Diethylene glycol

Classification/MAK value:	**10 ml/m^3 (ppm)** **44 mg/m^3** **peak limitation category II,2** **pregnancy risk group C**
Classification dates from:	**1995**
Synonyms:	bis(2-hydroxyethyl) ether 2,2′-dihydroxyethyl ether 3-oxapentane-1,5-diol 2,2-oxydiethanol
Chemical name (CAS):	diethylene glycol
CAS number:	111-46-6
Structural formula:	$HO–CH_2–CH_2–O–CH_2–CH_2–OH$
Molecular formula:	$C_4H_{10}O_3$
Molecular weight:	106.12
Density:	1.12 g/cm^3
Melting point:	–10.5°C
Boiling point:	245°C
Vapour pressure at 20°C:	0.027 hPa
1 ml/m^3 (ppm) = 4.403 mg/m^3	**1 mg/m^3 = 0.227 ml/m^3 (ppm)**

There is a toxicological evaluation available for diethylene glycol from the BG Chemie (1989b) (German Employers' Liability Insurance Association for the chemical industry). This describes in detail numerous studies which are only summarized or presented in table form here.

1 Toxic Effects and Modes of Action

Single, lethal oral doses of diethylene glycol produce narcotic, hepatotoxic and nephrotoxic effects, and also metabolic acidosis. Repeated doses lead to kidney and liver damage, bladder stones and cystitis. Cleavage of the ether bridge appears to be possible—at

least after repeated doses—as oxalate crystals also occurred in some experiments in which the percentage of monoethylene glycol in the test substance was below 0.01 %.

The well-documented genotoxicity studies provide no evidence of a genotoxic potential. After long-term exposure to diethylene glycol, bladder tumours (mostly papillomas) were formed in male rats as sequelae to persistent bladder stones. There is, however, no evidence of a direct carcinogenic effect of diethylene glycol or its metabolites.

Adverse effects on fertility, prenatal toxicity and testicular atrophy, as are observed after exposure to ethoxyethanol and methoxyethanol and their alkoxyacetic acid metabolites, were not observed with diethylene glycol.

2 Mechanisms of Action

The kidney damage (tubule cell necrosis, protein cylinders, nephrosis) is the result of the nephrotoxic effect of the main metabolite, 2-hydroxyethoxyacetic acid. In addition, the metabolites glyoxylic acid and oxalic acid are also thought to have nephrotoxic effects as in some experiments with single and repeated doses, calcium oxalate crystals led to oxalate nephrosis and bladder stones and in some cases also induced bladder tumours.

Sudden death after diethylene glycol intoxication is probably caused by myocardial damage (Ogbuihi *et al.* 1991).

3 Toxicokinetics and Metabolism

After ingestion diethylene glycol was rapidly and quantitatively absorbed by rats and distributed in all tissues (Heilmair *et al.* 1993). After a single, 12-hour application of diethylene glycol to the skin of rats in doses of 50 mg/kg body weight, about 10 % of the dose was absorbed (Mathews *et al.* 1991).

In the acutely toxic dose range, oxalic acid was found in the urine of male rats (Durand *et al.* 1976) and oxalate crystals in the kidneys (Hebert *et al.* 1978). After a single high dose of diethylene glycol, no metabolism to either monoethylene glycol or oxalate was observed in rats (Heilmair *et al.* 1993; Lenk *et al.* 1989; Mathews *et al.* 1991; Wiener and Richardson 1989).

In long-term experiments an increase was observed in the level of oxalate excreted in the urine of male rats (Gaunt *et al.* 1976). This indicates that the ether bridge can, in principle, be split; however, the oxalic acid concentrations in the blood and kidneys after administration of diethylene glycol remain lower than after administration of the same amounts of ethylene glycol (Winek *et al.* 1978).

After a single oral or intravenous dose of ^{14}C-labelled diethylene glycol of 1.1 g/kg body weight, no ether cleavage products were found in the urine of male rats, only the administered substance, and after 6 and 12 hours about 20 % and 32 % of the dose was

recovered as 2-hydroxyethoxyacetic acid. Contamination with monoethylene glycol has been suggested in other studies as the source of oxalic acid. After inhibition of alcohol dehydrogenase (ADH) with pyrazole the authors found almost exclusively diethylene glycol in urine and no 2-hydroxyethoxyacetic acid. The acute toxicity was also lowered by pyrazole, which indicates that the metabolites are the cause of the nephrotoxic effects (Wiener and Richardson 1989).

