volume, specific gravity, creatinine, lactate dehydrogenase, leucine aminopeptidase, β -galactosidase, leucocytes, erythrocytes, nitrite, protein (albumin), glucose, ketone, urobilinogen, bilirubin, and pH. (Freundt & Weis 1989).

Fischer 344 rats (50 animals of each sex per group) were given 0, 1.25 or 2.5% (equal to 0, 1088/1038, or 2350/2275 mg/kg b.w./day in males/females, respectively) of DEG in the drinking water for 108 weeks. Survival of male rats in the high-dose group was lower than in the other groups (36/50, 36/50, 31/50) while survival of females was similar in the three groups (31/50, 32/50, 30/50). Body weights of male rats decreased slightly with dose (434, 406, 392 g at 108 weeks) while body weights of females were similar in the three groups (322, 326, 323 g at 108 weeks). Water intake increased with dose (75/78, 87/83, 94/91 ml/kg b.w./day in males/females, respectively) and the average DEG intake was directly dose related in both males and females. No significant differences between the three groups were found for organ weights (brain, heart, lung, liver, spleen, kidney, and testis) at week 108. Haematological analyses and clinical chemistry parameters showed no significant differences between the treated and control rats. The histological examination of the organs did not reveal any changes except for tumours, see section 4.7. (Hiasa et al. 1990).

In a 28-days study (OECD guideline 407), Wistar rats were given DEG (purity not specified in IUCLID) in the feed at concentrations of 0, 500, 2500, 10000, or 40000 mg/kg feed (equivalent to 0, 50, 250, 1000, or 4000 mg/kg b.w./day assuming that an adult young rat consumes 100 g feed/kg b.w./day). The animals were observed for 3 weeks after the dosing period. The control group and the highest dose group consisted of 10 animals of each sex and the other dose groups consisted of 5 animals of each sex. In the highest dose group, a significant increase in the concentration of oxalic acid in the urine was observed in both sexes and oxalate crystals were observed in the urine of the males; these changes were reversible within the post-observation period. No effects were observed at the lower dose levels. The NOEL was considered, according to IUCLID, to be 10000 mg/kg feed (equivalent to 1000 mg/kg b.w.). (BASF 1988 – quoted from IUCLID 2000).

Wistar rats (15 animals of each sex per group) were given 0, 0.4, 2.0, or 4.0% DEG (containing less than 0.01% monoethylene glycol) in the feed (equal to 0, 300/400, 1600/1800, or 3000/3700 mg/kg b.w./day in males and females, respectively) for 99 days. At the highest dose level (4.0%), 6 male rats died (or were killed during the study) with signs of renal damage (tubular necrosis, mainly of the proximal convoluted tubules in five rats, and hydropic degeneration of the proximal tubule in the remaining animal). The bladder and ureters were distended in all six animals and calculi (containing calcium oxalate crystals) were found in the bladders together with marked haematuria. High-dose male animals showed a significantly decreased body weight gain; at the lower dietary levels, the body weights were lower (not significant) in male animals whereas no differences were observed for female rats. The water intake was increased (significant for males) at 4% in both sexes and in males at 2%. Male rats showed haematological changes (significant increase in the packed cell volume, haemoglobin concentration, and total erythrocytes at 4%; significant increase in haemoglobin concentration at 2%); no differences were observed in female rats. Rats of both sexes at the high-dose level produced an increased volume of more dilute urine following a prolonged period of water deprivation. Oxalate crystals were found in the urine from both sexes at the 2% (males:

3/15; females: 7/15) and 4% (males: 6/8 (survivors); females: 13/15) dietary levels and in females (7/15) from the 0.4% level. When expressed in relation to body weight, most of the organ weights in males at the high-dose level were significantly increased; no significant changes were observed for males at the lower dietary levels or in females. The histopathological findings were confined to the kidney and liver. Lesions in the liver were seen only in the females at the highest dose level (hydropic degeneration in 6/15 and necrosis in 1/15). The most marked change in the kidneys was tubular necrosis at the highest dose level only (6/9 males (survivors) and 9/15 females); hydropic degeneration was observed in 1/15 mid-dose males and in 3/9 high-dose males.

In a second experiment investigating oxalate excretion in the urine, rats (10 animals of each sex per group) were given diets containing 0, 0.085, 0.17, 0.4, or 2.0 % DEG (equal to 0, 50/60, 100/130, 230/290, or 1200/1500 mg/kg b.w./day in males and females, respectively) for 225 days. Water intake was significantly increased in high-dose males. The concentrations of urinary oxalic acid was significantly increased at the 2% dose level from week 13 in males and from week 9 in females; at the 0.4% dose level from week 13 in males and at week 19 in females; and at the 0.17% dose level at week 19 in males; no changes were detected at the lowest dose level of 0.085%. Oxalate crystals were found in the urine of the treated groups (males: 1/10, 4/10, 5/10, 9/10, 2/10; females: 1/10, 1/10, 3/10, 8/10, 10/10 for controls, 0.085, 0.17, 0.4, or 4% dietary levels, respectively); this finding was significant at the two highest dose levels only. There were no differences between treated and control rats in the results of the haematological examinations. Histological examination of the kidneys (only organ examined) did not reveal any differences between the treated and control animals.

