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Appendices 1-18 to: Report on the Health Effects of Selected Pesticide Coformulants

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Contents

Appendix 1: Manganese (II) sulphate, Manganese (II) sulphide

1 General description

Because limited data are available specifically for manganese sulphate and manganese sulphide, and most manganese compounds seem to cause the same adverse effects, this paper includes several studies with other inorganic manganese compounds.

Manganese displays oxidation states: +2, +3, +4, +6 and +7. Compounds in which manganese is in its +2 oxidation state are the most stable ones, but bivalent manganese can be readily oxidized to the +3 and +4 states. (Beliles 1994).

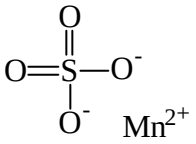
In this paper, the following manganese compounds are addressed:

Manganese (II) sulphate ($MnSO_4$)
Manganese (II) sulphide (MnS),
Manganese (II) chloride ($MnCl_2$)
Manganese (II, III) tetroxide (Mn_3O_4)
Manganese (III) trioxide (Mn_2O_3)
Manganese (III) phosphate ($MnPO_4$)
Manganese (IV) dioxide (MnO_2)

1.1 Identity

Molecular formula: a) $MnSO_4$
b) MnS

Structural formula: a)



b)

$Mn^{2+}\ S^{2-}$

Molecular weight: a) 151.00
b) 87.00

CAS-no.: a) 7785-87-7
b) 18820-29-6

Synonyms: a) Manganese sulphate
Sulphuric acid, manganese (2^+) salt (1:1)
b) Manganese monosulphide

1.2 Physical / chemical properties

Description:	a) Manganese sulphate forms several hydrates. The article of commerce is usually a mixture of the tetra- and pentahydrates. The monohydrate exists in the form of pale red crystals. b) Manganese sulphide is a pink, green or brown-green powder. Three crystalline modifications exist: α-form, green cubic crystals; β-form, red cubic crystals; γ-form, red hexagonal crystals. In moist conditions manganese sulphide readily oxidizes in air to the sulphate.
Melting point:	a) 700°C
Boiling point:	a) 850°C (decomposes)
Density:	a) 3.25 g/ml
Vapour pressure:	-
Concentration of saturated vapours:	-
Solubility:	a) Water: 520 g/l (at 5°C), 700 g/l (at 70°C) b) Insoluble in water. Soluble in diluted acids.
References:	ATSDR (2000), ChemFinder (2001), HSDB (2000), Merck Index (1996)

2 Toxicokinetics

Manganese is a natural component of the environment and an essential element in the nutrition of humans and animals. No formal recommended dietary allowance exists for manganese. However, the Scientific Committee for Food of the EU has estimated 1 to 10 mg Mn/day as an acceptable range of intake. This level is equivalent to the usual daily intake. Manganese is involved in basic enzymatic processes including oxidative phosphorylation, decarboxylation, hydrolysis, Krebs cycle reactions, and the metabolism of carbohydrates. The concentration of manganese in the body is under normal circumstances held relatively constant by alterations in the rate and amount of manganese absorbed from the gastrointestinal tract, and by its distribution and rate of excretion (homeostasis). In children under one year of age, the homeostatic mechanisms may not have fully developed. (ATSDR 2000, Beliles 1994, SCF 2000).

2.1 Absorption

Manganese absorption occurs primarily through the diet. However, absorption via the lungs can be significant for occupationally exposed persons or for those exposed to excess levels of airborne manganese, such as downwind of a manganese point source (ATSDR 2000).

2.1.1 Inhalation

Inhalation of inorganic manganese may be in the form of aerosols or attached to particles, which may reach the lungs depending on the aerosol or particle size, shape, density, hygroscopicity, and electric charge. The deposition pattern in the respiratory tract of inhaled inorganic manganese is related to particle size. In humans, large particles (aerodynamic diameter of 5-30 μm) are mainly deposited in the upper airways while smaller particles (5 μm or less) may reach the lower airways. Particles that are deposited in the lower airways are probably mainly absorbed, while particles deposited in the upper airways may be moved by mucociliary transport to the throat, where they are swallowed and enter the stomach. A study has reported that more than 60% of particulate manganese trioxide inhaled by human volunteers was transferred to the gastrointestinal tract. No studies measuring the absolute amount of absorbed manganese following inhalation have been found. Manganese chloride is more readily absorbed than manganese dioxide following intratracheal instillation indicating that the absorption following inhalation is higher for more soluble forms of inorganic manganese. (ATSDR 2000, Beliles 1994).

2.1.2 Oral intake

Of ingested inorganic manganese only about 3-5% is absorbed from the gastro-intestinal tract in humans. There are many factors influencing the absorption such as water solubility of the compounds in question, age, and the level of manganese and other trace elements such as iron in the gut. (ATSDR 2000, Beliles 1994).

Studies with rodents show that the absorption and/or retention of manganese are higher in neonates, but returns to the level of older animals at approximately post-gestational day 17-18. Available studies do not provide adequate data to determine when this transition takes place in human infants. (ATSDR 2000, Beliles 1994).

In persons with iron deficiency, manganese absorption is increased probably because iron and manganese are absorbed by the same transport system in the gut. In some animal studies, calcium and phosphorus have depressed the manganese uptake. In rats fed a diet deficient in manganese, the absorption was at least two-fold higher than in rats ingesting an adequate amount of manganese. (ATSDR 2000, Beliles 1994).

2.1.3 Dermal contact

Manganese uptake across intact skin would be expected to be extremely limited (ATSDR 2000).

2.2 Distribution

In healthy humans, tissue concentrations of manganese range mainly between 0.1 and 1 mg Mn/kg wet weight with the highest levels in the liver, pancreas, and kidney and the lowest levels in bone and fat. (ATSDR 2000, Beliles 1994).

2.2.1 Inhalation

Seventy male CD rats were exposed nose-only for 90 minutes to an aerosol of radiolabelled manganese chloride particles at 0.54 mg Mn/m^3. Half of the animals had both nostrils patent while the other half had the right nostril plugged to prevent nasal deposition of manganese chloride on the occluded side. At 0, 1, 2, 4, and 8 days post-exposure, the left and right sides of the nose and brain, including the olfactory pathway and striatum were sampled and analysed for the level of manganese. High levels of manganese were observed in the olfactory bulb and tract/tubercle on the side or sides with an open nostril within 1-2 days following inhalation consistent with direct olfactory transport of inhaled manganese from the nasal cavity to the brain bypassing the blood-brain barrier. The difference between the manganese level on the occluded and unoccluded side of the olfactory pathway was more than 90%. Only minor differences in the manganese level were seen between the left and right sides of the striatum in unilaterally occluded rats. Besides the brain, other organs (lungs, liver, kidneys, pancreas) also had increased tissue manganese concentrations following inhalation exposure. (Brenneman et al. 2000).

Groups of 6 adult male Sprague-Dawley rats were exposed to airborne manganese phosphate particles at 0, 0.03, 0.3 or 3 mg Mn/m^3 for 6 hours/day for 2 weeks. Increased manganese concentrations were reported in olfactory bulb, lung, and striatum at the middle dose and also in cerebellum, femur, skeletal muscle, plasma, and erythrocytes at the high dose at the cessation of exposure. The liver manganese concentration was unaffected by inhalation exposure to manganese phosphate. (Vitarella et al. 2000).

Sprague-Dawley rats got an intranasal instillation with 0.004 mg Mn/kg b.w. of manganese chloride. The olfactory bulb contained the vast majority of measured manganese at 1, 3, and 7 days post-dosing (90, 69, and 47%, respectively) with a value decreasing to 16% at 12 weeks. At the same time-points, the basal forebrain contained 6, 21, and 28% of measured manganese, respectively. Manganese levels in the cerebral cortex, hypothalamus, striatum, and hippocampus were also maximal at 7 days post-dosing. Liver and kidney each contained about 1% of the measured manganese during the first 7 days. (Tjälve et al. 1996 – quoted from ATSDR 2000).

Following intranasal injection of manganese chloride into one nostril, manganese was dose-dependently accumulated in the olfactory bulb and tubercle (Gianutsos et al. 1997 – quoted from ATSDR 2000).

Rats were instilled intratracheally with manganese chloride or manganese dioxide at 1.22 mg Mn/kg b.w. once a week for 4 weeks. Compared to control rats, an increase of the manganese level was measured in striatum, blood, cortex, and cerebellum. (Roels et al. 1997 – quoted from ATSDR 2000).

Preferential accumulation of manganese in specific locations of the brain (including the caudate nucleus, globus pallidus, and the substantia nigra) was noted in 1 monkey exposed to an aerosol of manganese chloride (9-17 mg Mn/m^3) several hours/day for 3-5 months (Newland et al. 1989 – quoted from ATSDR 2000).

2.2.2 Oral intake

Dietary manganese, absorbed mainly as Mn(II), enters portal circulation from the gastrointestinal tract and is bound to α-macroglobulin or albumin in the plasma. After transportation to the liver, the major portion of Mn(II) is secreted in bile, but some may be oxidized to Mn(III), which enters the circulation conjugated with plasma transferrin. (Aschner & Aschner 1991 – quoted from ATSDR 2000).

Studies in animals indicate that prolonged oral exposure to manganese compounds results in increased manganese levels in the tissues, but that the magnitude of the increase diminishes over time (ATSDR 2000).

Manganese in the form of manganese chloride or manganese dioxide is distributed more readily to the brain following intratracheal exposure compared to oral exposure by gavage (Roels et al 1997 – quoted from ATSDR 2000).

Mice and rats chronically fed manganese sulphate generally had elevated tissue levels of manganese. The manganese levels in the liver and kidney were higher than the levels in the brain. (NTP 1993 – quoted from ATSDR 2000).

Excess manganese (following oral exposure in humans with liver diseases) was accumulated in certain regions of the brain (basal ganglia, especially the globus pallidus and the substantia nigra) (ATSDR 2000).

2.2.3 Dermal contact

No data were found.

2.2.4 Other routes

Studies with intraperitoneal and intravenous injections of inorganic manganese to monkeys, calves and rats have been performed. The distribution of manganese may vary depending on the route of exposure. For instance it has been shown that calves injected intravenously had a higher concentration of manganese in the liver and pancreas than calves fed manganese. Rats injected intraperitoneally had more manganese in the pancreas and less in the liver than rats fed manganese. Administration of manganese dioxide intraperitoneally to rats resulted in greater concentration of manganese in the brain than did manganese chloride. In a monkey study with intraperitoneal administration, manganese was found in all tissues studied with the highest level in pancreas, kidney and liver and the lowest level in the blood. (ATSDR 2000).

2.3 Elimination

In general, biliary excretion is the main pathway by which absorbed manganese reaches the intestines where most of the element is excreted in the faeces. However, some of the manganese in the intestine is reabsorbed through enterohepatic circulation. Small amounts of manganese can also be found in urine, sweat, and milk. (ATSDR 2000).

2.3.1 Inhalation

In humans inhaling manganese chloride or manganese trioxide, about 60% of the material originally deposited in the lungs was excreted in the faeces within 4 days. In several studies, occupationally exposed men had significantly higher urine manganese levels compared to unexposed men. (ATSDR 2000).

Rats instilled intratracheally with manganese chloride or manganese tetroxide excreted about 50% of the dose in the faeces within 3-7 days (Drown et al. 1986 – quoted from ATSDR 2000).

Monkeys exposed to an aerosol of manganese chloride excreted most of the manganese with a half-life of less than half a day. However, a portion of the compound was retained in the lung and brain and was cleared with a half-life of 12-250 days. No further details were given. (Newland et al. 1987 – quoted from ATSDR 2000).

2.3.2 Oral intake

Humans who ingested tracer levels of radioactive manganese excreted the manganese with a half-life of 13-37 days (ATSDR 2000).

2.3.3 Dermal contact

No data were found.

2.3.4 Other routes

Rats exposed to manganese chloride by intravenous injection excreted 50% of the dose in the faeces within 1 day and 85% by day 23. Only minimal levels were excreted in the urine (<0.1% of the dose within 5 days). (Klaassen 1974, Dastur et al. 1971 – both quoted from ATSDR 2000).

2.4 Mode of action

The central nervous system is the primary target of manganese toxicity. In humans manganese causes neurochemical and neuropathological changes predominantly in the globus pallidus in the basal ganglia resulting in symptoms similar to but still different from parkinsonism. Although it is known that manganese is a cellular toxicant that can impair transport systems, enzyme activities, and receptor functions, the principal manner in which manganese neurotoxicity occurs has not been clearly established. Several possibilities have been proposed. These include enhancement of the oxidation of dopamine and other catecholamines, which increases production of free radicals and reactive oxygen species, thereby causing oxidative stress and damage to the cell, following the depletion of cellular antioxidant defence systems. Other hypotheses involve damage predominantly to the mitochondria. (ATSDR 2000).

3 Human toxicity

3.1 Single dose toxicity

No data were found.

3.2 Repeated dose toxicity

3.2.1 Inhalation

3.2.1.1 Respiratory effects

Inhalation exposure to manganese dusts such as manganese dioxide or manganese tetroxide has lead to an inflammatory response in the lungs mainly of workers exposed to fairly high concentrations (Abdel Hamid et al. 1990, Akbar-Khanzadeh 1993, Kagamimori et al. 1973, Lloyd Davies 1946, Nogowa et al. 1973, Roels et al. 1987a, WHO 1987 – all quoted from ATSDR 2000).

3.2.1.2 Cardiovascular effects

Three occupational studies indicate that manganese may affect the cardiovascular system following inhalation by lowering the blood pressure. Specific data on exposure levels are lacking. (Saric & Hrustic 1975, Jiang et al. 1996a, Hobbesland et al. 1997b – all quoted from ATSDR 2000).

3.2.1.3 Endocrine effects

Only two studies have measured endocrine effects in humans exposed to inorganic manganese. Foundry workers exposed for approximately 10 years to 0.04-1.1 mg Mn/m^3 (particulate matter) and 0.05-0.9 mg Mn/m^3 (fumes) had elevated serum prolactin and cortisol levels. (Alessio et al. 1989 – quoted from ATSDR 2000).

Workers from a ferroalloy plant also had elevated serum prolactin levels (Smargiassi & Mutti 1999 – quoted from ATSDR 2000).

3.2.1.4 Neurological effects

From studies of humans exposed to manganese dusts (mainly manganese dioxide) in mines and factories, clear evidence exist that exposure to manganese for longer periods can lead to a series of serious and ultimately disabling neurological effects. This disease, termed manganism, typically begins with feelings of weakness and lethargy typically after several years of exposure but some individuals may begin to show signs after as few as 1-3 months of exposure. As the disease progresses, a number of other neurological signs such as slow and clumsy gait, speech disturbances, a mask like face, and tremors may become manifest. These signs are frequently accompanied by apathy and dullness along with impotence and loss of libido. (Abdel-Hamid et al. 1967, Emera et al. 1971, Mena et al. 1967, Nelson et al.