After administration of single oral doses of ^{14}C-diethylene glycol of 1, 5 and 10 ml/kg body weight (1.1, 5.6, 11.2 g/kg body weight) to male rats, the radioactivity in the blood was found to decrease with a half-life of about 3.5 hours; 73 %–96 % of the total radioactivity was excreted with the urine. As a result of the diuretic effect, the two higher doses of diethylene glycol were excreted at a faster rate than was the low dose. The main metabolite found was 2-hydroxyethoxyacetic acid.

4 Effects in Man

4.1 Single exposures

An alcoholic survived ingestion of 150–200 ml diethylene glycol (about 2.4–3.2 g/kg body weight) because of treatment with peritoneal dialysis and compensation of the acidosis. Oxalaturia was also observed in this patient. It is still unclear whether ethanol has a protective effect as a result of competition for the ADH which delays the oxidation of diethylene glycol to 2-hydroxyethoxyacetic acid (Auzépy *et al.* 1973).

4.2. Repeated exposure

In 1937 mass intoxication with the death of 71 adults and 41 children occurred in the USA as a result of a sulfonamide preparation containing 72 % diethylene glycol as solvent. After repeated intake, first of all headaches, dizziness and vomiting occurred and later clinical signs of progressive kidney damage and finally kidney failure; 248 persons survived, among them 48 children. The doses ingested by adults ranged from about 16 g to 190 g. The average total dose survived by adults was 60 g diethylene glycol. The smallest lethal dose calculated from these cases of intoxication was about 1 ml/kg body weight (1.1 g/kg body weight) (Calvery and Klumpp 1939; Geiling and Cannon 1938).

A publication reported the death of 7 South African children who died after taking a sedative containing diethylene glycol. No information was given on the amounts ingested. Metabolic acidosis and damage to the liver and kidneys was also observed here (Bowie and McKenzie 1972).

In other cases of intoxication with preparations containing diethylene glycol, other substances which cause kidney damage were taken up in addition to diethylene glycol (Pandya 1988), or extensive burns occurred (Cantarell *et al.* 1987), which also affect the

kidneys, so that diethylene glycol was possibly only one of several reasons for the kidney failure.

With a deduced smallest lethal dose of about 1 g/kg body weight, the toxicity of diethylene glycol in man is much higher than the acute toxicity in animals (see Section 5.1.2). The cases of intoxication described, however, involved combinations of diethylene glycol with drugs, which in the presence of diethylene glycol were possibly more readily available intracellularly. Higher sensitivity of man than of rodents, as is known with monoethylene glycol, can, however, not be excluded.

4.3 Local effects on skin and mucous membranes

In an occlusive patch test carried out on 50 test persons with healthy skin, diethylene glycol at a concentration of 20 % in Vaseline was tolerated for 48 hours without irritation. At higher concentrations skin irritation occurred (Meneghini *et al.* 1971).

5 Animal Experiments and *in vitro* Studies

5.1 Acute toxicity

5.1.1 Inhalation

Four-hour exposure to a concentration of diethylene glycol of about 4500 mg/m^3 (maximum possible aerosol concentration, mean particle size 2.6–3.1 µm) was not lethal for rats (data from summary; Cascieri *et al.* 1991). Also no deaths were observed in rats after 6-hour (data from summary; Union Carbide 1991) and 8-hour inhalation exposure to a saturated vapour atmosphere or to an aerosol produced at 70°C (Union Carbide undated, cited from Rowe and Wolf 1982).

5.1.2 Ingestion

Diethylene glycol was found to be of low toxicity (BG Chemie 1989b).

For rats, mice and dogs the oral LD_{50} was between 10000 and 27000 mg/kg body weight. For guinea pigs the oral LD_{50} was 7.76 ml/kg body weight (about 8700 mg/kg body weight) and for rabbits 4.40 ml/kg body weight (about 4930 mg/kg body weight). The main symptoms were initial diuresis, followed by dehydration, proteinuria and uraemic coma, and central nervous symptoms. Histological examination revealed tubule necrosis and oxalate crystals in the kidneys and central lobe necrosis in the liver.