Based upon these two studies, the authors concluded that no detectable oxalate crystalluria was associated with dietary levels of 0.085 and 0.17%. At levels of 0.4% and above, there were indications of effects on renal function, but this was accompanied by histological evidence of kidney damage only with dietary levels of 2% or more.

(BIBRA 1976).

Osborne-Mendel rats (12 males per group) were maintained for 2 years on diets containing 1, 2, or 4% DEG (equivalent to 500, 1000, 2000 mg/kg b.w./day assuming that an adult rat consumes 50 g feed/kg b.w./day). Reduced growth was seen in all treated groups; the effect was marked in high-dose animals, but significant only during the fast growing period for the rats in mid- and low-dose groups. Food consumption was not affected. Extensive lesions (dose-related) were observed in the lower urinary tract. Bladder stones composed of calcium oxalate occurred in all high-dose rats except one and in lesser numbers of mid- and low-dose animals. Lesions in the kidneys were moderate to marked in high-dose animals, slight to moderate in mid-dose animals, and slight or absent in low-dose animals; microscopically, the main lesions were varying degrees of focal tubular atrophy and hyaline cast formation, and less often hydropic degeneration, calcification and glomerular atrophy. Hepatic lesions were observed with a moderate amount of damage at 4%, a small amount at 2%, and almost none at 1%; microscopically, hydropic degeneration varying from very slight to marked was seen in slightly over half of the rats on the 4% level and in a few of those on lower levels. (Fitzhugh & Nelson 1946).

When rats were given DEG (containing only 0.031% of ethylene glycol) in the diet at 0, 2, or 4% (equivalent to 0, 1000 or 2000 mg/kg b.w./day assuming that an adult rat consumes 50 g feed/kg b.w./day) for 2 years, no oxalate stones were observed in the 2% dose group, while 8 of 20 male rats in the 4% dose group developed stones. No stones were formed in any of the female rats, which, according to the authors, indicate that stone formation is sex-related. (Weil et al. 1965).

4.4.2.2 *Mice*

Swiss CD-1 mice (8 animals of each sex per group) were given 0, 1, 2.5, 5, 7.5, or 10% of DEG in drinking water for 14 days. In the highest dose group (10%), 3 males and 2 females died. Water intake and body weight gain were significantly decreased from 5%. No effects were observed below 5%. (Williams et al. 1990 – quoted from IUCLID 2000).

Mice were exposed to 0.03, 0.3, or 3% DEG in drinking water for up to 4 months (lowest concentration equivalent to about 50 mg/kg b.w./day according to A&H). Prolonged coagulation time (dose-related) was noted at all dose levels after 14 days of exposure. After 3.5 months of exposure, the two higher dose groups had a 50% reduction in serum titer of tetanus antibodies, indicating (according to A&H) reduced immune defence. (Huber et al. 1986 - quoted from A&H 1993).

4.4.2.3 *Rabbits*

Changes in the electroretinograms were observed in rabbits given 4% DEG in drinking water (equivalent to about 7000 mg/kg b.w./day according to A&H) for 3 months (Rossa & Weber 1987 - quoted from A&H 1993).

4.4.2.4 *Guinea pigs*

Guinea pigs given DEG (1200 mg/kg b.w.) by gavage daily for 2 to 11 days developed degenerative changes in the myocardium (Ogbuihi et al. 1991 - quoted from A&H 1993 and from Toxline).

4.4.3 **Dermal contact**

When DEG was applied to the skin of rabbits 1 hour per day for 30 days, all rabbits (3 per dose groups) receiving 0.32 ml/kg b.w./day or more died within 2 or 3 weeks; the lower dose (0.16 ml/kg b.w./day) had no effects on mortality (Hanzlik et al. 1947 - quoted from A&H 1993).

4.5 Toxicity to reproduction

4.5.1 **Inhalation**

No data have been found.

4.5.2 **Oral intake**

4.5.2.1 *Rats*

Sprague-Dawley rats (30 animals of each sex per group) were exposed to DEG via the drinking water at doses of 150, 500, or 1500 mg/kg b.w./day for 73 days prior to mating and continuing to termination. Significantly increased relative kidney weights were noted in F0 and F1 high-dose males. DEG had no adverse effects on the reproductive capability of the F0 generation or the development, survival and growth of the F1 generation to weaning. (Rodwell et al. 1987).

In rats given 1 ml of a 20% aqueous solution of DEG per 100 g of body weight (2200 mg/kg b.w.) daily over a period of 12 weeks, DEG had no influence on the reproductive ability of the animals or on their offspring (Wegener 1953 - quoted from Cavender & Sowinski 1994).

CD rats (25/group) were administered DEG by gavage at doses of 0, 1.0, 4.0, or 8.0 ml/kg b.w./day (0, 1100, 4500, or 8900 mg/kg b.w./day) during gestation days 6 to 15. Maternal effects were observed in mid- and high-dose animals and included reduced body weight and food consumption, increased water consumption and kidney weights, and interstitial nephritis and renal tubule damage (high-dose group only). No foetal malformations were observed at any dose level. Litter weights were reduced and the incidence of skeletal variations were increased in foetuses at 8.0 ml/kg b.w./day. (Neeper-Bradley et al. 1992 - quoted from Toxline).

In a teratogenicity study (OECD guide-line 414) in Wistar rats, DEG did not reveal any maternal toxicity, embryotoxicity or teratogenicity when administered at dose levels of 0, 200, 1000, or 5000 mg/kg b.w./day (gavage) from gestation day 6 to 15 (RCC Research & Consulting Company 1985 - quoted from IUCLID 2000).