1993, Rodier 1955, Saric et al. 1977a, Schuler et al. 1957, Shuqin et al. 1992, Smyth et al. 1973, Tanaka & Lieben 1969, Whitlock et al. 1966 – all quoted from ATSDR).

Neurological symptoms may improve in some cases (Shuqin et al. 1992, Smyth et al. 1973 – both quoted from ATSDR 2000). However, in most cases the symptoms are irreversible (Cotzias et al. 1968, Huang et al. 1998 – both quoted from ATSDR 2000).

Frequently psychological disturbances such as hallucinations and psychosis emerge (Cook et al. 1974 – quoted from ATSDR 2000). As the disease progresses, patients develop severe hypertonia and muscle rigidity and may be permanently disabled (Rodier 1955 – quoted from ATSDR 2000).

In neurobehavioral tests, people with manganism, generally have significantly poorer eye-hand coordination, hand steadiness, reaction time and postural stability, and a lower level of cognitive flexibility (Chia et al. 1993, 1995, Crump & Rosseau 1999, Iregren 1990, Lucchini et al. 1995, 1999, Mergler et al. 1994, Roels et al. 1987, 1992, 1999, Wennberg et al. 1991 – all quoted from ATSDR 2000).

One occupational study has reported a lack of significant neurological effects (Gibbs et al. 1999 – quoted from ATSDR 2000).

Reliable dose-response data on the inhalation exposure levels leading to neurological injury in humans are not extensive. However, recent epidemiological data indicate that concentrations of manganese in respirable dust in the range of 0.027 to 0.215 mg Mn/m^3 and in total dust in the range of 0.14 to 1.59 mg Mn/m^3 in the workplace can result in measurable neurological effects. (Roels et al. 1992 – quoted from ATSDR 2000).

People in a Canadian community near a former manganese alloy production plant were exposed to environmental manganese pollution. Both men and women were adversely affected by exposure to manganese as evidenced in performance in neurobehavioral tests and increased neuropsychiatric symptoms. The poorest performance occurred in those with the highest blood manganese level and neurological effects associated with higher levels of blood manganese were more likely to be observed in persons older than 50 years of age. (Baldwin et al. 1999, Beuter et al. 1999, Bowler et al. 1999, Mergler et al. 1999 – all quoted from ATSDR 2000).

3.2.2 Oral intake

3.2.2.1 Neurological effects

Only limited evidence exist that oral exposure to excess manganese in humans leads to neurological effects similar to those reported for inhalation exposure. Although people exposed to excess manganese via food or drinking water for a longer period exhibited neurological symptoms resembling manganism, data were not sufficient in any of the studies to conclude that the symptoms could be attributed solely to manganese exposure. (Banta & Markesbery 1977, Cawte et al. 1987, Goldsmith et al. 1990, He et al. 1994, Holzgraefe et al. 1986, Iwami et al. 1994, Kawamura et al. 1941, Kilburn

1987, Kondakis et al. 1989, Zhang et al. 1995 – all quoted from ATSDR 2000).

Neurological effects in children are described in the chapter on reproductive and developmental effects.

3.2.3 Dermal contact

No data were found.

3.3 Toxicity to reproduction

3.3.1 Inhalation

Decreased libido and impotence are common symptoms in male workers with clinical signs of manganism following exposure to high levels of manganese dusts in the workplace for 1-35 years (Emera et al. 1971, Jing et al. 1996b, Mena et al. 1967, Rodier 1955, Schuler et al. 1957 – all quoted from ATSDR 2000).
In one of the newer studies, the mean total manganese dust concentration was 0.145 mg Mn/m^3 as manganese dioxide (Jing et al. 1996b).

The number of children born to occupationally exposed males (inhalation of 0.97 mg Mn/m^3 of manganese dust for 1-19 years) was lower than average (Lauwerys et al. 1985 –quoted from ATSDR 2000).

In another occupational study with 70 men, inhalation of manganese dust as manganese dioxide at a median concentration of 0.71 mg Mn/m^3 in total dust for an average of 6.2 years had no effect on the fertility (Gennart et al. 1992 – quoted from ATSDR 2000).

One study has reported increased semen liquefaction time, decreased sperm count and decreased sperm viability in workers exposed to manganese dioxide dust at a total concentration of 0.14 –5.5 mg Mn/m^3 for one or more years. Workers exposed to manganese fumes at 6.5 – 82.3 mg Mn/m^3 for one or more years had decreased sperm viability. However, an increased level of other metals may have influenced the reproductive effect seen. (Wu et al. 1996 – quoted from ATSDR 2000).

The reported reproductive effects may in part occur as a secondary result of neurotoxicity (ATSDR 2000).

3.3.2 Oral intake

Incidences of stillbirths and malformations have been studied in an Australian aboriginal population living on an island where environmental levels of manganese are high. However, data from a suitable control group are lacking, and the study population is so small that it is not possible to judge if the incidence of developmental abnormalities (and neurological disorders) is higher than average. The route of exposure was assumed to be primarily oral but inhalation exposure was not ruled out. (Kilburn 1987 – quoted from ATSDR 2000).

Two studies in school children showed that increased exposure (at least 0.24 mg Mn/l water for 3 years or more) to manganese in drinking water and food

was associated with poorer performance in school and on neurobehavioral tests as compared to children exposed to lower levels (at most 0.04 mg Mn/l water). The children with increased manganese exposure had significantly decreased serum levels of serotonin, noradrenaline, dopamine and acetylcholinesterase. The manganese level of the hair was inversely related to the performance in the neurobehavioral tests. However, the children may have been exposed to other metals that may have influenced the developmental effects seen (He et al. 1994, Zhang et al. 1995 – both quoted from ATSDR 2000).

A higher manganese level has also been found in the hair of learning disabled children. The route of excess exposure may be through ingestion of increased amounts, metabolic disturbances, improper balance of other nutrients (such as iron), or decreased ability to clear manganese. (Collipp et al. 1983, Pihl & Parkes 1977 – both quoted from ATSDR 2000).

3.3.3 Dermal contact

No data were found.

3.4 Mutagenic and genotoxic effects

An increase in chromosomal aberrations was found in a group of workers, which had been exposed to a mixture of manganese, nickel, and chromium by inhalation for 10-24 years. The median manganese concentrations during the survey were 0.18 mg Mn/m^3 for respirable dust and 0.71 mg Mn/m^3 for total dust. No information was available on the genotoxicity of manganese alone. (Elias et al. 1989 – quoted from ATSDR 2000).

3.5 Carcinogenic effects

No data were found.

4 Animal toxicity

4.1 Single dose toxicity

Acute inhalation exposure to high concentrations of manganese dusts (manganese dioxide) can cause an inflammatory response in the lung (Maigetter et al. 1976, Shiotsuka 1984 – both quoted from ATSDR 2000).

Oral doses of manganese salts given by gavage can cause death in animals (LD_{50}-values for rats in the range of 275 to 1082 mg Mn/kg b.w.) (Holbrook et al. 1975, Kostial et al. 1978, 1989, Singh & Junnarkar 1991, Smyth et al. 1969 – all quoted from ATSDR 2000).

4.2 Repeated dose toxicity

4.2.1 Inhalation

4.2.1.1 Respiratory effects

Inhalation exposure to manganese dusts such as manganese dioxide or manganese tetroxide has in several studies lead to an inflammatory response in the lungs of animals exposed for periods ranging from 1 day to 10 months at manganese concentrations ranging from 0.7 to 69 mg Mn/m^3 (Bergstrom 1977, Camner et al. 1988, Shiotsuka 1984, Suzuki et al. 1978, Ulrich et al. 1979a, 1979b – all quoted from ATSDR 2000).

4.2.1.2 Neurological effects

In several animal studies, intermediate or chronic inhalation exposure of monkeys and rats to manganese dusts has not produced neurological signs similar to those seen in humans (Bird et al. 1984, EPA 1983c, Ulrich et al. 1979a, 1979b – all quoted from ATSDR 2000).
However, in a chronic study with rhesus monkeys, decreased levels of dopamine were found in several regions of the brain (caudate and globus pallidus) (Bird et al. 1984 – quoted from ATSDR 2000).

Behavioural tests have detected signs of neurological effects in mice, although these are only seen at relatively high exposure levels of about 60-70 mg Mn/m^3 (Lown et al. 1984, Morganti et al. 1985 – both quoted from ATSDR 2000).

4.2.2 Oral intake

4.2.2.1 Gastrointestinal effects

Adverse gastrointestinal effects including hyperplasia and erosion of the forestomach have been reported in $B6C3F_1$ mice fed manganese sulphate at a dose level of 585 mg Mn/kg b.w. per day for males and 731 mg Mn/kg b.w. per day for females for 2 years but not in F344 rats fed similar doses (NTP 1993 - quoted from ATSDR 2000).

4.2.2.2 Haematological effects

Some alterations in haematological parameters (including decreases in red blood cell, leukocyte, and neutrophil counts) have been reported in rats and mice following oral exposure. The effects varied according to species, duration of exposure and form of manganese administered. (ATSDR 2000).

No significant haematological effects were observed in F344 rats or $B6C3F_1$ mice exposed for 2 years to average oral doses of 331 or 905 mg Mn/kg b.w. per day (as manganese sulphate), respectively (Hejtmancik et al. 1987a, 1987b - quoted from ATSDR 2000).

4.2.2.3 Hepatic effects

Minor histological and weight changes of the liver have been reported in mice and rats fed high doses of manganese chloride, manganese tetroxide, and manganese sulphate for different time periods (ATSDR 2000).

A decreased liver weight occurred in male F344 rats fed 33 mg Mn/kg b.w. per day as manganese sulphate for 13 weeks. However, no hepatic changes were reported in a 2-year study in which F344 rats were fed up to 232 mg Mn/kg b.w. per day as manganese sulphate, and $B6C3F_1$ mice were fed up to 731 mg Mn/kg b.w. per day as manganese sulphate. (NTP 1993 – quoted from ATSDR 2000).

4.2.2.4 Renal effects

An increased severity of chronic progressive nephropathy was noted in male F344 rats fed 200 mg Mn/kg b.w. per day as manganese sulphate for 2 years (NTP 1993 – quoted from ATSDR 2000).
However, in no other animal studies were any significant renal histopathological changes observed including another 2-year study where $B6C3F_1$ mice and F344 rats were fed 905 or 331 mg Mn/kg b.w. per day as manganese sulphate, respectively (Hejtmancik et al. 1987a, 1987b - quoted from ATSDR 2000).

4.2.2.5 Endocrine effects

In a 2-year $B6C3F_1$ mouse study, thyroid follicular hyperplasia and dilation were observed in males fed 584 mg Mn/kg b.w. per day as manganese sulphate, and thyroid follicular hyperplasia was observed in females fed 64 mg Mn/kg b.w. per day. However, no endocrine effects were observed in F344 rats fed up to 232 mg Mn/kg b.w. per day as manganese sulphate for 2 years or in F344 rats and $B6C3F_1$ mice fed up to 1950 Mn/kg b.w. per day as manganese sulphate for 13 weeks. (NTP 1993 – quoted from ATSDR 2000).

4.2.2.6 Immunological effects

In a 13-week F344 rat study with feeding of manganese sulphate, an increased neutrophil count at 33 mg Mn/kg b.w. per day in males, a decreased lymphocyte count at 130 mg Mn/kg b.w. per day in males, and a decreased total leukocyte count at 155 mg Mn/kg b.w. per day in females were observed. However, no immunological effects were observed in F344

rats fed up to 232 mg Mn/kg b.w. per day or in B6C3F$_1$ mice fed up to 731 Mn/kg b.w. per day as manganese sulphate for 2 years. (NTP 1993 – quoted from ATSDR 2000).

4.2.2.7 Neurological effects

Oral studies in animals exposed to manganese have sometimes revealed biochemical or neurobehavioral evidence of neurological effects (Bonilla 1978b, 1980, Bonilla & Prasad 1984, Chandra 1983, Chandra & Shukla 1981, Deskin et al. 1981, Eriksson et al. 1987a, Gianutsos & Murray 1982, Gray & Laskey 1980, Komuara & Sakamoto 1991, 1992, Kristensson et al. 1986, Lai et al. 1984, Nachtman et al. 1986, Subhash & Padmashree 1991 – all quoted from ATSDR 2000).

Although decreased motor activity (Gray & Laskey 1980, Komura & Sakamoto 1991 – both quoted from ATSDR 2000) has been observed in mice at high oral doses (280 mg Mn/kg b.w. per day for 100 days), motor deficits similar to clinical effects in humans are seldom observed in rodents and tend to be transient effects (Kristensson et al. 1986 – quoted from ATSDR 2000).

However, neurological effects (weakness, muscular rigidity, and marked degeneration with de-pigmentation of neurons in the region of substantia nigra) similar to those seen in workers exposed to manganese have developed in monkeys given 25 mg Mn/kg b.w. per day (as manganese chloride) for 18 months (Gupta et al. 1980 – quoted from ATSDR 2000 and SCF 2000).

Neurological effects in neonatal animals are described in the section on reproductive and developmental effects.

4.2.3 Dermal contact

No data were found.

4.2.4 Other routes

Monkeys exposed to manganese injected either intravenously or subcutaneously exhibited neurological symptoms very similar to those observed in workers exposed to manganese. In addition, they accumulated manganese in the basal ganglia, as do humans exposed to excess manganese. (Eriksson et al. 1992, Newland & Weiss 1992, Olanow et al. 1996 – all quoted from ATSDR 2000).

4.3 Toxicity to reproduction

4.3.1 Inhalation

Female mice exposed to manganese dioxide by inhalation for 18 weeks (before conception and through gestation) had an increased number of pups per litter (Lown et al. 1984 – quoted from ATSDR 2000).

In utero exposure to manganese (as manganese dioxide) did not alter gross locomotor activity, different behavioural parameters and learning performance in mice pups. The dams had been exposed to manganese at an average concentration of 61 mg Mn/m^3 7 hours/day, 5 days/week for 16

weeks prior to conception and to either filtered air or manganese during the gestation period. The only effect observed was a reduced weight in pups of mothers exposed to manganese before conception and filtered air after conception. (Lown et al. 1984 – quoted from ATSDR 2000).

Rabbits exposed to a single dose of 158 mg Mn/kg b.w. of manganese dioxide by intratracheal instillation experienced severe degenerative changes in the seminiferous tubules leading to infertility over a period of 1-8 months (Chandra et al. 1973, Seth et al. 1973 – both quoted from ATSDR 2000).

4.3.2 Oral intake

No gross, histopathological or organ weight changes were observed in reproductive organs of F344 rats fed manganese sulphate in doses up to 232 mg Mn/kg b.w. per day or $B6C3F_1$ mice fed up to 731 mg Mn/kg b.w. per day for 2 years, or in F344 rats fed up to 618 mg Mn/kg b.w. per day or $B6C3F_1$ mice fed up to 1950 mg Mn/kg b.w. per day for 13 weeks (NTP 1993 – quoted from ATSDR 2000).