After single exposures of rats to 700 mg/kg body weight, the lactate dehydrogenase activity in urine was increased. After 2000 mg/kg body weight β-galactosidase activity

and the urine volume were also increased and the creatinine concentration and pH value decreased. After 8000 mg/kg body weight the leucine-aminopeptidase activity was increased and the specific weight of the urine decreased. All effects were reversible on the third day after exposure at the latest. Doses of 200 mg/kg body weight produced no effects (Freundt and Weis 1989).

5.1.3 Dermal absorption

For rabbits the LD_{50} after dermal application under occlusive conditions was 11.9 ml/kg body weight (about 13300 mg/kg body weight) (BG Chemie 1989b).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

Sixteen female mice (strain not stated) were exposed to a vapour-aerosol mixture produced at 30–35°C with a diethylene glycol concentration of 4–5 mg/m^3 (about 0.92 ml/m^3), 2 hours a day for 7 months, with a follow-up period of 2.5–11 months. The animals were found to have bronchitis and interstitial pneumonia, changes in the liver and kidneys described as "protein dystrophy" and round cell infiltration in the kidneys (Sanina *et al.* 1968).

Exposure of mice for three months to 5 mg/m^3 led to oedema, hyperaemia, focal haemorrhages in the brain and the destruction of neurons (Marchenko 1973).

The results of these two studies can hardly be evaluated because the documentation is inadequate.

5.2.2 Ingestion

The numerous studies in which diethylene glycol was administered orally are presented in detail in the publication from the BG Chemie (1989b) and summarized in Table 1.

Most of these studies are, however, older and limited in their significance by the presence of differing amounts of monoethylene glycol in the administered substance. Therefore, only the most recent studies are described in detail here.

In a feeding study with guinea pigs which were given diethylene glycol (monoethylene glycol content 0.4 %) for 2 to 11 days in doses of 1200 mg/kg body weight, kidney and myocardial damage was observed. Such damage did not occur in control animals which received with the diet only monoethylene glycol in doses of 4.5 mg/kg body weight (corresponding to the monoethylene glycol level in the diethylene glycol) (Ogbuihi *et al.* 1991). It was, however, not established whether the diethylene glycol was metabolized to monoethylene glycol, so that it is not certain whether diethylene glycol is really cardiotoxic.

Table 1. Toxicity of diethylene glycol after repeated oral doses

Species number/dose	Dose; type of administration; duration	Dose (mg/kg body weight and day): effects	References
rat 17–35, not specified	0.5, 1, 3, 5 %; drinking water (about 300, 600, 3500, 6000 mg/kg body weight and day); 33–124, 33–174, 15–95, 1–6 days	600: NOEL from 3500: deaths, swollen, vacuolated and necrotic renal cells, anuria, cell degeneration with vacuoles in the liver	Kesten *et al.* 1937
rat 2–7 ♂/♀	1680, 7560, 10080, 20070 mg/kg body weight and day; gavage; 2–8 days	1680: NOEL from 7560: thirst, diuresis, kidney failure, coma, death after 2–5 days, hydropic degeneration of the renal tubular epithelium, in some cases necrosis	Geiling *et al.* 1937; Cannon 1937
rat 3–31 ♂/♀	1120–28000 mg/kg body weight and day; gavage; 1–35 days	from 1120: kidney damage, tubule necrosis, liver oedema from 3360: renal vein thrombosis, hydropic degeneration of the liver from 11200: lethal, hydropic degeneration of the kidneys	Harris 1949
rat 10♂, 10♀	112, 560, 1120, 2240 mg/kg body weight and day; gavage; 50 days	normal body weight gains, not lethal, no substance-specific findings in liver, heart and kidneys	Loeser 1954
rat 10 ♂	8400 mg/kg body weight and day (purity > 98 %); gavage; up to 60 days	liver and kidney damage, deaths	Weatherby and Williams 1939
rat 5 ♀	0.125, 0.25, 0.5, 1, 2, 4 % (purified substance); drinking water; 9 weeks	0.125 %: NOEL from 0.25 %: reductions in body weight gains from 1 %: slight myocardial damage, no substance-specific findings in liver, lungs, kidneys from 4 %: mortality, irritation in the gastro-intestinal tract	Holck 1937
rat not specified	200 mg/kg body weight and day (analytically pure); drinking water; 90 days	no effects on kidney function	Freundt and Weis 1989
rat 15 ♂, 15 ♀	0.4, 2, 4 %; in the diet (300, 1500, 3000 mg/kg body weight and day); 99 days	from 300: hyperoxaluria 1500: kidney function impaired, increased relative kidney weights 3300: nephrosis, liver damage, 6 ♂ died	Gaunt *et al.* 1976