4.5.2.2 Mice

DEG was evaluated for reproductive toxicity in CD-1 mice (20 animals of each sex per exposed groups; 40 animals of each sex in the control group) using a continuous breeding protocol by administration in the drinking water at 0, 0.35, 1.75, or 3.5% (equal to 0, 610, 3060, or 6130 mg/kg b.w./day). Exposure of the breeding pairs to 3.5% DEG for 14 days produced significant decreases in the number of litters per pair, live pups per litter, proportion of pups born alive, and live pup weight. There was also a significant increase in the cumulative days to litter and a significant decrease in the number of pairs producing the third, fourth, and fifth litters. Slight maternal (F_0) toxicity (a 7% decrease in body weight) was observed at the high-dose level. There were no gross or histopathological lesions in the organs examined from the male and female F_0 mice. A crossover mating trial of the F_0 mice to determine the affected sex was inconclusive, but suggested that offspring development was compromised in high-dose females (3.5%). No effects on reproductive parameters were observed at lower dose levels (0.35 and 1.75%). The F_1 generation, at 3.5% DEG, had decreased body weights at birth and exhibited poor postnatal survival. At 1.75%, body weights of both sexes were depressed at weaning, at onset of mating, and at necropsy; however, no adverse effects on reproduction were observed. At the highest dose level, 12% (14/114) of the live-born pups and 95% (18/19) of pups found dead on postnatal day 0 had craniofacial malformations including exencephaly and cleft palate. (Williams et al. 1990, Lamb IV et al. 1997).

DEG was administered by gavage to Swiss (CD-1) mice (26-31 animals per group) on gestational days 6 to 15 at dose levels of 0, 1250, 5000, or 10000 mg/kg b.w./day. No significant effect on maternal weight gain was observed. Renal lesions were noted in high-dose animals (3/28) and mean maternal kidney weight was increased in the mid- and high-dose groups. Exposure had no effect on pre- or post-implantation loss. The only significant developmental effect was a decrease in foetal body weight in the high-dose group. Examination of the foetuses for external, visceral and skeletal malformations did not reveal any significant effects. (Bates et al. 1991).

No evidence of developmental effects was observed in mice administered 11180 mg/kg b.w. of DEG daily by gavage on days 7 to 14 of gestation. The variables

recorded were the number of litters, survival, litter size, and birth weights and weight gain of pups. (Hardin et al. 1987, Schuler et al. 1984).

4.5.2.3 Rabbits

No signs of maternal toxicity or embryo- and foetotoxicity, including teratogenicity, were observed in Himalayan rabbits (15/group) after oral administration (gavage) of DEG in daily doses of 0, 100, 400, or 1000 mg/kg b.w./day from gestations days 7 to 19. The study was performed according to OECD guideline 414. (BASF 1989 – quoted from IUCLID 2000; Hellwig et al. 1995 - quoted from Toxline).

4.6 Mutagenic and genotoxic effects

4.6.1 *In vitro* studies

DEG was negative when tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 at concentrations from 1 to 111.8 mg/plate with and without metabolic activation (Hengler & Slesinski 1984 – quoted from IUCLID 2000).

A number of other Ames tests with negative results are also included in IUCLID (2000); however, details are generally not specified.

DEG showed a negative result when tested in the gene conversion test in *Saccharomyces cerevisiae* D7; a positive result for aneuploidy was obtained in *Saccharomyces cerevisiae* D61M without metabolic activation; no further details are given (Krug et al. 1986 – quoted from IUCLID 2000).

DEG was negative for gene mutation (HGPRT) in Chinese Hamster Ovary (CHO) cells with and without metabolic activation when tested at concentrations from 30 to 50 mg/ml (Slesinski et al. 1989 – quoted from IUCLID 2000).

DEG was also negative in the Sister Chromatid Exchange assay when tested in CHO cells with and without metabolic activation at concentrations from 30 to 50 mg/ml (Slesinski et al. 1989 – quoted from IUCLID 2000).

DEG did not induce chromosomal aberrations when tested in CHO cells with and without metabolic activation at concentrations from 10 to 50 mg/ml (Guzzi & Slesinski 1989 – quoted from IUCLID 2000).

4.6.2 *In vivo* studies

DEG was negative in the micronucleus assay (species not given) following oral administration of a daily dose (4% of the oral LD₅₀) for 7 days but positive following a single intraperitoneal dose (60% of the LD₅₀); no further details are given (Krug et al. 1986 – quoted from IUCLID 2000).

Slight increases in the incidence of chromosomal damage were observed when DEG was given to hamsters by a single intraperitoneal injection at 1.25 mg/kg. When administered by the oral route (single dose of 7.5 g/kg b.w.; 2% in water for 3 weeks; 5% in the diet for 12 weeks), equivocal results were obtained. No further details are given. (Yoshida et al. 1986 - quoted from IUCLID 2000).

4.7 Carcinogenic effects

In the 2-year study by Fitzhugh & Nelson (1946) on Osborne-Mendel rats (12 males per group) maintained on diets containing 1, 2, or 4% DEG (equivalent to 500, 1000, 2000 mg/kg b.w./day assuming that an adult rat consumes 50 g feed/kg b.w./day), bladder tumours (papillary which were generally benign and intramural which showed varying degrees of malignancy) occurred in about half of the mid- and high-dose rats with none of the low-dose rats. Non-neoplastic findings are described in 4.4.2.1.