Groups of female Sprague-Dawley rats were fed manganese sulphate in their diet at doses of 0.75, 4.5, 10, 29, 94 or 187 mg Mn/kg b.w. per day for 8 weeks prior to mating and until gestational day 21. No effect was found on maternal weight gain, implantation number, resorptions, or percentage of dead foetuses. The pups from dams administered 94 mg Mn/kg b.w. per day had significantly decreased weights as compared to other groups. No gross malformations were observed in the foetuses. Manganese doses of 94 and 187 mg Mn/kg b.w. per day resulted in a significant increase in liver manganese concentrations in pregnant females compared to non-pregnant females. Also pregnant rats had consistent liver iron concentrations, whereas non-pregnant rats suffered a dose-dependent decrease in liver iron concentrations. The highest dose in dams caused a significant increase in foetal manganese and a decrease in foetal iron content. (Jarvinen & Ahlstrom 1975 – quoted from ATSDR 2000).

Sperm head abnormalities and the percentage of abnormal sperm was significantly elevated in mice exposed by gavage to manganese sulphate at 23-198 mg Mn/kg b.w. per day for 21 days (Jordar & Sharma 1990 – quoted from ATSDR 2000).

Studies in mice and rats with oral intake of manganese tetroxide indicate that manganese ingestion at doses from about 1050 mg Mn/kg b.w. per day can lead to delayed maturation of the reproductive function in male offspring probably due to decreased testosterone secretion by Leydig cells (Gray & Laskey 1980, Laskey et al. 1982, Laskey et al. 1985 – all quoted from ATSDR 2000).
However, sperm count and fertility did not appear to be affected at manganese doses as high as 1050 mg Mn/kg b.w. per day (Laskey et al. 1982 – quoted from ATSDR 2000).
A diet low in iron worsened the reproductive effects (Laskey et al. 1982 – quoted from ATSDR 2000).

Studies in pregnant rats indicate that manganese ingested in the form of manganese chloride (Grant et al. 1997, Kontur & Fechter 1985, Pappas et al. 1997 – all quoted from ATSDR 2000) or manganese tetroxide (Laskey et al.

1982 – quoted from ATSDR 2000) via the diet or drinking water does not result in female reproductive effects at doses up to 620 mg Mn/kg b.w. per day (manganese chloride) or 1050 mg Mn/kg b.w. per day (manganese tetroxide) throughout gestation.
However, a dose of 33 mg Mn/kg b.w. per day of manganese chloride administered by gavage throughout gestation caused an increase in postimplantation loss in rats but not in rabbits (Szakmary et al. 1995 – quoted from ATSDR 2000).

A significant decrease in the number of pregnancies were observed in rats mated at day 90-100 postpartum following exposure to manganese tetroxide *in utero* and from day 14-15 postpartum to an oral dose of 3500 mg Mn/kg b.w. per day. No such effect was observed at the lower dose level of 1050 mg Mn/kg b.w. per day. (Laskey et al. 1982 – quoted from ATSDR 2000).

Several developmental studies have been performed with manganese chloride in rats exposed for different periods of time *in utero* and/or postnatally up to 60 days of age either via the drinking water (Kontur & Fechter 1988, Pappas et al. 1997, Ali et al. 1983a, 1985, Dorman et al. 2000 – all quoted from ATSDR 2000) or by gavage (Kristensson et al. 1986, Chandra & Shukla 1978, Deskin et al. 1980, 1981, Szakmary et al. 1995, Grant et al. 1997 – all quoted from ATSDR 2000).

Different neurochemical alterations such as a decreased dopamine level and changes in the level of enzymes involved in the synthesis or metabolism of the neurotransmitter have been observed after postnatal dosing mainly in the gavage studies at doses from about 1 mg Mn/kg b.w. per day (Kristensson et al. 1986, Chandra & Shukla 1978, Deskin et al. 1980, 1981 – all quoted from ATSDR 2000).

The dopamine level was not affected in two studies (Kontur & Fechter 1988, Pappas et al. 1997– both quoted from ATSDR 2000) where rats were dosed *in utero* (and in one of the studies also postnatally) via drinking water to the dams with 620 and 1240 mg Mn/kg b.w. per day, respectively.
However, the dopamine level was increased in rats dosed *in utero* and postnatally via drinking water to the dams with 420 mg Mn/kg b.w. per day (Ali et al. 1985 – quoted from ATSDR 2000) and in neonatal rats dosed with 22 mg Mn/kg b.w. per day with a micropipette for the first 21 days of their life (Dorman et al. 2000 – quoted from ATSDR 2000).

Retardation of development of the skeleton and internal organs as well as a significant increase in external malformations, such as clubfoot, was observed in rats exposed *in utero* via gavage to the dams of 33 mg Mn/kg b.w. per day. However, the external malformations were not observed in pups allowed to grow to 100 days of age indicating that these effects were self-corrected. No further details were given. (Szakmary et al. 1995 – quoted from ATSDR 2000).
No malformations were observed at a dose of 22 mg Mn/kg b.w. per day (Szakmary et al. 1995, Grant et al. 1997 – both quoted from ATSDR 2000).

In most of the studies, no clinical or behavioural signs of neurotoxicity were evident. Male pups exposed *in utero* and postnatally via drinking water to the dams with 620 mg Mn/kg b.w. per day, did not perform different than control rats in a number of behavioural tests that measured spontaneous

motor activity, memory, and cognitive ability (Pappas et al. 1997 – quoted from ATSDR 2000).
However, the offspring of rats who drank 240 mg Mn/kg b.w. per day had delayed air righting reflexes, and pups of dams fed a protein-deficient diet as well as administered manganese chloride had significant delays in age of eye opening and development of the auditory startle reflex (Ali et al. 1983a – quoted from ATSDR 2000).
In neonatal rats dosed with 22 mg Mn/kg b.w. per day with a micropipette for the first 21 days of their life, a significant increase in amplitude of the auditory startle reflex was induced (Dorman et al. 2000 – quoted from ATSDR 2000).

4.3.3 Dermal contact

No data were found.

4.3.4 Parenteral administration

Intraperitoneal injection of pregnant mice with 12.5 mg Mn/kg b.w. per day, as manganese sulphate, on days 8-10 of gestation resulted in exencephaly and embryo-lethality. No further details were given. (Webster & Valois 1987 – quoted from ATSDR 2000).

Similar degenerative changes in testes have been reported in rats and mice following intraperitoneal injection of manganese sulphate (Chandra et al. 1975, Singh et al. 1974 – both quoted from ATSDR 2000) and in rabbits following intravenous injection of manganese chloride (Imam & Chandra 1975 – quoted from ATSDR 2000).

Postimplantation loss has occurred in several studies in rats and mice injected subcutaneously or intravenously during gestation with manganese chloride at doses from about 1 mg Mn/kg b.w. per day (Colomina et al. 1996, Sanchez et al. 1993, Treinen et al. 1995 – all quoted from ATSDR 2000).

Increased skeletal abnormalities and delayed ossification has been found in the offspring of rats and mice injected subcutaneously or intravenously during gestation with manganese chloride at doses from about 1 mg Mn/kg b.w. per day. It was not stated whether maternal toxicity occurred in the studies. (Colomina et al. 1996, Grant et al. 1997, Sanchez et al. 1993, Treinen et al. 1995– all quoted from ATSDR 2000).

4.4 Mutagenic and genotoxic effects

4.4.1 *In vitro* studies

While manganese sulphate was negative in one Ames test with 5 strains (TA97, TA98, TA100, TA1535, TA1537) of *Salmonella typhimurium* with or without metabolic activation (Mortelsman et al. 1986 – quoted from ATSDR 2000), it was positive in another Ames test with strain TA97 without metabolic activation (Pagano & Zeiger 1992 – quoted from ATSDR 2000).

Manganese chloride was negative in Ames test in strains TA98, TA100, and TA1535 but it was positive in strain TA1537 (without metabolic activation)

and conflicting results were obtained for strain TA102 (Wong 1988, De Meo et al. 1991 – both quoted from ATSDR 2000).

Manganese sulphate was positive in a fungal gene conversion/reverse mutation assay (Singh 1984 – quoted from ATSDR 2000).

Manganese sulphate induced sister chromatid exchanges (with and without metabolic activation) and chromosomal aberrations (without metabolic activation) in Chinese hamster ovary cells (Galloway et al. 1987 – quoted from ATSDR 2000).

In *in vitro* studies without metabolic activation, manganese chloride produced gene mutations in cultured mouse lymphoma cells (Oberly et al. 1982 – quoted from ATSDR 2000), caused DNA damage in human lymphocytes in the single cell gel assay (De Meo et al. 1991 – quoted from ATSDR 2000), and caused cell transformations in Syrian hamster embryo cells (Casto et al. 1979 –quoted from ATSDR 2000).

4.4.2 *In vivo* studies

In vivo assays in mice showed that oral doses of manganese sulphate and potassium permanganate caused an increased incidence of micronuclei and chromosomal aberrations in bone marrow (Jordar & Sharma 1990 – quoted from ATSDR).

Oral doses of manganese chloride did not cause chromosomal aberration in the bone marrow or spermatogonia of rats (Dikshith & Chandra 1978 – quoted from ATSDR).

Manganese sulphate was negative for sex-linked recessive lethal mutations in male germ cells and manganese chloride for somatic mutations in fruit flies (Valencia et al. 1985, Rasmuson 1985 – both quoted from ATSDR 2000).

4.5 Carcinogenic effects

Chronic (2 years) oral exposure of F344 rats to manganese sulphate led to an increased incidence of pancreatic tumours (adenomas and carcinomas) in males dosed with up to 331 mg Mn/kg b.w. per day. Although the tumour incidence in the dosed groups was low and not dose-responsive (4 out of 50 in all 3 dose groups), the authors concluded that the tumours were compound related because the incidence of these tumours in the controls was zero. (Hejtmancik et al. 1987a – quoted from ATSDR 2000).

In $B6C3F_1$ mice, chronic oral exposure to manganese sulphate resulted in small increases in pituitary adenomas in females at 905 mg Mn/kg b.w. per day but not in males at 722 mg Mn/kg b.w. per day (Hejtmancik et al. 1987b – quoted from ATSDR 2000).

Chronic oral exposure of $B6C3F_1$ mice and F344 rats to manganese sulphate in doses up to 731 and 232 mg Mn/kg b.w. per day, respectively, resulted in a significantly increased incidence of follicular cell hyperplasia and a marginally increased incidence of thyroid gland follicular cell adenomas in high dose mice. No increased tumour incidence was found in any of the rats or in the

mice in the middle dose group (228 mg Mn/kg b.w. per day). (NTP 1993 – quoted from ATSDR 2000 and SCF 2000).

5 Regulations

5.1 Ambient air

Denmark (C-value): 0.001 mg Mn/m^3 (MST 2002).

WHO (2000): 0.00015 mg Mn/m^3.

US-EPA (1993): RfC 0.00005 mg Mn/m^3 (IRIS 2000).

5.2 Drinking water

Denmark: 0.05 mg Mn/l (MM 2001).

WHO (1996): 0.5 mg Mn/l.

US-EPA (1993): 0.05 mg Mn/l (HSDB 2000).

5.3 Soil

Denmark: -

The Netherlands: -

5.4 Occupational Exposure Limits

Denmark: 0.2 mg Mn/m^3 (At 2002).

ACGIH: 0.2 mg Mn/m^3 (HSDB 2000).

The occupational exposure limits in different countries around the world varies between 0.2 and 5 mg Mn/m^3 (IRIS 2000).

5.5 Classification

Manganese sulphate is classified for effects following repeated exposure (Xn;R48/20/22 - harmful: danger of serious damage to health by prolonged exposure through inhalation and if swallowed) and for environmental effects (N;R51/53 – toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment). (MM 2002).

5.6 IARC

-

5.7 US-EPA

Manganese is not classifiable with regard to human carcinogenicity (Group D) (EPA 1993b – quoted from ATSDR 2000).

5.8 ATSDR

Minimal risk level (MRL): 0.00004 mg Mn/m^3 (chronic inhalation).

6 Summary

6.1 Description

Manganese sulphate and manganese sulphide occur as powders or crystals of several hydrates. Manganese sulphate has a high water solubility (greater than 500 g/l) at room temperature, while manganese sulphide is insoluble in water. In moist conditions, manganese sulphide readily oxidizes in air to the sulphate.

Manganese is a natural component of the environment and an essential element in the nutrition of humans and animals.

6.2 Toxicokinetics

Manganese absorption occurs primarily through the diet. Of ingested inorganic manganese about 3-5% is absorbed in humans. The absorption is influenced by such factors as the water solubility of the compounds in question, age, and the amount of manganese as well as of other trace elements such as iron, calcium and phosphorus present in the gut. Absorption via the lungs can be significant for occupationally exposed persons or for those exposed to excess levels of airborne manganese. The absorption following inhalation is higher than that after oral administration for the more soluble forms of inorganic manganese.

In healthy humans, manganese is distributed to the tissues with the highest levels found in the liver, pancreas, and kidney and the lowest levels in bone and fat.
Excess manganese (following oral exposure in humans with liver diseases) was accumulated in certain regions of the brain (basal ganglia, especially the globus pallidus and the substantia nigra).
Mice and rats chronically fed manganese sulphate generally had elevated tissue levels of manganese. The manganese levels in the liver and kidney were higher than the levels in the brain.

Following inhalation in rats, most manganese was distributed to the olfactory bulb and the brain but also to other organs such as the lungs, the liver, the kidneys, and the pancreas. One study indicates that manganese may be distributed directly to the brain from the nasal cavity via the olfactory pathway by-passing the blood-brain barrier. Preferential accumulation of manganese in specific locations of the brain (including the caudate nucleus, globus pallidus, and the substantia nigra) was noted in one monkey exposed to an aerosol of manganese chloride several hours/day for 3-5 months.

Manganese is excreted with a half-life of 13-37 days in humans. In general, biliary excretion is the main pathway by which absorbed manganese reaches the intestines where most of the element is excreted in the faeces.
An inhalation study in monkeys showed that a portion of the manganese was retained in the lung and brain and was cleared with a half-life of up to 250

days. Small amounts of manganese can also be found in urine, sweat, and milk.

The central nervous system is the primary target of manganese toxicity. Several hypotheses have been proposed for the toxicological mechanism behind this toxicity.

6.3 Human toxicity

6.3.1 Single dose toxicity

No data were found.

6.3.2 Repeated dose toxicity

From studies of humans exposed to manganese dusts (mainly manganese dioxide) in mines and factories, clear evidence exist that inhalation exposure to high levels of manganese for longer periods can lead to neurological effects. The disease, termed manganism, is characterised by weakness, muscle rigidity, tremor, apathy, speech disturbances, a mask-like face, and slow clumsy movement of the limbs typically after several years of exposure but some individuals may begin to show signs after as few as 1-3 months of exposure. In most cases the symptoms are irreversible. People with manganism generally have significantly poorer eye-hand coordination, hand steadiness, reaction time and postural stability, and a lower level of performance in cognitive flexibility on neurobehavioral tests.