Table 1. continued

Species number/dose	Dose; type of administration; duration	Dose (mg/kg body weight and day): effects	References
rat 6 ♂, 6 ♀	1–20 %; drinking water; 3 months	2 %: NOEL from 5 %: lethal within 1 week, renal tubule damage	Loeser 1954
rat 6 ♂, 6 ♀	1–20 %; drinking water; 3 months	1 %: NOEL 2 %: increased relative kidney weights from 5 %: kidney damage from 10 %: exsiccosis, death	Bornmann 1954
rat 5, not specified	0.03, 0.1, 0.3, 1, 3, 10 %; drinking water (27, 100, 270, 1040, 8740, 6270 mg/kg body weight and day); 100 days	up to 270: no pathological findings 1040: pigment deposits in the spleen, no iron stain (Berlin-blue reaction) from 6270: 100 % mortality, death after 8–9 days	Haag and Ambrose 1937
rat 10 ♂, 10 ♀	0.3, 1 %; drinking water (600–2350 mg/kg body weight and day); 175 days	no effects	Weatherby and Williams 1939
rat 45 ♂; 45 ♀	2500, 5000 mg/kg body weight; twice a week (about 350 and 700 mg/kg body weight and day); gavage; 6 months	no effects	Loeser 1954
rat not specified	300 mg/kg body weight and day; gavage; 7 months	oedema, hyperaemia, and small haemorrhages in the brain	Marchenko 1973
rat 5 ♂	0.79, 1 %; drinking water (1310, 2560 mg/kg body weight and day); up to 23 months	1310: no effect 2560: deaths	Hanzlik *et al.* 1947
rat 6 ♂, 4 ♀	1.71, 3.42 %; in the diet (about 1300 and 2600 mg/kg body weight and day); 24 months	calcium oxalate bladder stones in 3 ♀ (dose group not specified), chronic cystitis, oxalate crystals in renal tubules, bile duct proliferation, slight hepatotoxicity	Morris *et al.* 1942

Table 1. continued

Species number/dose	Dose; type of administration; duration	Dose (mg/kg body weight and day): effects	References
mouse 8 ♂, 8 ♀	1, 2.5, 5, 7.5, 10 %; drinking water; 14 days	2.5 %: NOEL from 5 %: reductions in body weight gains; ♂: reduced water consumption, dehydration from 7.5 %: ♂: pilo-erection, tremor, lethargy, 3/8 died; ♀: dehydration 10 %: ♀: pilo-erection, tremor, lethargy, 2/8 died	Williams *et al.* 1990
mouse not specified	0.03 %, 0.3 %, 3 %; drinking water; 4 months	from 0.03 %: increased coagulation time from 0.3 %: resistance to infection and humoral immunity reduced	Huber *et al.* 1986
rabbit 1–2 ♂/♀	1680, 3360 mg/kg body weight and day; gavage; up to 9 days	from 1680: deaths as a result of kidney failure	Geiling *et al.* 1937
dog 3, not specified	8400 mg/kg body weight and day; gavage; up to 13 days	deaths, liver and kidney damage	Weatherby and Williams 1939
guinea pig 9 ♂	1200 mg/kg body weight and day (0.4 % ethylene glycol); gavage; 2–11 days	myocardial damage, kidney damage, no substance-specific findings in the liver	Ogbuihi *et al.* 1991