When rats were given DEG (containing only 0.031% of ethylene glycol) in the diet at 0, 2, or 4% (equivalent to 0, 1000 or 2000 mg/kg b.w./day assuming that an adult rat consumes 50 g feed/kg b.w./day) for 2 years, only one bladder tumour was found in a high-dose male rat, which also had bladder stones. To ascertain whether the tumours observed (in the study by Fitzhugh & Nelson 1946) in the urinary bladder of rats given large amounts of DEG developed as a result of the bladder stones produced, or if DEG was the primary carcinogen, calcium oxalate stones or glass beads were implanted into the bladders of rats which were maintained on a control diet for 2 years. Bladder tumours were only present with associated foreign body (calcium oxalate or glass bead). This led to the conclusion, by the authors, that DEG essentially free of ethylene glycol is not a primary carcinogen. (Weil et al. 1965).

In a study designed to study the promotive effect of oxalate crystals in the development of bladder cancer, male rats were given 2% DEG in food (equivalent to 1000 mg/kg b.w./day assuming that an adult rat consumes 50 g feed/kg b.w./day) for 32 weeks. No increase in the number of crystals or stones was observed in the urinary passages and no papillary or nodular hyperplasias, papillomas or carcinomas were observed in the bladder. No promotive effect of DEG was observed after pre-treatment with N-butyl-N-(4-hydroxybutyl)nitrosamine. (Masui et al. 1988 - quoted from A&H 1993).

In F344 rats (50 animals of each sex per group) given DEG 0, 1.25 or 2.5% in drinking water (equal to 0, 1088/1038, or 2350/2275 mg/kg b.w./day in males/females, respectively) for 108 weeks, the incidences of tumour-bearing males were 97% (39/40) in the 2.5% group, 97% (46/47) in the 1.25% group, and 97% (46/47) in the control group, while the corresponding figures for females were 57% (26/45), 62% (28/45), and 64% (31/48). Tumours primarily developed in the testis, uterus, pituitary gland, and thyroid. The incidences of tumours in all organs did not significantly differ between the three groups. Non-neoplastic findings are described in 4.4.2.1. In a second experiment, no renal promoting potential was evident for DEG, 2.5% in the drinking water for 30 weeks, after initiation with N-ethyl-N-hydroxyethylnitrosamine. The authors concluded that no evidence was obtained that DEG exerts either carcinogenic or promoting effects in rats. (Hiasa et al. 1990).

No tumours, either systemically or at the injection site, were seen in female NMRI mice given subcutaneous doses of 3, 10, or 30 mg DEG (about 75, 250, or 750 mg/kg b.w. according to A&H) once a week for two years (Dunkelberg 1987 - quoted from A&H 1993, Toxline, and from IUCLID 2000).

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: 2.5 ppm (11 mg/m³) (At 2005).

Germany: 10 ppm (44 mg/m³) (MAK 2005).

USA: 125 mg/m³ (TWA (8 hours), total vapour and aerosol),
10 mg/m³ (TWA (8 hours), aerosol only)
(AIHA 1985 - quoted from Toxline).

5.5 Classification

DEG is classified for acute toxicity (Xn;R22 – harmful if swallowed) (MM 2002).

5.6 IARC

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5.7 US-EPA

-

6 Summary and evaluation

6.1 Description

Diethylene glycol (DEG) is a colourless, practically odourless, viscous and hygroscopic liquid with a low vapour pressure. It is miscible with water.

6.2 Environment

DEG is released to the environment during its production and use. DEG has been detected in US drinking water supplies and in ground waters in the Netherlands; no other data on levels in the environment or in food have been found.

If released to the atmosphere, DEG is degraded by reaction with photochemically produced hydroxyl radicals, estimated half-life of about 13 hours. DEG may also undergo atmospheric removal by wet deposition.

If released to soil and water, DEG is expected to biodegrade quickly. DEG is not expected to adsorb to sediment or suspended organic matter or to volatilise from either soil or water. It is expected to display a very high mobility in soil.

6.3 Human exposure

No data have been found.

6.4 Toxicokinetics

DEG is rapidly and almost completely absorbed in rats following oral administration. Absorption following dermal contact is indicated by the occurrence of toxic effects; no data are available regarding inhalation. Following absorption DEG is rapidly distributed and excreted primarily in the urine with the parent compound accounting for 60 to 80% and the metabolite 2-hydroxyethoxyacetic acid (2-HEAA) accounting for 10 to 30% of the total amount excreted through the kidneys.

6.5 Toxicological mechanism

No data have been found.

6.6 Human toxicity

Several deaths due to the use of DEG in medicinals and from accidents have been reported; the acute lethal dose for humans has been estimated to be about 1 g/kg b.w. Clinical symptoms include nausea, dizziness and pain in the region of the kidneys, initial polyuria followed by oliguria, anuria and death in a uraemic coma. Depression of the central nervous system is the main effect after exposure to single high doses.

DEG is not particularly irritating to eyes or skin and was not shown to be sensitising in a Patch-test. Prolonged dermal exposure can result in skin maceration.