Recent epidemiological data indicate that concentrations of manganese in respirable dust in the range of 0.027 to 0.215 mg Mn/m^3 and in total dust in the range of 0.14 to 1.59 mg Mn/m^3 in the workplace can result in measurable neurological effects.

Occupational studies indicate that manganese inhaled in high concentrations may elevate the serum prolactin and cortisol levels, lower the blood pressure, and cause inflammation in the lungs.

Only limited evidence exist that oral exposure to excess manganese in humans leads to neurological effects similar to those reported for inhalation exposure.

6.3.3 Toxicity to reproduction

Decreased libido and impotence are common symptoms in male workers with clinical signs of manganism following exposure to high levels of manganese dusts. In addition increased semen liquefaction time, decreased sperm count, decreased sperm viability, and decreased fertility have been reported in some studies.

Two studies of school children showed that increased oral exposure to manganese in drinking water and food was associated with poorer performance in school and on neurobehavioral tests as compared to children exposed to lower levels. However, the children may have been exposed to other metals that may have influenced the developmental effects seen.

Incidences of stillbirths and malformations have been studied in an Australian aboriginal population living on an island where environmental levels of manganese are high. However, data from a suitable control group are lacking.

6.3.4 Mutagenic and genotoxic effects

An increase in chromosomal aberrations was found in a group of workers, which had been exposed to a mixture of manganese, nickel, and chromium by inhalation for 10-24 years.

6.3.5 Carcinogenic effects

No data were found.

6.4 Animal toxicity

6.4.1 Single dose toxicity

Acute inhalation exposure to high concentrations of manganese dusts can cause an inflammatory response in the lung. Oral doses of manganese salts given by gavage can cause death in animals.

6.4.2 Repeated dose toxicity

Oral and inhalation studies in animals exposed to manganese have sometimes revealed biochemical or neurobehavioral evidence of neurological effects. Monkeys exposed to manganese via inhalation, oral intake or injection exhibit neurological symptoms very similar to those observed in workers exposed to manganese. They accumulate manganese in the basal ganglia, as do humans exposed to excess manganese. However, motor deficits similar to clinical effects in humans are seldom observed in rodents and tend to be transient effects.

Animal studies indicate that manganese inhaled in high concentrations may cause inflammation in the lungs. Mice and/or rats exposed orally to high doses of manganese sulphate for 2 years may experience gastrointestinal (e.g. hyperplasia and erosion of the forestomach), renal (increased severity of chronic progressive nephropathy) and/or endocrine (thyroid follicular hyperplasia and dilation) effects.

6.4.3 Toxicity to reproduction

A high systemic concentration of manganese may cause reproductive and developmental effects as evidenced by degenerative changes in testes of male animals injected, instilled or gavaged with manganese and by embryo-lethality, postimplantation loss, and structural abnormalities and delays in pups in studies where manganese was injected during gestation at doses from about 1 mg Mn/kg b.w. per day or gavaged at doses from about 33 mg Mn/kg b.w. per day.

Studies in mice and rats with oral intake of manganese indicate that ingestion at doses from about 1050 mg Mn/kg b.w. per day can lead to delayed maturation of the reproductive function in male offspring probably due to decreased testosterone secretion by Leydig cells. However, sperm count and

fertility did not appear to be affected at manganese doses as high as 1050 mg Mn/kg b.w. per day. A diet low in iron worsened the reproductive effects.

No female reproductive effects were found in rats fed manganese sulphate in their diet at doses up to 187 mg Mn/kg b.w. per day (highest dose tested) for 8 weeks prior to mating and until gestational day 21 or in pregnant rats ingesting other inorganic manganese substances at even higher doses.

No gross malformations were observed in the foetuses of the rats fed manganese sulphate in their diet at doses up to 187 mg Mn/kg b.w. per day (highest dose tested) for 8 weeks prior to mating and until gestational day 21. The highest dose did cause a significant increase in foetal manganese and a decrease in foetal iron content.

In most studies, no biochemical or behavioural signs of neurotoxicity were evident in pups. Male pups exposed *in utero* and postnatally via drinking water to the dams with 620 mg Mn/kg b.w. per day, did not perform different than control rats in a number of behavioural tests that measured spontaneous motor activity, memory, and cognitive ability, and did not have any neurochemical alterations. In neonatal rats dosed with 22 mg Mn/kg b.w. per day with a micropipette for the first 21 days of their life, the dopamine level was increased and a significant increase in amplitude of the auditory startle reflex was induced. A decreased dopamine level and changes in the level of enzymes involved in the synthesis or metabolism of the neurotransmitter have been observed after postnatal dosing but mainly in the gavage studies at doses from about 1 mg Mn/kg b.w. per day.

In utero exposure to manganese did not alter gross locomotor activity, different behavioural parameters and learning performance in mice pups whose mothers had been exposed to manganese at an average concentration of 61 mg Mn/m^3 7 hours/day, 5 days/week for 16 weeks prior to conception and to either filtered air or manganese during the gestation period. The only effect observed was a reduced weight in pups of mothers exposed to manganese before conception and filtered air after conception.

6.4.4 Mutagenic and genotoxic effects

In vitro, manganese sulphate was negative in one Ames test with 5 strains of *Salmonella typhimurium* with or without metabolic activation. It was positive in another Ames test with strain TA97 without metabolic activation, in a fungal gene conversion/reverse mutation assay, in the sister chromatid exchange test in CHO cells with and without metabolic activation, and in a test for chromosomal aberrations in CHO cells without metabolic activation.

In vivo assays in mice showed that oral doses of manganese sulphate caused an increased incidence of micronuclei and chromosomal aberrations in bone marrow. Manganese sulphate was negative for sex-linked recessive lethal mutations in male germ cells in fruit flies.

6.4.5 Carcinogenic effects

Exposure to high oral doses of manganese sulphate for 2 years may cause small increases in thyroid gland follicular cell adenomas (in mice at 731 mg Mn/kg b.w. per day), pituitary adenomas (in female mice at 905 mg Mn/kg

b.w. per day), and pancreatic adenomas and carcinomas (in male rats at all doses up to 331 mg Mn/kg b.w. per day). However, rats and mice exposed to about 230 mg Mn/kg b.w. per day for 2 years showed no increases in tumour incidence in one of the studies.

Manganese is an essential element in the nutrition of humans and animals. The concentration of manganese in our body following oral exposure is normally well regulated. However, the homeostatic system may become overloaded which may result in adverse effects. Most of the observed adverse effects following exposure to manganese have been observed in workers exposed by inhalation.

Neurotoxicity following inhalation is the critical effect of manganese. Clear evidence exist from studies of human workers that inhalation exposure to manganese for longer periods can lead to serious neurological effects (resembling parkinsonism) that in most cases are irreversible. These effects may occur at concentrations of manganese in respirable dust in the range of 0.027 to 0.215 mg Mn/m^3 and in total dust in the range of 0.14 to 1.59 mg Mn/m^3. Studies in rats indicate that manganese may be distributed directly to the brain from the nasal cavity via the olfactory pathway, which may in part explain why it is mainly neurological effects that are seen after inhalation exposure. In addition, one study in monkeys that had been exposed to an aerosol of manganese chloride showed that most manganese was excreted fairly quickly but a portion of the manganese was retained in the lungs and brain and was cleared with a longer half-life.
Other effects observed in workers exposed to manganese by inhalation included an elevated serum prolactin and cortisol level, a lowered blood pressure, and inflamed lungs (also seen in rodents). The different systemic effects seen in rodents exposed orally to manganese occurred at relatively high concentrations.
Monkeys exposed to manganese exhibit neurological symptoms resembling those observed in workers exposed to manganese but in rodents this is seldom the case. For this reason, studies in rodents may not necessarily be relevant models for the toxicity of manganese in humans even though neurochemical changes are sometimes revealed in rodents exposed to manganese.

Only limited evidence exist that oral exposure to excess manganese in humans lead to neurological effects similar to those reported for inhalation exposure.

Manganese has the potential to cause reproductive and developmental effects as evidenced by reproductive effects seen in workers exposed to manganese by inhalation and by reproductive and developmental effects seen in rodent studies. However, most of the reproductive and developmental effects seen in rodent studies occurred after exposure via gavage or injection where a high systemic concentration of manganese would be found. In most studies, no biochemical or behavioural signs of neurotoxicity were evident in pups even though the absorption and/or retention of manganese are known to be higher in neonates. However, the dopamine level and the amplitude of the auditory startle reflex was increased in neonatal rats dosed with 22 mg Mn/kg b.w. per day with a micropipette for the first 21 days of their life. A reduced weight of

pups of mothers exposed to 61 mg Mn/m^3 for 16 weeks before conception was the only effect observed in these pups.

It is a cause of concern that manganese was positive in several *in vitro* and *in vivo* genotoxic assays. Small increases in thyroid gland follicular cell adenomas, pituitary adenomas, and pancreatic adenomas and carcinomas did occur in rodents exposed orally to relatively high doses of manganese for 2 years.

7 References

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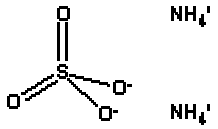
Appendix 2: Diammonium sulphate

8 General description

8.1 Identity

Molecular formula: $H_8N_2O_4S$

Structural formula:



Molecular weight: 132.14

CAS-no.: 7783-20-2

Synonyms: Ammonium sulphate
Mascagnite
Sulphuric acid, diammonium salt

8.2 Physical / chemical properties

Description: Colourless orthorhombic crystals or white granules with no odour.

Melting point: Decomposes above 235°C

Density: 1.77 g/ml (at 20°C)

Vapour pressure: -

Concentration of saturated vapours: -

Solubility: Water: 754-770 g/l (at 20°C)
Insoluble in acetone, alcohol and ammonia.

References: ChemFinder (2001), HSDB (2000), IUCLID (2000), Merck Index (1996).

Other chemical substances mentioned in this paper:

Sodium sulphate (Na_2SO_4)
Sodium bisulphate ($NaHSO_4$)
Ammonium bisulphate (NH_4HSO_4)
Sulphuric acid (H_2SO_4)
Sulphur dioxide (SO_2)
Ammonium nitrate (NH_4NO_3)
Ozone (O_3)

9 Toxicokinetics

9.1 Absorption, distribution, and elimination

9.1.1 Inhalation

Inhaled diammonium sulphate particles (0.2 mg/m^3) with a mass median aerodynamic diameter (MMAD) of 0.3 to 0.6 µm reached the lungs of Syrian hamsters. However, a substantial proportion of the compound was found in the nose. (US Department of Commerce - quoted from IUCLID 2000).

The clearance of diammonium sulphate (inhaled at a concentration of 0.2 mg/m^3) from the hamster lungs (via the blood and urinary tract) has been determined to be about 20 minutes. The results of clearance studies with guinea pigs and rabbits suggested that there was no species difference. No further details were given. (US Department of Commerce - quoted from IUCLID 2000).

9.1.2 Oral intake

Systemic effects and death following oral exposure to diammonium sulphate indicate that it may be absorbed from the gastrointestinal tract (IUCLID 2000, Sato et al. 1999).

Soluble sulphate salts are rather slowly absorbed from the alimentary tract. Because of their osmotic activity, they draw water into the lumen of the bowel and produce purging. (Gosselin et al. 1984 – quoted from HSDB 2000).

9.1.3 Dermal contact

No data have been found.

9.1.4 Other routes

No data have been found.

9.2 Mode of action

Acute intoxication with ammonium may affect the cerebral nervous system by inhibition of the energy metabolism such as a decrease in ATP in the brain or by inhibition of glutamic acid metabolism resulting in a disturbance of neurotransmitters (Jablonska 1975, Hindfelt et al. 1977, McCandless & Schenker 1981, Schenker & Brady 1988 - all quoted from Sato et al. 1999).

10 Human toxicity

10.1 Single dose toxicity

10.1.1 Inhalation

Groups of 9-15 healthy non-smoking male volunteers between the ages of 18 and 40 were exposed to diammonium sulphate aerosols (or to other gaseous or aerosol pollutants alone or to mixtures of these) at a concentration of 0 or 0.133 mg/m^3 (MMAD = 0.55 μm) for 4 hours. During the exposure each person had two 15 minutes exercise sessions on a treadmill. Environmental conditions were mildly stressful with a temperature of 30°C and a relative humidity of 60%. A battery of 19 measurements of pulmonary function was performed just prior to exposure; 2 hours into the exposure, following the first exercise session; 4 hours into the exposure, following the second exercise session; and 24 hours after exposure. Diammonium sulphate under the conditions of the experiment had no effect on the pulmonary function. Ozone in a concentration of 0.4 ppm affected the pulmonary function. The effect of exposure to a combination of ozone and diammonium sulphate seemed slightly greater (although not statistically significant) than exposure to ozone alone. (Stacy et al. 1983).

Twenty healthy non-smoking male and female volunteers aged 21-34 years were exposed by inhalation to clean air, diammonium sulphate aerosols at a concentration of 0.5 mg/m^3 (MMAD = 1.0 μm), 1 ppm sulphur dioxide and combined diammonium sulphate/sulphur dioxide. Exposures lasted for 4 hours which included two light to moderate exercise stints for 15 minutes each. The study ran for 3 days/week for 3 consecutive weeks where each volunteer served as his or her own control. Clean air was breathed on day 1 and 3 of each week. Diammonium sulphate (week 1), sulphur dioxide (week 2) or the combination (week 3) was breathed on day 2. No significant changes were measured in pulmonary function or bronchial reactivity following exposure to diammonium sulphate, sulphur dioxide or the combination. The most common symptoms were upper respiratory and eye irritation. Three volunteers reported upper respiratory irritation during the exposure to diammonium sulphate alone and nine during the combined exposure. Two volunteers reported eye irritation during diammonium sulphate exposure alone and three during the combined exposure. (Kulle et al. 1984).

Eight healthy and nine asthmatic non-smoking volunteers aged 60-76 years were exposed by inhalation to clean air, diammonium sulphate at a concentration of 0.070 mg/m^3 and sulphuric acid. Exposures to each of the test atmospheres (clean air, diammonium sulphate, or sulphuric acid) were separated by at least 1 week and lasted for 40 minutes composed of 30 minutes at rest and 10 minutes of light exercise on a treadmill. Diammonium sulphate exposure had no effect on pulmonary function parameters (forced expiratory volume in one second, forced vital capacity, and total respiratory resistance) in either group. (Koenig et al. 1993).