A study with rats (strain not stated) which were fed diethylene glycol containing less than 0.01 % monoethylene glycol for up to 225 days produced a NOEL (no observed effect level) for diethylene glycol of 850 mg/kg feed (about 50 mg/kg body weight and day of diethylene glycol). First of all groups of 15 male and 15 female rats (see Table 1) received diethylene glycol in the diet in concentrations of 4000, 20000 and 40000 mg/kg for 99 days (300, 1500 and 3300 mg/kg body weight and day). In a second experiment groups of 10 male and 10 female rats received 850, 1700, 4000 and 20000 mg/kg feed (50, 100, 300 and 1500 mg/kg body weight and day) for 225 days. In the 3300 mg/kg body weight group 6 rats died of kidney failure. The surviving animals were found to have nephrosis and slight liver damage. In animals given 300 and 1500 mg/kg body weight, oxalate crystals were found in the urine and slight disturbances in kidney function were observed. Doses as low as 100 mg/kg body weight still increased excretion of oxalate in the male animals by 13 %–23 %, according to the authors a finding of questionable significance. 50 mg/kg body weight had no effect. From this last value, and applying a safety factor of 100, the authors deduced a tolerable daily dose of 38 mg for a person weighing 70 kg (Gaunt *et al.* 1976).

In a 28-day study diethylene glycol doses of 500, 2500, 10000 and 40000 mg/kg feed (about 38, 188, 750 and 3000 mg/kg body weight and day) were given to groups of 25 male and 25 female Wistar rats; 10 male and 10 female animals served as a control group. The exposure period was followed by an observation period of 3 weeks for 5 male and 5 female animals from the highest dose group and the control group. After 3000 mg/kg body weight the amount and concentration of oxalic acid in the urine of the male animals was increased and oxalate crystals were formed. This finding was reversible within the follow-up period. In the female animals a slight increase in the oxalic acid concentration was also recognizable, but there was no precipitation of crystals. In addition, the absolute brain weights of the female animals were found to be reduced relative to the control values. At the end of the follow-up period this was no longer apparent. The NOEL in this study, therefore, was about 750 mg/kg body weight and day (BG Chemie 1988).

The reasons for the considerable difference in the NOEL in the two studies described above are unclear. A reduction in the NOEL after an exposure period of more than 28 days is rare. The extent to which the metabolism of diethylene glycol to oxalic acid varies remains uncertain. This has already been shown by studies of the metabolism and acute toxicity. Also in the earlier studies of chronic toxicity (Table 1) and carcinogenicity there are differences regarding the formation of oxalate crystals and bladder stones (see Section 5.7).

5.2.3 Dermal absorption

After dermal exposure of mice to 2800 mg/kg body weight and day for about 2 months, oedema, hyperaemia, focal haemorrhages in the brain, destruction of neurons and glia proliferation were observed in all animals (Marchenko 1973).

The inadequate documentation of the results does not allow evaluation of these findings.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

Diethylene glycol produced no skin irritation in rats and guinea pigs even after repeated application (BG Chemie 1989b).

5.3.2 Eyes

No irritation of the rabbit eye was observed (BG Chemie 1989b).

5.4 Allergenic effects

In a maximization test with guinea pigs there was no evidence of sensitizing effects (BASF 1991).

5.5 Reproductive toxicity

5.5.1 Fertility studies

In an older fertility study, 10 male and 10 female albino rats received gavage doses of diethylene glycol of 2 ml/kg body weight in a 20 % aqueous solution daily for eight weeks. Afterwards the animals were mated and 5 female animals were exposed further until they produced a litter or until the offspring were weaned. Again, 10 male and 10 female animals of the F_1 generation were mated. No effects were observed on fertility, litter size or development of the offspring (Wegener *et al.* 1953).

Groups of 30 male and 30 female CD rats received diethylene glycol with the drinking water in doses of 150, 500 and 1500 mg/kg body weight and day for 73 days before mating and during the gestation period. On day 20 of gestation 15 animals were killed and the foetuses examined for skeletal and visceral malformations. 15 animals were allowed to produce a litter and rear the offspring normally. Also in this study fertility and prenatal, perinatal and postnatal development were not impaired, apart from an increase in the liver weights of the male rats of the F_0 and F_1 generations in the 1500 mg/kg group. In the treated male animals of the high and middle dose groups, body weight gains during the first week of treatment were reduced (Rodwell *et al.* 1987).