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

6.7 Animal toxicity

6.7.1 Single dose toxicity

The acute toxicity of DEG in experimental animals are essentially similar for all species and is based on a dose-dependent increase in urine excretion, a narcotic effect, and the development of metabolic acidosis.

When rats were exposed to DEG (aerosol) at the maximum attainable concentration of 4400-4600 mg/m³ for 4 hours, no deaths were observed. An essentially saturated atmosphere (about 170°C) or a fog (about 70°C) caused no deaths of rats exposed for 8 hours.

The reported oral LD₅₀-values for different species are above 4000 mg/kg b.w. with the rabbit being the most sensitive species.

Reported dermal LD₅₀-values are 11900 and 13100 mg/kg b.w. for the rabbit.

6.7.2 Irritation

DEG produces no significant skin and eye irritation; prolonged contact over an extended period of time may produce skin maceration.

When rats were exposed to DEG (aerosol) at the maximum attainable concentration of 4400-4600 mg/m³ for 4 hours, nasal discharge or lachrymation was observed, which persisted for several days.

6.7.3 Sensitisation

DEG was not sensitising when tested in the guinea pig maximization test (EEC guideline).

6.7.4 Repeated dose toxicity

Repeated oral administration of high doses to rats results in damage of the kidneys and the liver.

One study (Freundt & Weis 1989) of Sprague-Dawley rats reported no changes in renal function or in kidney weight when 200 mg/kg b.w./day (the only dose level tested) was administered in the drinking water for 90 days; histological examinations were not performed.

Increased oxalate excretion (both sexes) and oxalate crystals (males) were observed in the urine of Wistar rats when DEG was administered in the feed (at 40000 mg/kg feed/day) for 28 days (OECD guideline 407) (BASF 1988 – quoted from IUCLID 2000); these findings were reversible within the 3-week post-observation

period. A NOAEL of 10000 mg/kg feed/day (equivalent to 1000 mg/kg b.w./day) was considered.

Mortality (males), decreased body weight gain (males), increased water intake (males), haematological changes (males), increased relative organ weights (males), and histopathological changes in kidneys (males and females) and liver (females) were observed in Wistar rats given 4% (equal to 3000/3700 mg/kg b.w./day for males/females, respectively) DEG in the diet for 99 days (BIBRA 1976). Oxalate crystals were found in the urine from both sexes from the 2% dietary level (equal to 1600/1800 mg/kg b.w./day for males/females, respectively) and in females from the 0.4% dietary level (equal to 400 mg/kg b.w./day). In a second experiment investigating oxalate excretion in the urine of the same strain of rats (BIBRA 1976), no detectable oxalate crystalluria was associated with dietary levels of DEG (essentially free of ethylene glycol) of up to 0.17% (equal to 100/130 mg/kg b.w./day for males/females, respectively) for 225 days; indications of effects on renal function was observed at dietary levels from 0.4% (equal to 230/290 mg/kg b.w./day for males/females, respectively).

In a 2-year drinking water study (Hiasa et al. 1990) in Fischer 344 rats, survival of male animals was lower at 2.5% (equivalent to 2350 mg/kg b.w./day) than in the other male groups but similar to that of females. Body weights of male rats decreased slightly with dose while body weights of females were similar in the three groups. Water intake increased with dose in both males and females. No differences were observed for organ weights or upon histological examination of the organs.

Formation of calcium oxalate stones in the bladder has been reported in a 2-year dietary study (Fitzhugh & Nelson 1946) of male Osborne-Mendel rats at dietary levels from 1% (equivalent to 500 mg/kg b.w./day); lesions in the kidneys (focal tubular atrophy, hyaline cast formation, hydropic degeneration - 4%: moderate to marked; 2%: slight to moderate; 1%: slight or absent) and liver (hydropic degeneration - 4%: moderate amount of damage; 2%: small amount; 1%: almost none) were observed at 4%.

Another 2-year dietary study (Weil et al. 1965) testing DEG essentially free of ethylene glycol reported that formation of stones was only seen in male rats at a dietary level of 4% (equivalent to 2000 mg/kg b.w./day).

In one study of mice (Huber et al. 1986 – quoted from A&H 1993), a reduced immune defence was observed following administration in the drinking water at concentrations from 0.3% for 3.5 months.

Water intake and body weight gain were significantly decreased in mice given DEG in the drinking water at concentrations for 5% for 14 days (Williams et al. 1990 – quoted from IUCLID 2000).

Changes in the electroretinograms have been observed in rabbits given 4% of DEG in the drinking water (about 7000 mg/kg b.w./day) for 3 months.

Guinea pigs given DEG (1200 mg/kg b.w.) by gavage daily for 2 to 11 days developed degenerative changes in the myocardium.

6.7.5 Toxicity to reproduction

No adverse effects on the reproductive capability of the F0 generation or the development, survival and growth of the F1 generation to weaning were observed in Sprague-Dawley rats exposed to DEG in the drinking water at doses of up to 1500 mg/kg b.w./day for 73 days prior to mating and continuing to termination; increased relative kidney weights were noted in F0 and F1 high-dose males at the highest dose level.

In rats given 2200 mg/kg b.w. of DEG daily over a period of 12 weeks, no influence on the reproductive ability of the animals or on their offspring was noted. In a continuous breeding study in CD-1 mice, reproductive effects (decreases in the number of litters per pair, live pups per litter, proportion of pups born alive, and live pup weight) and developmental effects (decreased body weights at birth and poor postnatal survival) were noted following administration of 3.5% of DEG in the drinking water (equal to 6130 mg/kg b.w./day), but not at lower concentrations (up to 1.75%, equal to 3060 mg/kg b.w./day); slight maternal toxicity (a 7% decrease in F₀ body weight) was observed at 3.5%.