Sixteen healthy and seventeen asthmatic non-smoking volunteers with a mean age of 27 were exposed by inhalation to sodium chloride aerosols (control), diammonium sulphate aerosols at a concentration of about 0.1 or 1.0 mg/m^3 (MMAD = 0.5-1 μm) and other sulphates (sodium bisulphate, ammonium bisulphate, sulphuric acid) by double blind randomisation. Exposures to each of the test atmospheres (control, diammonium sulphate, sodium bisulphate, ammonium bisulphate, or sulphuric acid) were separated by at least 3 hours and lasted for 16 minutes. To determine if sulphate inhalation caused increased reactivity to a known bronchoconstrictor, all volunteers inhaled carbachol following each 16 minutes exposure. Diammonium sulphate (and the other sulphates) produced a small but significant decrease in flow rates on maximum and partial expiratory flow-volume curves and potentiated the bronchoconstrictor action of carbachol at 1 mg/m^3 both in asthmatic and healthy volunteers. The most significant changes were seen with the most acidic sulphates (sulphuric acid and ammonium bisulphate), which also produced a significant decrease at 1 mg/m^3 in the mean specific airway conductance and in the forced vital capacity in one second in asthmatic volunteers. No significant changes on the pulmonary function occurred following exposure to 0.1 mg/m^3 of sulphates. (Utell et al. 1982).

10.1.2 Oral intake

An 85-year old woman was found dead lying on the ground outside her house. The autopsy could not determine the definite cause of her death since routine poison analysis revealed no toxicological substances in her blood and the pathological examination showed nothing unusual. However, examination at the police laboratory of the solution in the beer can found next to her showed that it was very likely diammonium sulphate. Measurements showed a significant increase of ammonium and sulphate ions in serum and gastric contents. Therefore it was concluded that the cause of death was acute intoxication due to ingestion of diammonium sulphate. (Sato et al. 1999).

10.1.3 Dermal contact

No data have been found.

10.1.4 Other routes

No relevant data have been found.

10.2 Repeated dose toxicity

Ten healthy and six asthmatic non-smoking volunteer men with a mean age of 42.3 were exposed by inhalation to purified air, diammonium sulphate aerosols at a concentration of 0.1 mg/m^3 (MMAD = 0.3 μm) and other sulphates (ammonium bisulphate, sulphuric acid) in a blind experimental fashion with 1-2 days of exposure to air followed by 2-3 consecutive days of sulphate aerosol exposure. Exposures lasted for 2 hours with light exercise on bicycle ergometers for the first 15 minutes of each half hour. No consistent changes in pulmonary function or in symptoms occurred in the healthy or asthmatic men when exposed to diammonium sulphate aerosols. (Avol et al. 1979).

10.3 Toxicity to reproduction

No data have been found.

10.4 Mutagenic and genotoxic effects

No data have been found.

10.5 Carcinogenic effects

No data have been found.

11 Animal toxicity

11.1 Single dose toxicity

11.1.1 Inhalation

No mortality or signs of gross toxicity was observed in 6 young adult male rats that were exposed to diammonium sulphate aerosols at a concentration of 1000-1200 mg/m^3 (particle size averaged 2-3 μm) 8 hours/day for 3 consecutive days. Eight of 20 guinea pigs exposed to 800-900 mg/m^3, 1/6 exposed to 600-700 mg/m^3, and 0/6 exposed to 500-600 mg/m^3 for 8 hours died during exposure. The survivors recovered with no noticeable after effects. (Pepelko et al. 1980).

No effect on pulmonary macrophage numbers and shape was found in hamsters exposed to 0.86 mg/m^3 of diammonium sulphate with a MMAD of 0.3 μm for 12 hours (US Department of Commerce - quoted from IUCLID 2000).

Groups of 6-8 adult sheep were exposed by inhalation to diammonium sulphate aerosols in a concentration of 0.1 or 4 mg/m^3 (MMAD = 0.5 or 1.5 μm) or to other sulphates (ammonium bisulphate, sulphuric acid) for 4 hours. The sheep first inhaled physiologic buffer, then the known bronchoconstrictor carbachol, and finally half an hour later sulphate aerosols. Some sheep were exposed to more than one salt. These exposures were separated by at least 1 week. The mean pulmonary flow resistance was not altered following exposure to diammonium sulphate. The airway responsiveness to inhaled carbachol was enhanced at some time within the 24-hour period immediately following exposure to diammonium sulphate with a MMAD of 1.5 μm. (Abraham et al. 1983).

A group of five male, mixed breed rabbits were exposed by oral inhalation to clean air, diammonium sulphate aerosols in a concentration of 2.0 mg/m^3 (MMAD = 0.4 μm) and to other sulphates (ammonium bisulphate, sodium sulphate). Exposures for individual rabbits were separated by at least 1 week and lasted for 1 hour. The bronchiolar mucociliary clearance was not affected by the treatment with diammonium sulphate. (Schlesinger 1984).

The bronchiolar or tracheal mucociliary clearance was not affected either in donkeys, rats or sheep inhaling diammonium sulphate with a MMAD of 0.1-0.4 for 1-4 hours in doses of 0.3-3.0, 3.6 or 1.1 mg/m^3, respectively (Schlesinger et al. 1978, Phalen et al. 1980, Sackner et al. 1981 – all quoted from Schlesinger 1984).

11.1.2 Oral intake

The oral LD_{50}-values reported for diammonium sulphate range from 2840 to 4250 mg/kg b.w. for rats (3 values reported) and from 610 to 640 mg/kg b.w. for mice (2 values reported) (IUCLID 2000).

Groups of 2-3 Japanese white rabbits were anaesthetised and dosed with 0 or 1500 mg/kg b.w. of diammonium sulphate in saline through a gastric probe. The three rabbits dosed with diammonium sulphate showed similar symptoms such as mydriasis, irregular respiratory rhythms, local and general convulsions, until they fell into respiratory failure with cardiac arrest about an hour after the dosing. Electroencephalogram showed slow, suppressive waves initially and 15-20 minutes later a high-amplitude slowing wave pattern in correspondence to local convulsions. Electrocardiogram did not show any change of wave pattern except for bradycardia immediately after the general convulsions and until cardiac arrest. The blood pressure fell down rapidly after the general convulsions. The concentration of ammonium and sulphate ions in serum increased remarkable and blood gas analysis showed severe metabolic acidosis. Biochemical analysis of blood and histological examination of brain, heart, lung, spleen, kidney, liver and stomach revealed no significant changes except for a moderate increase in K^+ just before cardiac arrest. (Sato et al. 1999).

11.1.3 Dermal contact

Diammonium sulphate was not irritating to intact or abraded rabbit skin that was tested for 20 or 8 hours, respectively. No further details were given. (BASF 1969 – quoted from IUCLID 2000).

11.1.4 Other routes

11.1.4.1 Ocular irritation

Diammonium sulphate was not irritating to rabbit eyes. No further details were given. (BASF 1969 – quoted from IUCLID 2000).

11.2 Repeated dose toxicity

11.2.1 Inhalation

Young male Sprague-Dawley rats were pre-treated intratracheally with either physiologic saline (to model normal lungs) or porcine pancreatic elastase (to model chronic pulmonary impairment). Groups of 30 rats from each pre-treatment group were exposed to filtered air (control), to 1 ppm sulphur dioxide, to wet diammonium sulphate aerosols in concentrations of 0.5 mg/m^3 (MMAD = 0.44 µm), or to combined sulphur dioxide and diammonium sulphate for 5 hours/day, 5 days/week for 4 or 8 months. Half of the rats exposed for 8 months were held for an additional 3-month recovery period.
At 4 months, bronchial epithelial hyperplasia and alveolar mean and median cord (a long flexible structure) length significantly increased in the saline/diammonium sulphate and saline/diammonium sulphate/sulphur dioxide groups but decreased in the elastase/diammonium sulphate and elastase/ diammonium sulphate/sulphur dioxide groups compared to air controls. The number of non-ciliated epithelial cells in the bronchioles was significantly increased in the saline/diammonium sulphate group and focal haemosiderosis was significantly increased in the elastase/diammonium sulphate/sulphur dioxide group.
At 8 months, alveolar interstitial fibrosis tended to be greater in aerosol-exposed rats with either normal or impaired lungs compared to air controls,

but was significant only in the saline/diammonium sulphate exposed group. Other significant effects observed at 8 months following exposure to diammonium sulphate and compared to air controls included increased lung volume, emphysema and focal haemosiderosis in elastase treated rats and an increased count of non-ciliated epithelial cells in the bronchioles in saline treated rats.
Effects of elastase treatment (e.g. greater lung volumes, emphysema, and alveolar interstitial fibrosis) were the only effects that persisted throughout the recovery period. In addition, a significant increase in alveolar mean and median cord length in saline/diammonium sulphate treated rats was observed. No significant pathological changes were observed in the nasal cavities of any group at any time point. The pulmonary function of rats exposed for 4 months to diammonium sulphate alone was significantly decreased compared to air control for a few measured parameters (residual volume/total lung capacity, quasistatic compliance, nitrogen-slope) in either saline or elastase treated rats. No significant changes in the pulmonary function were observed in rats exposed to both diammonium sulphate and sulphur dioxide. Immunological studies of peripheral lymphocytes and spleen cells of rats exposed to diammonium sulphate for 4 months revealed no significant depressive effects on the immune system.
The authors concluded that changes related to exposure to sulphur dioxide and/or diammonium sulphate were minimal and transient. No additive effect appeared to occur between diammonium sulphate and sulphur dioxide. The authors were mainly concerned about the increased alveolar fibrosis (observed after 8 months of exposure but not after 4 months of exposure or after 3 months of recovery) associated with diammonium sulphate exposure and the implications it might have in progressive fibrotic disease in human lungs. (Smith et al. 1989).

Male Sprague-Dawley rats and Hartley strain guinea pigs were instilled intratracheally with either sterile saline or porcine pancreatic elastase dissolved in saline. Groups of 15-29 animals from each pre-treatment group were exposed to filtered air (control), to diammonium sulphate aerosols in concentrations of 1.0 mg/m^3 (MMAD = 0.4 μm), or to ammonium nitrate for 6 hours/day, 5 days/week for 5 or 20 days. Pulmonary function evaluations conducted in guinea pigs showed no significant effects of diammonium sulphate (or ammonium nitrate) exposure. Compared with air-exposed animals, rats exposed to diammonium sulphate aerosols had increased values of residual volume and functional residual capacity and a decreased nitrogen-slope in saline as well as elastase treated rats. Diammonium sulphate exposure aggravated elastase-induced emphysema in the rat. Guinea pigs (but not rats) exposed to diammonium sulphate had hypertrophy and hyperplasia of non-ciliated epithelial cells in the alveoli and bronchioles. Alveolar septa of saline/diammonium sulphate exposed animals were affected by interstitial thickening caused by an increased collagen content and an increased number of interstitial cells. The alveolar cord length was increased in saline/diammonium sulphate exposed animals. (Loscutoff et al. 1985, Busch et al. 1984).

Groups of 6 male Sprague-Dawley rats were exposed to diammonium sulphate aerosols in concentrations of 0, 1 or 5 mg/m^3 (MMAD = 0.5-0.6 μm), ozone in concentrations of 0, 0.20, 0.64 or 0.96 ppm or mixtures of both for 23.5 hours per day for 2-7 days. Exposure to diammonium sulphate alone showed no effect in several biochemical and morphometric assays for

lung injury (e.g. protein content of lung lavage fluid, collagen synthesis rates, soluble proline content, fibroblast content, lung lesions). Ozone alone caused lung injury as evidenced by significantly differences between the control rats and the rats exposed to ozone in the assays for lung injury. A synergistic effect of the combination of ozone and diammonium sulphate (at a dose of 5 mg/m^3, but not at 1 mg/m^3) was observed in the form of significantly greater increases in the measured parameters as compared with results observed in rats exposed to ozone alone. Based on these and other studies with combinations of ozone or nitrogen dioxide with different aerosols, the authors conclude that acidity and size of the particles in the aerosol is responsible for the synergy. (Last 1991).

Groups of 10 adult male rats were exposed to diammonium sulphate aerosols in concentrations of 0 or 300 mg/m^3 (particle size averaged 1-2 µm) for 8 hours/day for 1, 3, 7 or 14 days. No significant differences could be detected between control and exposed rats in arterial blood gases, pH and standard bicarbonate, in body weight and wet lung weight, in vital capacity and residual volume of the lungs, or in histological examination of the trachea, bronchial lymph nodes and lungs. (Pepelko et al. 1980).

Groups of 7-12 male guinea pigs were exposed to diammonium sulphate aerosols in concentrations of 0, 0.2, 0.4 or 2.0 mg/m^3 (MMAD = 0.7-0.9 µm) for 2 hours/day, 5 days/week for 7½ weeks. The animals were successively 3 times a week for 2½-3 weeks exposed to aerosols for 2 hours, and after 30 minutes, to an albumin spray for 30 minutes. Breathing curves were continuously recorded during the sensitisation periods (periods with exposure to albumin) and for 5 minutes before exposure to albumin. The experiments showed that the degree of asthmatic dyspnoea (immediate type induced by inhalation of albumin) was dose-dependently increased by exposure to diammonium sulphate aerosols (only significant at 2 mg/m^3). The aerosols alone did not alter the breathing curves at the concentrations studied. (Kitabatake et al. 1991).

11.2.2 Oral intake

Groups of 10 F344 rats of each sex were fed 0, 0.38, 0.75, 1.5 or 3% of diammonium sulphate in their CRF-1 powder diet for 13 weeks. The only effect observed was diarrhoea during the administration period in the males dosed with 3% diammonium sulphate. No changes indicating toxicity were observed in body and organ weights, in blood and biochemical parameters, or in the histopathological examination. According to the authors, the NOEL in this study was 1.5% (equal to 886 mg/kg b.w. per day) in males but 3% (equal to 1975 mg/kg b.w. per day) in females. (Takagi H et al. 1999 – quoted from Toxline Plus).

11.2.3 Dermal contact

No data have been found.

11.2.4 Other routes

No data have been found.

11.3 Toxicity to reproduction

No data have been found.

11.4 Mutagenic and genotoxic effects

11.4.1 *In vitro* studies

Diammonium sulphate was not mutagenic when tested (in accordance with OECD guideline 471) in 4 strains (TA98, TA100, TA1535 and TA1537) of *Salmonella typhimurium* in a concentration of 20 – 5000 µg/plate in Ames tests with and without metabolic activation systems (BASF 1983 – quoted from IUCLID 2000).

In a cytogenetic assay without metabolic activation systems, an isotonic solution of diammonium sulphate did not cause chromosomal aberrations in V79 hamster cells. However, cells treated with a hypotonic diammonium sulphate solution (or other hypotonic solutions) had an increase in aberration frequencies. Post-treatment with a hypertonic solution of diammonium sulphate increased the mutagenicity of ethylmethanesulfonate, a known mutagen. (Nowak 1987, 1988 - both quoted from IUCLID 2000).

In two other cytogenetic assays performed in human lymphocytes and CHO cells, respectively, diammonium sulphate did not cause chromosomal aberrations (Obe & Kamra 1986, Obe et al. 1986 - both quoted from IUCLID 2000).