In a 2-generation study with CD-1 mice, which were mated continually during the experiment, groups of 40 male and 40 female animals received diethylene glycol in the drinking water in concentrations of 0.35 %, 1.75 % and 3.5 % (on average 610, 3060 and 6130 mg/kg body weight and day). Exposure took place during the 7 days before mating, the 98-day mating period and the following 3 weeks. The animals were then mated with

untreated animals. In the highest dose group there was a decrease in the body weights of the dams. In addition, reductions were observed in the number of litters and litter size, in birth weights and postnatal survival. Craniofascial malformations (exencephaly, cleft palates) also occurred in the offspring. The fertility index was not impaired, but the number of litters per pair was decreased. In the 3060 mg/kg group reductions in body weight gains were observed in the offspring. As a result of the toxic effects in the highest dose group, only the F_1 generation of the 3060 mg/kg group was exposed to this dose for a further 74 days and then mated. With the animals of the F_2 generation no further effects were detected apart from decreased birth weights (Williams *et al.* 1990).

5.5.2 Developmental toxicity

In a teratogenicity study diethylene glycol was administered to groups of 15 rabbits in gavage doses of 100, 400 and 1000 mg/kg body weight and day, on days 7 to 19 after mating. No effects were seen on the dams or on foetal development (BG Chemie 1989a).

Maternal toxicity with typical kidney damage in the two high dose groups was found in groups of 25 CD rats given 1, 4 or 8 ml/kg body weight and day (1120, 4480, 8960 mg/kg body weight and day) from days 6 to 15 of gestation. With 8960 mg/kg body weight 3 animals died on day 11 of gestation. The weights of the foetuses were reduced, the incidence of some skeletal variations increased. Doses of 4480 mg/kg body weight also led to an increase in the incidence of variations. 1120 mg/kg body weight and day produced no effects (data from summary; Neeper-Bradley *et al.* 1993).

In the litters of Syrian hamsters given single doses of 2520 to 4480 mg/kg body weight by intraperitoneal injection on day 8 of gestation, in addition to dose-dependent resorption, defects of the neural crest (exencephaly, myelomenigocele and cranial bleb) were observed. Maternal toxicity with some deaths was observed at all doses (Renwick and Cameron 1992).

A screening study with CD-1 mice (Chernoff-Kavlock assay) which received oral doses of 11.2 g/kg body weight from days 7 to 14 of gestation revealed, in addition to 4 % maternal mortality, reductions in body weight gains in the offspring (no further details; Schuler *et al.* 1984).

CD-1 mice given gavage doses of 1250, 5000 or 10000 mg/kg body weight and day from days 6 to 15 of gestation (26–31 animals per group) were found to have increased kidney weights in the two highest dose groups. After 10000 mg/kg body weight 3 of 28 animals were found to have tubular lesions and 1 animal became moribund. In this dose group foetal weights were reduced. Malformations were not observed (Bates *et al.* 1991).

5.6 Genotoxicity

5.6.1 *In vitro*

In *Salmonella* mutagenicity tests, diethylene glycol was not found to be mutagenic either with or without metabolic activation in the plate incorporation test or the pre-incubation test (Pfeiffer and Dunkelberg 1980; Yoshida *et al.* 1986; Zeiger *et al.* 1987).

Diethylene glycol was investigated in *Salmonella* mutagenicity tests with the strains TA98, TA100, TA102 and TA104. With TA104 in the presence of S9 mix, the number of revertants was increased by a factor of 2.2. No gene conversion, mitotic crossing-over or reversion were found in *Saccharomyces cerevisiae* D7. With strain D61M, however, in the absence of S9 mix, an increase was observed in mitotic aneuploidy (data from summary; Krug *et al.* 1986a, 1986b).

Diethylene glycol was not found to be genotoxic in CHO cells (a cell line derived from Chinese hamster ovary cells) in the HPRT test (test for hypoxanthine guanine phosphoribosyl transferase), in the SCE test (test for sister chromatid exchange) or in the chromosomal aberration test. Concentrations of up to 50 mg/ml were used (data from summary; Slesinsky *et al.* 1986).