Developmental toxicity (reduced litter weights and increased incidence of skeletal variations), but no malformations, was observed in offspring of CD rats administered DEG (during gestation days 6 to 15) by gavage (8900 mg/kg b.w./day); maternal effects (reduced body weight and food consumption, increased water consumption and kidney weights, and interstitial nephritis and renal tubule damage (high-dose group only)) were observed from 4500 mg/kg b.w./day.

In a teratogenicity study (OECD guide-line 414) in Wistar rats, DEG did not reveal any maternal toxicity, embryotoxicity or teratogenicity when administered DEG at dose levels of up to 5000 mg/kg b.w./day (gavage) from gestation day 6 to 15.

When DEG was administered by gavage to Swiss (CD-1) mice (gestation days 6-15), the only significant developmental effect was a decrease in foetal body weight in the high-dose group (10000 mg/kg b.w./day); examination of the foetuses for malformations did not reveal any significant effects. Maternal effects, increased kidney weight and renal lesions, were observed from 5000 and at 10000 mg/kg b.w./day, respectively.

No evidence of developmental effects was observed in mice administered 11180 mg/kg b.w. of DEG daily by gavage on days 7 to 14 of gestation.

No signs of maternal toxicity or embryo- and foetotoxicity, including teratogenicity, were observed in Himalayan rabbits after oral administration (gavage, gestations days 7 to 19) of DEG in daily doses of up to 1000 mg/kg b.w./day (OECD guideline 414).

6.7.6 Mutagenic and genotoxic effects

DEG was negative when tested in the Ames test (several tests in *Salmonella typhimurium*), in one test for gene conversion in *Saccharomyces cerevisiae* D7, and in Chinese Hamster Ovary (CHO) cells (gene mutation, SCE, and chromosomal aberration). A positive result has been reported for aneuploidy in *Saccharomyces cerevisiae* D61M (one test). Most of the assays were performed with and without metabolic activation.

DEG was negative in the micronucleus assay (species not specified) following oral administration (7 days), whereas a positive result was obtained following a single intraperitoneal dose. Equivocal results have been obtained for chromosomal damage following oral administration to hamsters.

6.7.7 Carcinogenic effects

In a 2-year study in male Osborne-Mendel rats, bladder tumours (papillary which were generally benign and intramural which showed varying degrees of malignancy) occurred in about half of the animals maintained on diets containing 2 or 4% DEG (equivalent to 1000 or 2000 mg/kg b.w./day); no tumours were observed at the lowest dietary level of 1% (equivalent to 5000 mg/kg b.w./day).

In another 2-year dietary study in rats, only one bladder tumour was found in a high-dose male rat (4% DEG (containing only 0.031% of ethylene glycol), equivalent to 2000 mg/kg b.w./day), which also had bladder stones. In a second study, calcium oxalate stones or glass beads were implanted into the bladders of rats, which were maintained on a control diet for 2 years; bladder tumours were only present with associated foreign body (calcium oxalate or glass bead).

No evidence of carcinogenic effect was found in F344 rats given DEG of up to 2.5% in drinking water (equivalent 2350/2275 mg/kg b.w./day in males/females, respectively) for 108 weeks and no renal promoting potential was evident for DEG (2.5% in the drinking water for 30 weeks) after initiation with N-ethyl-N-hydroxy-ethylnitrosamine.

In a study designed to study the promotive effect of oxalate crystals in the development of bladder cancer, no papillary or nodular hyperplasias, papillomas or carcinomas were observed in the bladder and no increase in the number of crystals or stones was observed in the urinary passages in male rats given 2% DEG in food (equivalent to 1000 mg/kg b.w./day) for 32 weeks.

6.8 Evaluation

Diethylene glycol (DEG) is rapidly and almost completely absorbed in rats following oral administration, and distributed, metabolised, and excreted primarily in the urine with the parent compound accounting for 60 to 80% and the only metabolite identified (2-hydroxyethoxyacetic acid, 2-HEAA) accounting for 10 to 30% of the total amount excreted through the kidneys.

The metabolic cleavage of the ether linkage of DEG suggested in some studies, which would result in the formation of ethylene glycol (EG) and thus in a metabolic pathway similar to that of EG (with the formation of oxalic acid via glycol aldehyde, glycolic acid, and glyoxylate) has not been confirmed in metabolism studies involving single exposures to pure DEG. Therefore, the excretion of oxalic acid observed in some older studies in experimental animals (see below) has been considered to be a result of contamination of DEG with EG from which DEG is synthesised. However, no data are available regarding the metabolism of DEG following repeated exposures for longer durations. Furthermore, one of the repeated dose toxicity studies (BIBRA 1976) has reported increased excretion in the urine of rats administered DEG essentially free of EG (less than 0.01%) whereas other repeated dose toxicity studies (Weil et al. 1965, Hiasa et al. 1990) have not reported such a finding.

Overall, the available toxicokinetic studies indicate that DEG is not metabolised to EG; however, uncertainty exists regarding metabolism of DEG following repeated exposure for longer time and thus, formation of EG from DEG cannot be fully excluded.