Diammonium sulphate tested negative in the yeast gene mutation assay in Saccharomyces cerevisiae D4 with and without metabolic activation systems (US Department of Commerce 1975 - quoted from IUCLID 2000).

11.4.2 *In vivo* studies

No data have been found.

11.5 Carcinogenic effects

Groups of Syrian hamsters received 5 mg of a known carcinogen (benzo(a)pyrene) by intratracheal intubation once a week for 15 weeks. One group of the animals was simultaneously exposed to 0.2 mg/m^3 of diammonium sulphate 6 hours/day for 5 days/week for the 15 weeks. Other groups received benzo(a)pyrene or diammonium sulphate alone. There was no effect of diammonium sulphate inhalation on benzo(a)pyrene carcinogenesis. (US Department of Commerce - quoted from IUCLID 2000).

12 Regulations

12.1 Ambient air

-

12.2 Drinking water

Denmark:	0.5 mg ammonium/l 250 mg sulphate/l	(MM 2001)

12.3 Soil

-

12.4 Occupational Exposure Limits

-

12.5 Classification

-

12.6 IARC

-

12.7 US-EPA

-

13 Summary

13.1 Description

Diammonium sulphate occurs as colourless orthorhombic crystals or white granules with no odour. The water solubility is high (754-770 g/l).

13.2 Toxicokinetics

When hamsters inhaled diammonium sulphate at a concentration of 0.2 mg/m^3, a substantial proportion of the compound was found in the nose. The clearance from the lungs was determined to be about 20 minutes. The results of clearance studies with guinea pigs and rabbits suggested that there was no species difference.

Systemic effects and death following oral exposure to diammonium sulphate indicate that it may be absorbed from the gastrointestinal tract.

Acute intoxication with ammonium may affect the cerebral nervous system by inhibition of the energy metabolism or by inhibition of glutamic acid metabolism.

13.3 Human toxicity

13.3.1 Single dose toxicity

In several studies healthy and asthmatic non-smoking volunteers aged 18-76 have been exposed by inhalation to diammonium sulphate aerosols in concentrations between 0.07 and 1.0 mg/m^3 (and with MMAD of 0.5-1 μm) for up to 4 hours. In some of the studies diammonium sulphate was administered in combination with ozone, sulphur dioxide or carbachol (a known bronchoconstrictor). In all studies different measurements of pulmonary function was performed. In doses up to 0.5 mg/m^3 consistent changes in pulmonary function or in symptoms occurred in neither healthy nor asthmatic volunteers. At a concentration of 1 mg/m^3, diammonium sulphate produced a small but significant decrease in flow rates on maximum and partial expiratory flow-volume curves and potentiated the bronchoconstrictor action of carbachol both in asthmatic and healthy volunteers. The effect of exposure to a combination of ozone and diammonium sulphate seemed slightly greater (although not statistically significant) than exposure to ozone alone. The combination of diammonium sulphate and sulphur dioxide produced no significant changes on the lung function at the doses studied. Upper respiratory and eye irritation was reported in one of the studies in some of the volunteers exposed to 0.5 mg/m^3 of diammonium sulphate.

One case-report of death caused by acute intoxication due to ingestion of diammonium sulphate exists.

13.3.2 Repeated dose toxicity

No consistent changes in pulmonary function or in symptoms occurred in healthy or asthmatic men when exposed to diammonium sulphate aerosols at a concentration of 0.1 mg/m^3 (MMAD = 0.3 μm) for 2 hours a day for 2-3 days.

13.3.3 Toxicity to reproduction

No data have been found.

13.3.4 Mutagenic and genotoxic effects

No data have been found.

13.3.5 Carcinogenic effects

No data have been found.

13.4 Animal toxicity

13.4.1 Single dose toxicity

The reported LC_{50}-value (exposure duration: 8 hours) of diammonium sulphate is higher than 1200 mg/m^3 (particle size averaged 2-3 μm) for rats and higher than 900 mg/m^3 (particle size not stated) for guinea pigs. The LD_{50}-value following oral exposure is reported to be 2840-4250 mg/kg b.w. for rats and 610-640 mg/kg b.w. for mice. Three rabbits dosed with 1500 mg/kg b.w. of diammonium sulphate through a gastric probe all died.

The airway responsiveness to inhaled carbachol (a known bronchoconstrictor) was enhanced in sheep exposed to 0.1 or 4 mg/m^3 of diammonium sulphate with a MMAD of 1.5 μm (but not of 0.5 μm) for 4 hours.

The bronchiolar or tracheal mucociliary clearance was not affected either in donkeys, rats, rabbits, or sheep inhaling diammonium sulphate aerosols for 1-4 hours in doses of 0.3-3.6 mg/m^3.

Diammonium sulphate was not irritating to intact or abraded rabbit skin or to rabbit eyes.

13.4.2 Repeated dose toxicity

Pulmonary histology

In rats exposed to 0.5 mg/m^3 (MMAD = 0.44 μm), the authors were mainly concerned about the increased alveolar fibrosis observed after 8 months of exposure (but not after 4 months of exposure or after 3 months of recovery). Increased alveolar collagen content was also observed in rats and guinea pigs exposed to 1 mg/m^3 (MMAD = 0.4 μm) for 20 days.
A significant increase in alveolar cord length was observed in rats exposed to 0.5 mg/m^3 (MMAD = 0.44 μm) for 4 months (but not after 8 months of exposure) and after 3 months of recovery. Increased alveolar cord length was

also observed in rats and guinea pigs exposed to 1 mg/m^3 (MMAD = 0.4 μm) for 20 days.
The number of non-ciliated epithelial cells in the bronchioles increased in rats exposed to 0.5 mg/m^3 (MMAD = 0.44 μm) for 4 and 8 months (but not after recovery). Guinea pigs (but not rats) exposed to 1 mg/m^3 (MMAD = 0.4 μm) for 20 days, had hypertrophy and hyperplasia of non-ciliated epithelial cells in the alveoli and bronchioles.
No significant changes could be detected in histological examination of the trachea, bronchial lymph nodes and lungs of rats, which had been exposed to 300 mg/m^3 of diammonium sulphate aerosols (particle size averaged 1-2 μm) for 1-14 days. Rats exposed to 1 or 5 mg/m^3 (MMAD = 0.5-0.6 μm) of diammonium sulphate aerosols for 2-7 days did not show any histological changes of the lungs either.

Pulmonary function

The pulmonary function of rats exposed for 4 months to 0.5 mg/m^3 (MMAD = 0.44 μm) of diammonium sulphate was significantly decreased for a few measured parameters (residual volume/total lung capacity, quasistatic compliance, nitrogen-slope).
Rats exposed to 1.0 mg/m^3 (MMAD = 0.4 μm) of diammonium sulphate aerosols for 20 days had increased values of residual volume and functional residual capacity and a decreased nitrogen-slope.
No significant changes could be detected in pulmonary function of rats, which had been exposed to 300 mg/m^3 of diammonium sulphate aerosols (particle size averaged 1-2 μm) for 1-14 days or in guinea pigs exposed for 20 days to 1.0 mg/m^3 (MMAD = 0.4 μm).

Interactions

Diammonium sulphate exposure at 0.5-1.0 mg/m^3 aggravated the effects induced by elastase pre-treatment in the rats exposed for 20 days, 4 months and 8 months. A synergistic effect of the combination of ozone and diammonium sulphate was observed in rats exposed to diammonium sulphate for 2-7 days at a concentration of 5 mg/m^3 (but not at 1 mg/m^3) (MMAD = 0.5-0.6 μm). The degree of asthmatic dyspnoea (immediate type induced by inhalation of albumin) was dose-dependently increased in guinea pigs exposed to 0.2-2.0 mg/m^3 (MMAD = 0.7-0.9 μm) diammonium sulphate aerosols (only significant at 2 mg/m^3) for 7½ weeks.

Immunological studies of peripheral lymphocytes and spleen cells of rats exposed to 0.5 mg/m^3 (MMAD = 0.44 μm) of diammonium sulphate for 4 months revealed no significant depressive effects on the immune system.

Diarrhoea in the males at the highest dose was the only observed effect in rats fed diammonium sulphate in their diet in doses up to 1975 mg/kg b.w. per day for 13 weeks.

13.4.3 Toxicity to reproduction

No data have been found.

13.4.4 Mutagenic and genotoxic effects

Diammonium sulphate tested negative in Ames tests and in a yeast gene mutation assay with and without metabolic activation systems, and in cytogenetic assays in V79 hamster cells, in human lymphocytes, and in CHO cells.

Addition of diammonium sulphate increased the mutagenicity of a known mutagen (ethylmethanesulphonate).

13.4.5 Carcinogenic effects

Diammonium sulphate inhaled by hamsters at a concentration of 0.2 mg/m^3 for 15 weeks did not affect the carcinogenic potential of the known carcinogen benzo(a)pyrene.

14 Evaluation

The critical effects of diammonium sulphate are the local effects observed in the lungs:
A lot of studies in humans and animals have evaluated the pulmonary effects of exposure to diammonium sulphate aerosols. Statistically small changes in the pulmonary function have been measured in some (but not all of the studies) in both humans and animals. Histological examination of the lungs of exposed rats and guinea pigs revealed transient increased alveolar fibrosis, alveolar cord length, and hypertrophy and hyperplasia of non-ciliated epithelial cells in the alveoli and bronchioles in some (but not all of the studies). In addition, diammonium sulphate seems to potentiate the effect of the bronchoconstrictor carbachol (studied in humans), ozone (studied in humans and rats), albumin used to induce asthmatic dyspnoea (studied in guinea pigs), and elastase used to model chronic pulmonary impairment (studied in rats).
All of the observed effects occurred at doses around 1 mg/m^3 of diammonium sulphate with a MMAD of about 0.5 μm. However, in rats exposed to 300 mg/m^3 of diammonium sulphate with an average particle size of 1-2 μm, no pulmonary changes were observed. The deposition of aerosols in the lungs is dependent on several factors (e.g. particle size and ventilation rate), which might have been different in the different studies and possibly can explain the apparent inconsistency in results.

Upper respiratory irritation was reported in one of the human studies in some of the volunteers exposed to 0.5 mg/m^3 of diammonium sulphate.

Diammonium sulphate was, according to the citations in IUCLID (2000), not irritating to rabbit skin and eyes in the two studies reported. However, the description of the studies is limited (e.g., information on the concentration of the applied chemical is not stated); therefore, the studies are considered inadequate with respect to an evaluation of the irritant potential of diammonium sulphate.

Death following acute exposure to high oral doses of diammonium sulphate has been reported in a human case report and in animal studies.

The only effect observed in rats fed diammonium sulphate in their diet for 13 weeks was diarrhoea in the males in the highest dose group exposed to 1975 mg/kg b.w. per day.

None of the tests for mutagenicity and genotoxicity indicate any potential for diammonium sulphate to possess such properties
No adequate carcinogenic studies with diammonium sulphate have been found.

No data have been found for reproductive and developmental effects.

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Appendix 3: Dimethyl ether (DME)

16 General description

16.1 Identity

Molecular formula:	C_2H_6O
Structural formula:	$H_3C\text{-}O\text{-}CH_3$
Molecular weight:	46.07
CAS-no.:	115-10-6
Synonyms:	Methyl ether Oxybismethane Methoxymethane Methyloxide Dimethyl –13C2 (gas) Wood ether

16.2 Physical / chemical properties

Description:	Colourless, flammable gas with a slight ethereal odour.
Melting point:	-138.5 °C
Boiling point:	- 23.7 °C
Density:	0.669 g/ml (at 20°C)
Vapour pressure:	3982 mmHg (530.8 kPa) (at 20°C)
Concentration of saturated vapours:	-
Conversion factor:	1 ppm = 1.91 mg/m^3 (at 20°C and 760 mmHg) 1 mg/m^3 = 0.523 ppm
Solubility:	Water: 328 g/l (at 20°C)
$LogP_{o/w}$:	- 0,18
References:	A&H (1995), Berkhout (1997), Kirwin & Galvin (1993), IUCLID (2000), Merck Index (1996).

17 Toxicokinetics

Dimethyl ether (DME) is rapidly taken up via the lungs after inhalation or intratracheal administration (Eckard & Kemper 1979 - quoted from A&H 1995).

Pharmacokinetic studies in rats have shown that after inhalation DME is rapidly distributed to various organs and tissues. The steady state level is reached within 30 minutes. No tissue storage of DME was seen. (Daly & Kennedy 1987).

The DME content in various tissues after exposure to 1000 ppm (1900 mg/m^3) in rats is reported to be 14-22 µg/g tissue. The DME concentrations in organs and tissues were reported to fall rapidly after termination of exposure, though somewhat more slowly in fat and muscle than in blood and other tissues. (Bohnenn 1979 - quoted from A&H 1995).

Elimination was described as a two-phase process. The half times in blood were reported to be 10 minutes for the α phase and 90 minutes for the β phase (Daly & Kennedy 1987).

No data on absorption, distribution or elimination of DME after oral intake or dermal contact were found.

17.1 Mode of action

No information is available on the mechanisms of DME in causing CNS effects and other physiological effects in humans or animals.

18 Human toxicity

18.1 Single dose toxicity

18.1.1 Inhalation

Only two very old studies describing the effects of DME to humans have been found.

DME concentrations of 50 % were inhaled by humans in the laboratory (around 500000 ppm (960000 mg/m^3) according to the citation in IUCLID). The gas was most unpleasant to inhale (being distinctly suffocating, even when taken with a high percentage of oxygen). (Brown 1924 - quoted from Kirwin & Galvin 1993, IUCLID 2000).

In human subjects, 50000 and 75000 ppm (96000 and 143000 mg/m^3) of DME caused feelings of mild intoxication after 12 minutes exposure. At 82000 ppm (157000 mg/m^3) some incoordination developed after 21.5 minutes, and a complaint was made of indistinct vision. At 100000 ppm (191000 mg/m^3), no objective symptoms occurred during the first 15 minutes. Distinct signs of incoordination developed after 21 minutes of exposure. The experiment continued for 64 minutes, with the subject being unable to do simple tasks. At 144000 ppm (275000 mg/m^3), symptoms first occurred after 7 minutes with the subject loosing consciousness after 26 minutes. Inhalation of 200000 ppm (382000 mg/m^3) caused unconsciousness within 17 minutes. (Davidson 1925 - quoted from IUCLID 2000).

18.1.2 Oral intake

No data have been found.

18.1.3 Dermal contact

Liquid DME will cause severe frostbite if spilled on the skin (IUCLID 2000).

18.2 Repeated dose toxicity

No data have been found.

18.3 Toxicity to reproduction

No data on reproductive or developmental effects in humans related to DME exposure have been found.

18.4 Mutagenic and genotoxic effects

No data on mutagenic or genotoxic effects in humans related to DME exposure have been found.

18.5 Carcinogenic effects

No data on carcinogenic effects in humans related to DME exposure have been found.