An SOS chromotest yielded negative results (von der Hude *et al.* 1988).

5.6.2 *In vivo*

In hamsters given a single intraperitoneal dose of diethylene glycol of 1.25–5 g/kg body weight or 2 % diethylene glycol in the drinking water for 3 weeks, a slight increase in chromosomal aberrations in the form of gaps was described (Yoshida *et al.* 1986). An increase in the incidence of gaps, however, is not evidence of chromosomal damage.

Intraperitoneal injection of 60 % of the LD_{50} (species not specified) led to a positive result in a micronucleus test (data from summary; Krug *et al.* 1986a, 1986b). Daily oral administration of 4 % of the oral LD_{50} for 7 days, however, induced no increase in the incidence of micronuclei (data from summary; Krug *et al.* 1986b). Because of the inadequate documentation of the results these findings cannot be used to assess the genotoxicity of diethylene glycol.

5.7 Carcinogenicity

Groups of 12 male Osborne-Mendel rats received 1 %, 2 % and 4 % diethylene glycol in the diet (about 750, 1500 and 3000 mg/kg body weight and day) for 2 years. In the highest dose group, marked reductions in body weight gains, typical liver and kidney damage and increased mortality were observed. After doses of 750 and 1500 mg/kg body weight only mild to moderate effects were described. In addition, there was a dose-dependent increase in the number of bladder stones (0, 2, 7 and 11 of 12 animals). Among the histologically different bladder tumours which 5 animals from the high dose group devel-

oped, there was one metastasizing carcinoma. In the 1500 mg/kg group, 6 of 7 animals with bladder stones had a bladder tumour. The level of monoethylene glycol was not specified (Fitzhugh and Nelson 1946).

After administration of diethylene glycol containing 0.031 % monoethylene glycol in concentrations of 2 % and 4 % in the diet (about 1500 and 3000 mg/kg body weight and day) to groups of 15–20 male and female Carworth Farm rats, bladder stones only occurred in the males of the high dose group. One of these animals developed a bladder tumour (Weil *et al.* 1965).

Concentrations of 2.5 % or 1.25 % diethylene glycol (purity 97 %, uptake about 2600 and 1200 mg/kg body weight and day) in the drinking water of groups of 50 male and 50 female F344 rats for 108 weeks led in both dose groups to increased drinking water consumption and slight reductions in body weight gains. After 2600 mg/kg body weight, survival was increasingly reduced from about week 60. In this group a renal cell carcinoma was found at the end of the experiment. After 1200 mg/kg body weight one renal cell carcinoma and one nephroblastoma developed. In the control group no kidney tumours were detected. Oxalate nephrosis and bladder stones were not found. The authors also carried out an initiation-promotion study in which the initiation with *N*-ethyl-*N*-hydroxyethyl-nitrosamine was followed by a promotion phase with 2.5 % diethylene glycol for 30 weeks with groups of 16–19 animals. The diethylene glycol treatment led to a non-significant increase in the incidence of kidney tumours from 43 % to 68 %. For nitrilotriacetic acid there was an increase to 82 %, which was statistically significant. The incidence of adenomatous hyperplasia remained unchanged in the diethylene glycol group at 78 % compared with 81 % in the *N*-ethyl-*N*-hydroxyethyl-nitrosamine group. After treatment with nitrilotriacetic acid, however, 100 % of the animals had adenomatous hyperplasia. These results show that diethylene glycol is neither carcinogenic nor tumour promoting (Hiasa *et al.* 1990).

The same authors carried out another promotion study with diethylene glycol which cannot be evaluated because administration of the substance was discontinued after only 2 days as a result of high mortality (Hiasa *et al.* 1991).

After inhalation of diethylnitrosamine, subsequent partial hepatectomy and promotion with diethylene glycol in doses of 10 g/kg feed, no increase was found in the number of glutathione-*S*-transferase-positive foci in the livers of rats (Ito *et al.* 1988).

In another initiation-promotion study, 19 F344 rats received first of all for 4 weeks 0.05 % *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine in the drinking water and then for 32 weeks 2 % diethylene glycol in the diet. The control animals received only the initiation substance. As a result of the exposure to diethylene glycol, mild crystalluria occurred but there was no increase in tumours. The degree of contamination with monoethylene glycol was not specified (Masui *et al.* 1988).