The available data on human toxicity are very limited and include only case stories following the use of DEG in medicinals and from accidental intake. In virtually all of the case stories, the kidney is the target organ and in fatal cases, the ultimate

cause of death was kidney failure. Ingestion of about 1000 mg/kg b.w. can lead to severe intoxication with fatal consequences.

DEG is of low acute toxicity in experimental animals following oral administration with reported LD₅₀-values for different species being above 4000 mg/kg b.w. Following inhalation (4400-4600 mg/m³ (aerosol) for 4 hours), no deaths were observed in rats.

The acute toxicity data in humans and animals indicate that humans are more sensitive to DEG than experimental animals are.

DEG has not shown a particularly irritating potential to eyes or skin of neither humans nor experimental animals. Prolonged dermal exposure can result in skin maceration and has been observed in both humans and experimental animals.

Rats exposed to DEG (aerosol) at the maximum attainable concentration of 4400-4600 mg/m³ for 4 hours showed nasal discharge or lachrymation, suggestive of minor irritation, which persisted for several days.

DEG was not shown to be sensitising in a Patch-test in humans and was not sensitising when tested in the guinea pig maximization test (EEC guideline).

No data on toxicity following repeated administration of DEG in humans and no adequate data in experimental animals following inhalation or dermal contact have been found.

Two of the available studies in rats have shown that repeated oral administration of high doses of DEG resulted in damage of the kidneys (primarily tubular necrosis and hydropic degeneration) and the liver (hydropic degeneration and necrosis), which could be revealed by histopathological examinations (BIBRA (1976): in kidneys (including relative kidney weight) at 3000/3700 mg/kg b.w./day (males/females) and in the liver at 3700 mg/kg b.w./day in females only, for 99 days; Fitzhugh & Nelson (1946): from 1000 mg/kg b.w./day for 2 years, only male animals in the study). However, no histopathological changes were observed in rats in a 2-year drinking water study (Hiasa et al. 1990, including kidneys and liver) or in a dietary study for 225 days (BIBRA 1976, only the kidneys were examined) at the highest dose levels tested (up to about 2300 mg/kg b.w./day or up to 1200/1500 mg/kg b.w./day (males/females), respectively). The lowest NOAEL reported for renal effects (changes in renal function (evidenced by analysis of a number of urinary parameters) and relative kidney weight) in rats is 200 mg/kg b.w./day (the only dose level tested) in a 90-day drinking water study (Freundt & Weis 1989); however, histological examinations were not performed.

Increased water intake, which is among the first symptoms of the nephrotoxic effect of DEG, was observed in the 2 year drinking water study (Hiasa et al. 1990) at about 1050 and 2300 mg/kg b.w./day in both sexes; in the dietary 99-day study (BIBRA 1976) in males at 1600 and 3000 mg/kg b.w./day (only significant at 3000 mg/kg b.w./day) and in females at 3000 mg/kg b.w./day (not significant); and in the dietary 225-day study (BIBRA 1976) in males at 1200 mg/kg b.w./day.

Overall, a NOAEL of 2500 mg/kg b.w./day is considered for histopathological changes in the kidneys and the liver. If increased water intake is taken into account as an indication of adverse effects in the kidneys, a L/NOAEL of about 1050 mg/kg b.w./day is considered for renal effects based on the findings in Hiasa et al. (1990).

Equivocal results have been reported concerning excretion of oxalate in the urine of rats and formation of calcium oxalate crystals or stones:

Increased excretion of oxalate in the urine has been reported in a 28-day dietary study (BASF 1988 – quoted from IUCLID 2000, at about 4000 mg/kg b.w./day) in rats of both sexes, and at dietary levels from 100/290 mg/kg b.w./day in males and females, respectively, for 225 days (BIBRA 1976).

Excretion of calcium oxalate crystals in the urine has been reported in the 28-day dietary study (BASF 1988 – quoted from IUCLID 2000, at about 4000 mg/kg b.w./day) in male rats; in male and female rats given dietary levels of 1600/400 mg/kg b.w./day, respectively, for 99 days (BIBRA 1976); and at dietary levels from 230/290 mg/kg b.w./day in males and females, respectively, for 225 days (BIBRA 1976).

Formation of calcium oxalate stones in the bladder of rats has been reported in a 2-year dietary study (Fitzhugh & Nelson 1946) of male rats at dose levels from about 500 mg/kg b.w./day, whereas another 2-year dietary study (Weil et al. 1965) testing DEG essentially free of ethylene glycol reported that formation of stones was only seen in male rats at a dietary level of about 2000 mg/kg b.w./day.

The mechanism(s) behind the nephrotoxic effects of DEG are not known, but seems to be related to the diuretic effect of DEG followed by the subsequent reduction in urine volume and even anuria. Oxalate has been suggested to play a role in the nephrotoxicity of DEG similarly to what has long been accepted regarding the nephrotoxic effects of EG. However, none of the available studies of DEG have reported precipitation of calcium oxalate in the kidneys of rats. Furthermore, the renal lesions observed in rats exposed to DEG seem to be different from those observed in rats exposed to EG and for DEG, the same type of renal lesions are observed in both male and female rats whereas for EG, the renal lesions are only observed in male rats. Recently it has been suggested that the nephrotoxic effects of EG could be due to the metabolic acidosis (resulting from an accumulation of the EG metabolite glycolic acid) and/or from a metabolite-induced cytotoxicity (by the metabolites glycolaldehyde and/or glyoxylate). Metabolic acidosis is also observed in experimental animals following acute exposure to DEG and is reported to be correlated with formation of the DEG metabolite 2-hydroxyethoxyacetic acid (HEAA), the only metabolite identified in experimental animals exposed to DEG. Overall, oxalate is not considered to play a role in the nephrotoxicity observed following exposure to DEG and therefore, the increased oxalate excretion in the urine as well as formation of calcium oxalate crystals are not considered as being adverse effects following exposure to DEG.