19 Animal toxicity

19.1 Single dose toxicity

19.1.1 Inhalation

The LC_{50}-value in rats was reported to be 164000 ppm (313000 mg/m^3) after 4 hours of exposure (Daly & Kennedy 1987).

At exposure levels of 10000 ppm (19000 mg/m^3), rats showed slight sedation and at 50000 ppm (96000 mg/m^3) rats were asleep most of the time for exposures longer than 30 minutes (Daly & Kennedy 1987).

The LC_{50}-value in mice was reported to be 490000 ppm (936000 mg/m^3) after 15 minutes of exposure and 380000 ppm (~726000 mg/m^3) after 30 minutes exposure (Daly & Kennedy 1987).

The exposure to 120000 ppm (229000 mg/m^3) for 20-25 minutes was reported to cause light narcosis in mice (Meyer & Gottlieb-Billroth 1921 - quoted from A&H 1995).

Besides effects on the central nervous system (CNS) and the blood profile, DME was reported to cause weak cardiac sensitisation in dogs exposed for 5 minutes to 200000 and 300000 ppm (382000 and 573000 mg/m^3) DME, but not at 100000 ppm (~191000 mg/m^3) (Daly & Kennedy 1987).

19.1.2 Oral intake

No data have been found.

19.1.3 Dermal contact

No data have been found.

19.1.4 Other routes

Intraperitoneal doses of 5 mg/kg of DME produced reversible anaesthesia in mice (no further details are given) (Gosselin et al. 1984).

19.2 Repeated dose toxicity

19.2.1 Inhalation

Rats exposed to up to 50000 ppm (96000 mg/m^3) of DME (6 hours/day, 5 days/week, 2 weeks) showed the following effects: At 10000 ppm (19000 mg/m^3), slight sedation. At 50000 ppm (96000 mg/m^3), sedation, body weight gain suppression, minor haematological changes (a small increase in

the number of leucocytes and perhaps a decrease in the number of red blood cells), and organ weight changes (in relative liver and kidney weights), but no histopathological organ changes. All changes seen during the two-week exposure period were completely reversed following a two-week recovery period. (Daly & Kennedy 1987).
In the same study, 4-week inhalation exposures of DME to rats and hamsters were conducted. Rats were exposed to up to 10000 ppm (19000 mg/m^3) and hamsters to up to 20000 ppm (38000 mg/m^3). There were no toxicological changes seen and particularly no signs of sedation in the hamsters exposed to 20000 ppm (38000 mg/m^3) (Daly & Kennedy 1987).

In a 4-week study, rats were exposed to DME at concentrations of 1, 100, 1000, or 10000 ppm (1.9, 190, 1900, or 19000 mg/m^3) for 6 hours/day, 5 days/week. Observations were made of behaviour, growth, food intake, haematology, urine composition, organ weights, and gross as well as microscopic pathology. None of the parameters investigated revealed any distinct treatment related effects. (Kruysse 1976 - quoted from IUCLID 2000).

In a study in which rats were exposed to DME (0, 2000, 10000, or 20,000 ppm - 0, 3800, 19000, or 38000 mg/m^3) for up to 13 weeks, 6 hours/day, 5 days/week, altered blood profile (reduced percentage of lymphocytes and increase of neutrophilic white blood cells) was noted at the highest dose level (20000 ppm- 38000 mg/m^3). Male rats of the top-level group showed a minimal, though statistically significant, increase in serum glutamate pyruvate transaminase (SGPT) activity. Total serum protein was slightly, but statistically significantly lower in females of the top-level group than in females of the control group. The reporters did not consider these changes biologically significant and thus, it was concluded that exposure of rats to 20000 ppm (38000 mg/m^3) under the conditions of this study produced no effects of obvious toxicological significance. This conclusion was supported by the absence of chemically related significant gross toxic signs or abnormal histopathology. (Reuzel et al. 1981)

In a second 13-week study by Reuzel & Woutersen (1983 - quoted from IUCLID 2000), rats were exposed to DME at concentrations of 0, 1000, 5000, 10000 or 20000 ppm (0, 1900, 9600, 19000, or 38000 mg/m^3). Health condition, behaviour and body weight did not change due to DME exposure. No toxicologically relevant significance was ascribed to a temporary difference in white blood cell counts in males. Neutrophil counts were higher in male rats at all test levels. A dose-effect relation could not be found. In view of the fact that increased neutrophil counts were also found in previous sub-chronic study, it was stated in the final conclusion that the no-effect level in rats was 10000 ppm (19000 mg/m^3).

In a 13 week study in hamsters exposed to 0, 1000, 5000, 10000, or 20000 ppm (0, 1900, 9600, 19000, or 38000 mg/m^3) for 6 hours/day, 5 days/week, the following observations were made: Exposures did not affect health condition, behaviour and body weights of hamsters. White blood cell counts and in particular absolute lymphocyte counts had decreased both in male and female hamsters, which had been exposed to 20000 ppm (38000 mg/m^3). The difference concerning the absolute white blood cell counts was not statistically significant. The difference in absolute lymphocyte counts was only significant on day 56. A number of other measured haematological

values (no further information given) showed a significant difference compared with values found in the animals of the control group. For several reasons the investigators considered these differences to be of no importance. The investigators concluded that the no-effect level for DME in hamsters was 5000 ppm (9600 mg/m^3), as at concentrations of 20000 and 10000 ppm (38000 and 19000 mg/m^3) a decrease in white blood cell counts and lymphocyte counts occurred. (Reuzel & Woutersen 1983 - quoted from IUCLID 2000).

In a 30-week study, rats were exposed to 0.02%, 0.2%, or 2% v/v (0, 200, 2000, or 20000 ppm – 0, 380, 3800, or 38000 mg/m^3) of DME in air, 6 hours/day, 5 days/week. There was no evidence that exposure to DME at any dose level had a toxicologically significant effect on body weight or food consumption. There were no toxicologically significant clinical signs in animals from any of the groups during the 30 weeks of the experiment nor were there any significant ophthalmoscopic findings. (Collins et al. 1978).
The only abnormalities in blood chemistry concerned the SGPT and SGOT (serum glutamate oxaloacetate transaminase) levels. At 24 weeks, there were abnormally high SGPT values in a few rats in both the male and female high-dose groups, suggesting the possible onset of a hepatotoxic effect of DME. At week 27, SGPT levels showed no differences between groups for either sex. However, at the end of the study there was a statistically significant increase in SGPT for both males and females of the high dose group (20000 ppm - 38000 mg/m^3) when compared with the control group. There was a statistically significant increase in SGOT levels in the male medium dose group (2000 ppm- 3800 mg/m^3).
The only organ weight measurement that indicated a toxicological effect was that of the liver of the high dose male rats. There was a statistically significant reduction in liver weight relative to body weight in this group compared with the control group ($P < 0.05$). The unusual event, that decreased liver weights are accompanied with increased GPT levels, was explained as a possible hepatic fibrosis, which was insufficiently severe to be detected by routine histopathological examination. There were no histopathological changes seen that were considered to be related to exposure of the rats to DME.

Rats were exposed to DME by inhalation for 104 weeks, 6 hours/day, 5 days/week. The concentrations were 2000, 10000, or 25000 ppm (3800, 19000, or 48000 mg/m^3). There was no specific tissue damage, as indicated by either clinical function studies or morphologic studies of the tissues examined at the end of the study. Haematology and clinical pathology studies conducted at three-month intervals during the two years showed no evidence of change. Female animals in both the 10000 and 25000 ppm (19000 and 48000 mg/m^3) groups showed a slight decrease in mean survival time. The decrease was not statistically significant, but it was different from that seen either at the low level or in the control subjects. According to the authors, the no-effect level was 20000 ppm (38000 mg/m^3). (Daly & Kennedy 1987).
It should be noted that the no-effect level concluded by the authors is not one of the concentrations used in the study.

19.2.2 Other routes

No information on repeated dose toxicity following oral, dermal or other routes of exposure were found.

19.3 Toxicity to reproduction

19.3.1 Inhalation

Two developmental studies have been reported for DME.

In the first study, female rats were exposed to concentrations of 0, 1250, 5000, 20000, or 40000 ppm (0, 2400, 9600, 38000, or 76000 mg/m^3) of DME, 6 hours/day, from days 6 to 15 of gestation. Animals exposed to 5000 ppm (9600 mg/m^3) or more showed some evidence of narcotic effect, somewhat proportional to dose. At a concentration of 40000 ppm (76000 mg/m^3), these female animals showed a suppression of body weight gain. There was no evidence of any teratogenic effect on the foetuses. There was no increase in foetal resorption. At concentrations of 20000 and 40000 ppm (38000 and 76000 mg/m^3), there was some evidence that the foetuses were somewhat smaller, and that foetal variations such as ossification of the rib bones and some of the phalangeal bones in the extremities of these animals was somewhat retarded. These findings the reporters considered as variations reflecting developmental delay rather than a specific effect on the foetus. (Daly & Kennedy 1987).

In the second study, female rats were exposed to concentrations of 0, 20000, or 28000 ppm (0, 38000, or 53000 mg/m^3) of DME from days 6 to 15 of gestation. The researchers did not find any signs of narcosis in the maternal animals. Maternal body weight gain was normal. There was a slight increase in the number of extra ribs in the foetuses at both levels tested; the increase was statistically significant, yet there was no dose-response. No gross malformations were noted in these offspring, and embryo mortality was not reflected by increased resorptions. (Daly & Kennedy 1987).

19.3.2 Other routes

No information on reproductive and developmental effects following oral, dermal or other route of exposure were found.

19.4 Mutagenic and genotoxic effects

19.4.1 *In vitro* studies

DME was not mutagenic when tested in the Ames test with and without metabolic activation in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 at a concentration of 119000 ppm (Willems 1978 - quoted from IUCLID 2000).

Mutagenic effect of DME could not be shown in V79 Chinese hamster cells in suspension with 230, 460, 1150 and 3450 mg/l DME (Kramers et al 1981 - quoted from IUCLID 2000).

No induction of DNA-repair synthesis was observed at any dose level when primary rat liver cells were exposed to 230, 460, 1150, 2300, or 3450 mg/l DME (Kramers et al. 1981 - quoted from IUCLID 2000).

19.4.2 *In vivo* studies

Progeny examination led to the conclusion that DME was not mutagenic under the test conditions when Drosophila melanogaster was exposed to 8000 or 28000 ppm (15000 or 53000 mg/m^3) for 3 days or 28000 ppm (53000 mg/m^3) for 14 days (Kramers et al. 1981 - quoted from IUCLID 2000).

In the host mediated assay in mice, exposure to 10000 or 20000 ppm (19000 or 38000 mg/m^3) for 3 hours gave negative results (DGF 1992 - quoted from A&H 1995).

19.5 Carcinogenic effects

In a 104-week study in rats exposed to 2000, 10000, or 25000 ppm (3800, 19000, or48000 mg/m^3) of DME, there was no evidence of increased tumour formation in any of the tissues or organs of the animals (Daly & Kennedy 1987).

No data on carcinogenic effects following oral, dermal or other route of exposure were found.

20 Regulations

20.1 Ambient air

Denmark (C-value): 1 mg/m^3 (MST 2002).

20.2 Drinking water

Denmark: -

20.3 Soil

Denmark: -

20.4 Occupational Exposure Limits

Denmark: 1000 ppm (1885 mg/m^3) (At 2002).

Germany: 1000 ppm (1910 mg/m^3) (MAK 1993).

EU-SEG: 1000 ppm (1910 mg/m^3) (SEG 1991).

20.5 Classification

DME is classified as extremely flammable (Fx;R12) (MM 2002).

20.6 IARC

-

20.7 US-EPA

-

21 Summary

21.1 Description

Dimethyl ether (DME) is a colourless gas at room temperature and with a slight ethereal odour. The substance has a very high vapour pressure, and is soluble in water.

21.2 Toxicokinetics

DME is rapidly taken up after inhalation and distributed to various organs and tissues, where a steady state level is reached within 30 minutes. After end of exposure, the concentration of DME in organs and tissues falls very rapidly again. The elimination is described as a two-phase process. No tissue storage is seen.
No data on absorption, distribution or elimination of DME after oral intake or dermal contact were found.

21.3 Human toxicity

The only effects described after DME exposure to humans originate from very old studies, where human subjects have been exposed to very high acute doses of DME. The target organ after exposure to high concentrations of DME is the central nervous system, covering effects from incoordination, indistinct vision, and inability to do simple tasks and to unconsciousness (exposure levels from 75000 to 200000 ppm - 143000 to 382000 mg/m^3).

No information on toxic effects in humans following repeated dose exposure was found. Likewise no information on reproductive or developmental effects, as well as on mutagenic, genotoxic or carcinogenic effects in humans was found.

21.4 Animal toxicity

21.4.1 Single dose toxicity

The LC_{50}-values for DME in mice have been reported to be 490000 ppm (936000 mg/m^3) after exposure in 15 minutes and 380000 ppm (726000 mg/m^3) after exposure in 30 minutes. In rats, the LC_{50}-value has been reported to be 164000 ppm (313000 mg/m^3) after 4 hours exposure. The effects of DME in rats exposed to sub-lethal doses range from sedation to narcosis.
In dogs exposed to 200000 and 300000 ppm (382000 and 573000 mg/m^3), DME has been reported to be a light cardiac sensitiser.

21.4.2 Repeated dose toxicity

Short-term studies (2 weeks) in which rats were exposed to concentrations of DME of 50000 ppm (96000 mg/m^3) caused sedation, body weight gain

suppression, haematology and organ weight changes, but no histopathological organ changes. All changes were completely reversed after cessation of exposure.

In sub-chronic studies (13 or 30 weeks) with exposure of DME up to 20000 ppm (38000 mg/m^3) in rats and hamsters, the reported effects focus on changes in haematological parameters (white blood cells) for both rats and hamsters, and an increase of serum SGPT and SGOT values in rats, the last suggesting a possible onset of a hepatotoxic effect of DME. By histopathological examinations, no effects were observed in the liver. The no-effect level for haematological effects in the 13-week studies was reported to be 10000 ppm (19000 mg/m^3) for rats and 5000 ppm (9600 mg/m^3) for hamsters. In the 30-week study on rats, the no-effect level for increased levels of SGPT was reported to be 2000 ppm (3800 mg/m^3) and for increased levels of SGOT 200 ppm (380 mg/m^3).

In a lifetime study in rats exposed to concentrations up to 25000 ppm (48000 mg/m^3) of DME, the only effect reported was a decrease in mean survival time for female rats from 10000 ppm (19000 mg/m^3) . The decrease was not statistically significant, but different from that seen in the low dose group (2000 ppm - 3800 mg/m^3) and the control group. The no-effect level was reported to be 20000 ppm (38000 mg/m^3).

21.4.3 Toxicity to reproduction

In a developmental study, female rats were exposed to concentrations of 20000 or 28000 ppm (38000 or 53000 mg/m^3) of DME from days 6 to 15 of gestation. The researchers found a statistically significant increase in the number of extra ribs in the foetuses at both dose levels, these findings was not accompanied by maternal toxicity at any of the dose groups. There were no signs of teratogenicity or embryo mortality.