In groups of 100 female mice, which received subcutaneous injections of diethylene glycol of 3, 10 and 30 mg/animal, once a week for 106 weeks, no differences relative to the control values were found in body weight gain, mortality, tumour incidence or tumour spectrum (Dunkelberg 1987).

One of 74 mice which were treated with 2 drops of undiluted diethylene glycol (about 3 g/kg body weight) 3 times a week for 2 years on the shaved dorsal skin developed a

papilloma. No differences from the control group were observed (Vasileva *et al.* 1971, cited from BG Chemie 1989b).

Sixteen female mice were exposed to a vapour-aerosol mixture produced at 30–35°C with a diethylene glycol concentration in the air of 4–5 mg/m^3 (about 0.92 ml/m^3), 2 hours a day for 7 months, with a follow-up period of 2.5–11 months. The authors reported tumours in 10 of 12 animals, including a lymphosarcoma in the neck, 7 adenocarcinomas of the mammary gland and another mammary tumour. No tumours were found in the 20 controls (Sanina *et al.* 1968). An increased incidence of mammary tumours was not found in the other long-term studies. The descriptions of the experiments and documentation of the results do not allow more detailed analysis of this study. Contamination with ethylene oxide may have contributed to tumour formation.

After gavage or subcutaneous injection of diethylene glycol an increase in the total number of tumours and frequent occurrence of haemoblastomas was described in Wistar rats and mice (strain not specified). The rats received gavage doses of diethylene glycol (99.48 %) for 81–82 weeks, 3 times a week, or were injected once a week. The doses given to mice were 0.42 (oral) and 1.26 g/kg body weight (subcutaneous) and to rats 0.84 (oral) or 2.5 g/kg body weight (subcutaneous). Groups of about 90 animals of each sex were used for each experiment. The authors conclude from the result that diethylene glycol has a mildly carcinogenic effect (Maksimov *et al.* 1983). As only one dose group was used in each experiment, there are no dose-response relationships. It is also unclear whether the MTD (maximum tolerated dose) was reached or exceeded so that the study cannot be used to assess the carcinogenic potential of diethylene glycol.

The studies included in the evaluation yielded no evidence of a direct carcinogenic effect of diethylene glycol. With high doses, indirect effects as a result of persistent bladder stones or nephrotoxic organ changes cannot be excluded.

6 Manifesto (MAK value, classification)

Oral uptake of diethylene glycol can lead to kidney damage. With high doses, via formation of bladder stones, it can induce the development of bladder tumours.

There are no valid data available for inhalation toxicity after repeated exposure. Studies with oral uptake can be used to evaluate a provisional MAK value: diethylene glycol is absorbed readily from the gastro-intestinal tract, the main target organ is the kidney.

The NOEL found after oral administration to rats varies considerably (from 50–2350 mg/kg body weight). This could be a result of differing extents of metabolism of diethylene glycol to monoethylene glycol and oxalic acid. Whether differences between strains play a role is unclear. The lowest NOEL from a recent 225-day feeding study with rats is 50 mg/kg body weight and day.

As there is no experience of long-term exposure in man, but cases of intoxication have shown that man is about 10 times as sensitive as animals, a safety margin of 10 relative to the NOEL from the long-term animal experiment was chosen for derivation of the MAK

value. The MAK value was therefore set at 10 ml/m^3 (44 mg/m^3). This corresponds to an approximate uptake of 440 mg per person and day (or 6 mg/kg body weight and day) assuming 100 % absorption after inhalation. Even if it is assumed that 100 % of the diethylene glycol is cleaved to yield monoethylene glycol, a concentration which would lead to systemic effects of monoethylene glycol is not reached (DFG 1991).

Studies on the reproductive toxic effects after oral administration to rats, mice, rabbits and hamsters revealed embryotoxic and foetotoxic effects only after very high doses (several g/kg body weight) which also led to maternal toxicity. Diethylene glycol is therefore classified in Pregnancy risk group C.

Because of its systemic toxicity and because its biological half-life is more than 2 hours, diethylene glycol is classified in Peak limitation category II,2.

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