No data on toxicity to reproduction in humans have been found.

Reproductive (mice only) and embryotoxic effects and impairment of post-natal development have been observed only at maternally toxic doses (rat: about 8900 mg/kg b.w./day; mouse: about 6100 mg/kg b.w./day). DEG was not teratogenic after oral administration in the rat or mouse when tested at very high dose levels (rat: about 8900 mg/kg b.w./day; mouse: 10000 mg/kg b.w./day), or in the rabbit (1000 mg/kg b.w./day).

Most of the mutagenicity and genotoxicity tests available indicate that DEG is not a mutagenic or genotoxic substance although some positive results have been reported. No data in humans have been found.

No data on carcinogenic effects of DEG in humans have been found.

An increased incidence of bladder tumours have been observed in one 2-year dietary study of male rats (Fitzhugh & Nelson 1946), but not in another 2-year dietary study in rats (Weil et al. 1965) or in a 2-year drinking water study in rats (Hiasa et al. 1990). The purity of DEG was not reported in the study by Fitzhugh & Nelson (1946) and the tumours have been considered to be a consequence of mechanical irritation of the mucous membrane of the bladder due to the presence of calcium oxalate bladder stones. It has been shown by Weil et al. (1965) that implantation of calcium oxalate stones or glass beads into the bladders of rats can lead to the formation of bladder tumours independent of additional treatment with DEG. According to recent studies on the metabolism of DEG, oxalic acid, from which calcium oxalate stones can be formed, does not seem to be a metabolite of DEG, but it can-

not be fully excluded. Overall, it is considered that DEG essentially free of ethylene glycol is not a carcinogen.

6.8.1 Critical effect and NOAEL

The critical effect following exposure to DEG is considered to be the toxic effects observed in the kidney and the liver following oral administration of DEG to humans and rats, and respiratory tract irritation following inhalation.

Overall, a NOAEL of about 2300 mg/kg b.w./day (2350/2275 mg/kg b.w./day for males/females, respectively) is considered for histopathological changes in the kidneys and the liver, based on the results reported in the study by Hiasa et al. (1990). However, taken increased water intake into account as an indication of adverse effects in the kidneys, a LOAEL of about 1050 mg/kg b.w./day (1088/1038 mg/kg b.w./day for males/females, respectively) is considered for renal effects as water intakes increased with dose (75/78 (control), 87/83 (low-dose), 94/91 (high-dose) ml/kg b.w./day in males/females, respectively) although it is not stated whether the increases were significantly different from the controls.

No data are available on systemic toxicity following inhalation, however, there are no reason to expect that the systemic effects occurring following inhalation of DEG should be different from the effects occurring following oral administration. Therefore, the critical systemic effect following exposure to airborne DEG as a vapour or aerosol is also considered to be the effects on the kidney and the liver.

Equivocal results have been reported concerning formation of calcium oxalate crystals and excretion of oxalate in the urine. Overall, oxalate is not considered to play a role in the nephrotoxicity observed following exposure to DEG and therefore, the increased oxalate excretion in the urine as well as formation of calcium oxalate crystals are not considered as being adverse effects following exposure to DEG.

Rats exposed to DEG (aerosol) at the maximum attainable concentration of 4400-4600 mg/m³ for 4 hours showed nasal discharge or lachrymation, suggestive of minor irritation, which persisted for several days (Cascieri et al. 1991).

For the purpose of estimating a quality criterion in air, a LOAEL of 4400-4600 mg/m³ (4 hours exposure) is considered for respiratory tract irritation based on the findings in the study by Cascieri et al. (1991).

7 Quality criterion in air

7.1 Quality criterion in air

The quality criterion in air is calculated based on a LOAEL of 4400-4600 mg/m³ (4 hours exposure) for respiratory tract irritation in rats based on the findings in the study by Cascieri et al. (1991).

$$\begin{aligned} \text{QC}_{\text{air}} &= \frac{\text{LOAEL}}{\text{UF}_I \times \text{UF}_{II} \times \text{UF}_{III}} = \frac{4400-4600 \text{ mg/m}^3}{10 \times 10 \times 100} \\ &= 0.4 \text{ mg/m}^3 \end{aligned}$$

The uncertainty factor UF_I is set to 10 assuming that humans are more sensitive than animals. The UF_{II} is set to 10 to protect the most sensitive individuals in the population. The UF_{III} is set to 100 because of 1) using a LOAEL instead of a NOAEL, 2) the uncertainties in the establishment of a NOAEL for systemic (renal) effects, 3) short exposure duration in the study (4 hours), and 4) the study is only available in form of an abstract.

A quality criterion of 0.4 mg/m³ has been calculated.

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Evaluation of health hazards by exposure to Diethylene 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 diethylene 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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