In another developmental study, with exposures of 0, 1250, 5000, 20000, or 40000 ppm (0, 2400, 9600, 38000, or 76000 mg/m^3), to female rats from day 6 to 15 of gestation, the female animals showed evidence of narcotic effect from 5000 ppm (9600 mg/m^3) and suppressed body weight gain at 40000 ppm (76000 mg/m^3). There was no evidence of teratogenic effects or increase in foetal resorptions. Retarded ossification of the rib bones and some of the phalangeal bones in the extremities of the foetuses was considered as variations reflecting developmental delay rather than a specific effect on the foetuses.

21.4.4 Mutagenic and genotoxic effects

DME showed no signs of a mutagenic or genotoxic potential in 3 *in vitro* and 2 *in vivo* test systems.

21.4.5 Carcinogenic effects

In a lifetime study in rats exposed to DME at concentrations up to 25000 ppm (48000 mg/m^3), there was no increase in cancer in any of the tissues or organs of the animals.

22 Evaluation

As DME is a gas at ambient temperature, the main exposure routes for humans seem to be by inhalation and accidental dermal contact occurring from the use of DME as a carrier of active substances in aerosols.

The critical target organ at acute high concentrations is the CNS resulting in a narcotic effect. No contemporary human data are available in relation to CNS changes such as neurobehavioral disturbances.

Based on the limited and old data on human exposure to dimethyl ether it is not possible to estimate levels for the narcotic effects in humans.
The structurally related substance – diethyl ether – has been more extensively investigated according to narcotic effects, where exposure to concentrations of about 100000 ppm (308000 mg/m^3) induces anaesthesia, and about 15000 ppm (46200 mg/m^3) is the lowest anaesthetic concentration. (Strube 1995).

Available animal studies show a low order of acute and chronic toxicity, and any capability of dimethyl ether in being a genotoxic, carcinogenic or developmental toxicant has not been demonstrated.

Repeated dose toxicity studies have shown some effects of high concentrations of dimethyl ether on the liver (higher SGPT (= alanine amino transferase ALAT) and SGOT (= aspartate amino transferase (ASAT) values suggesting a possible onset of a hepatotoxic effect) and changes in white blood cell counts. The no-effect level in subchronic studies for haematological effects was reported to be 10000 ppm (19000 mg/m^3) for rats and 5000 ppm (9600 mg/m^3) for hamsters. In a 30-week study on rats, the no-effect level for increased levels of SGPT was reported to be 2000 ppm (3800 mg/m^3) and for increased levels of SGOT 200 ppm (380 mg/m^3). In a life-time study in rats, the no-effect level was stated to be 20000 ppm (38000 mg/m^3); haematological investigations showed no evidence of changes, whereas no information is given whether biochemical parameters (as e.g., SPGT and SGOT) were investigated.
Overall, the no-effect level for effects of dimethyl ether in repeated dose toxicity studies is considered to be 2000 ppm (3800 mg/m^3) based on the increased levels of SPGT observed at higher concentrations in the subchronic studies.

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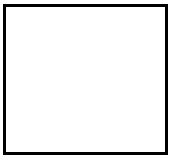
Appendix 4: Hexamethylenetetramine

24 General description

24.1 Identity

Molecular formula: $C_6H_{12}N_4$

Structural formula:



Molecular weight: 140.19

CAS-no.: 100-97-0

Synonyms: Ammonioformaldehyde
Formamine
Hexamethyleneamine
Hexamine
Hexilmethylenamine
HMT
HMTA
Methenamine
1,3,5,7-Tetraazaadamantane
1,3,5,7-Tetraazatricyclo(3.3.1.1(3,7))decane

24.2 Physical / chemical properties

Description: Hexamethylenetetramine occurs as hygroscopic and colourless crystals or as a white crystalline powder with no or mild ammonia odour.

Melting point: Sublimates at about 263°C without melting and with partial decomposition to mainly formaldehyde and ammonia.

Boiling point: Sublimates.

Density: 1.27 g/ml (at 25°C).

Vapour pressure: 0.004 mmHg (0.53 Pa) (at 25°C).

Concentration of saturated vapours: 5.3 ppm (calculated) at 25°C and 760 mmHg.

Conversion factor: 1 ppm = 5.83 mg/m^3 20°C
1 mg/m^3 = 0.17 ppm 1 atm

Solubility:	Water: 449 g/l (at 12°C).
References:	ChemFinder (2001), CIR (1992), Dreyfors et al. (1989), HSDB (2000), ICSC (1993), Loeper & Berzins (1995), Merck Index (1996).

25 Toxicokinetics

25.1 Absorption

Hexamethylenetetramine is rapidly absorbed in humans following oral administration. However, from 10 to 30 % of an orally administered dose of hexamethylenetetramine is hydrolysed in the gastric fluid to yield formaldehyde and ammonia. (CIR 1992, Loeper & Berzins 1995).

25.2 Distribution

Hexamethylenetetramine is distributed to blood and organs (not further specified) (Loeper & Berzins 1995).

It can pass the placenta and is detectable in the amniotic fluid and in milk (CIR 1992).

25.3 Elimination

The half-life for hexamethylenetetramine in 10 healthy volunteers administered 2 g per day for 8 days of two different formulations of tablets was about 4 hours. Following a single dose, approximately 82 % of the hexamethylenetetramine was recovered in the urine in 24 hours as the parent compound. During administration twice daily for 8 days, approximately 88 % of the dose was excreted as the parent compound within a 12-hour dosing period. (Klinge et al. 1982 – quoted from CIR 1992).

Part of hexamethylenetetramine is decomposed to formaldehyde and ammonia in the urine. The half-life of conversion of hexamethylenetetramine to formaldehyde is pH-dependent (about 20 hours at pH 5.0 and about 400 hours at pH 6.5). (Grady Strom & Won Jun 1993 – quoted from Loeper & Berzins 1995).

In slightly acidic environments, such as in sweat, hexamethylenetetramine partially degrades to formaldehyde and ammonia. (Loeper & Berzins 1995). In addition, hexamethylenetetramine has been reported to produce formic acid upon contact with the skin (Dreyfors et al. 1989).

25.4 Mode of action

No relevant data have been found.

26 Human toxicity

26.1 Single dose toxicity

An accidental overdose of 8 g of hexamethylenetetramine-mandelate (corresponding to a dose of 384 mg/kg b.w. of hexamethylenetetramine in a 10 kg child), which was ingested by a 2½-year-old boy, caused haemorrhagic cystitis (inflammation of the bladder) and mild azotaemia (increased concentration of nitrogen in the blood). The boy recovered without specific treatment. (Ross & Conway 1970 – quoted from Loeper & Berzins 1995).

26.2 Repeated dose toxicity

26.2.1 Inhalation

A cross-sectional study was performed with 33 employees of a hexamethylenetetramine producing plant to assess the health effects of hexamethylenetetramine on the airways and the skin of the workers. Sixteen of the employees (blue and white collar workers) with no or occasional low exposure to hexamethylenetetramine served as controls while 17 employees (baggers, shiftleaders, and executive staff) with exposure to far higher concentrations served as the exposed group. In addition, 4 out of 5 employees that had left the production for medical reasons during the last 10 years were included in the study. For each worker, anamnestic data were recorded and a physical examination of the skin and lungs was performed. In addition, total and specific IgE to four environmental allergens was measured, lung function and bronchial hyperresponsiveness was assessed, and skin prick tests and patch tests with known sensitising substances as well as with hexamethylenetetramine were performed. Measurements were made at different sites in the plant and/or in personal air space of inhalable dust, respirable dust, hexamethylenetetramine, formaldehyde, and ammonia to assess the exposure level.
All measured concentrations of dust and chemicals were in the range of 0.2 – 2.6 mg/m^3. Geometric mean hexamethylenetetramine concentrations as assessed by personal sampling were 0.3 mg/m^3 in shiftleaders and 0.6 mg/m^3 in baggers.
Irritant dermatitis of the hands, predominantly on the palmar parts, was observed in all highly exposed subjects, but also (to a lower extent) in two controls. No other differences were found between the exposed and the control group. Two workers in the exposed group and one in the control group had work-related symptoms. Of the two exposed workers, one person reported shortness of breath, rhinoconjunctivitis and dermatitis of the hands, face and neck that occurred after “accidents with high formaldehyde exposure”. The second worker had pre-existing hay fever and seasonal asthma and gave a history of work-related shortness of breath and rhinitis but he had a normal lung function and no bronchial hyperresponsiveness. One control worker reported work-related conjunctivitis, probably due to long-lasting computer work. No sensitisation to hexamethylenetetramine as assessed by skin prick and patch tests were found in exposed or control workers.

Two out of the four ex-workers (both former baggers) had a positive patch test (but a negative skin prick test) to hexamethylenetetramine. These two former workers had a negative patch test to formaldehyde and no airway symptoms. They reported eczema (located at exposed skin areas in one person and generalised in the other) and conjunctivitis during exposure after 2 weeks or 7 months but were free of symptoms after removal from exposure. The two former workers that tested negative in the patch tests, experienced eczema of the neck "shortly" after exposure and recurrent swelling of eyelids and wrists after one year, respectively. At the time of the study they both presented with eczema, although improved. (Merget et al. 1999).

Seven workers in the lacquer or plastics industries that had worked around epoxy resins, plastics, or paint developed asthma and other allergic symptoms (allergic coryza (nasal catarrh), contact dermatitis, allergic conjunctivitis). An intracutaneous skin test with 0.02 ml of a 1:100 dilution of hexamethylenetetramine gave positive reactions in all workers. The positive reaction was characterised by immediate wheal formation. In a provocative test the workers were inhaling the lacquer product in aerosol form and showed either 1) wheezing and heaviness on the chest or severe asthma, 2) allergic coryza, or 3) skin manifestations of allergy. Some of the workers also had positive reactions to ethylenediamine in both tests. It is unclear from the article which other chemicals the workers might have been exposed to. (Gelfand 1963).

Workers in a tire manufacturing plant were exposed daily to a hexamethylenetetramine-resorcinol mixture (which comprised 2-3 % of the total rubber mix) as well as to their reaction products which were thought to include formaldehyde, ammonia, cyanides and curatives. Fifty-two exposed workers completed a questionnaire and had their lung function measured. The workers suffered from acute symptoms such as itching, skin rashes, coughing, chest tightness, burning eyes and impaired breathing. The excess of symptoms (in comparison with a non-exposed group of workers) persisted when the effects of smoking and drinking were accounted for. Significant reductions in expiratory flow rates at low lung volumes were reported, indicating increased resistance in the small airways. (Gamble et al. 1976).

Workers in foundry where a moulding process utilizes a phenol-formaldehyde resin and hexamethylenetetramine as catalyst, suffered from nasal symptoms such as sneezing, rhinorrhoea, and obstruction as well as wheezy breathing. Twenty of 46 workers in the core shop or casting areas reported asthma or wheezy breathing. Of these, 11 developed the symptoms after starting work at the foundry. Workers involved in other working processes less frequently reported these symptoms. Most environmental measurements of chemical contaminant were below the threshold limits. However, the concentration of furfuryl alcohol (50 ppm in air) and formaldehyde (4 ppm in air) was above the limits in the general foundry. The authors suggest that both irritant and hypersensitivity mechanisms are present because of the onset of symptoms in relation to exposure to fumes and vapours. (Low & Mitchell 1985 – quoted from Loeper & Berzins 1995).

26.2.2 Oral intake

When hexamethylenetetramine is given orally at doses greater than 500 mg four times per day, gastrointestinal distress occur. Bladder irritation, painful and frequent micturition (urination), albuminuria, haematuria, and various rashes may result from doses of 4-8 g a day for longer than 3-4 weeks. (Goodman & Gilman 1975 – quoted from Loeper & Berzins 1995).

Adverse effects have been reported in less than 3.5 % of patients receiving hexamethylenetetramine and its salts as a drug. The most frequent adverse effect is gastrointestinal disturbances, including nausea, vomiting, diarrhoea, abdominal cramps, and anorexia. Rarely, hypersensitivity reactions including rash, pruritus, urticaria, and stomatitis have occurred. Other adverse effects reported rarely are headache, dyspnoea, generalized oedema, tinnitus, muscle cramps, dysuria, and microscopic or gross haematuria. (McEvoy 1997 – quoted from HSDB 2000).

26.2.3 Dermal contact

A maximization test has been performed using 25 adults, 8 men and 17 women, to assess the potential for contact sensitisation of a mascara, which contained 0.1 % hexamethylenetetramine. A pre-test using 25 adults who got an occlusive patch of test material applied to the volar aspect of the forearm for 48 hours showed that the test material was non-irritating. For induction, 0.3 g of test material was applied under an occlusive patch to the volar aspect of the forearm and was covered with a 15 mm aluminium chamber for five 48-hour periods. Sodium lauryl sulphate, 1 %, was used during the induction because the test material was found to be non-irritating during the pre-test. Following a 10-day non-treatment period a challenge was performed by applying an occlusive patch to a new site for 48 hours using the same procedure as for the induction. However, this time a 5 % aqueous solution of sodium lauryl sulphate was used. Observations were made upon removal of the patch and after 24 hours. No signs of sensitisation were observed following the challenge. (Ivy Research Laboratories Inc. 1980 – quoted from CIR 1992).

Hexamethylenetetramine has been reported to cause allergic eczema in rubber workers. After exposure to hot rubber containing 0.1 % hexamethylenetetramine, 60 workers suffered from acute dermatitis with itching and redness of exposed skin. Removal of the chemical from all rubber stock prevented further cases. (Cronin 1924 – quoted from IUCLID 2000).

Seven (2.0 %) out of 357 tested patients remitted to an occupational dermatology clinic in Finland during a 6-year period had a positive allergic patch test reaction to 2 % hexamethylenetetramine. In the same period 5.8 % (82/1414) were positive to 1 % formaldehyde. The study is not stating whether any cross-reaction between hexamethylenetetramine and formaldehyde was observed. (Kanerva et al. 1999).

A similar result was reported in another study where 1.9 % of 309 tested patients had a positive reaction to 2 % hexamethylenetetramine and 6.2-8.7 % of tested patients had a positive reaction to 1-2 % formaldehyde (Holness & Nethercott 1997).

One case report exists of a worker who had a positive patch test to hexamethylenetetramine but who tested negative to formaldehyde. The worker developed itchy eruptions, which were aggravated by sweating, on his hands, neck, and shoulders. When tested, negative results were obtained for a range of chemicals tested in open patch tests on the upper arm for 20 minutes. In closed patch tests, chemicals were applied for 24 hours and readings were taken 1 and 24 hours after removal. Only 1 % hexamethylenetetramine in petrolatum produced a positive reaction with erythema and papules. For 2 % aqueous formaldehyde a questionable positive result was observed at the 48-hour reading but at the 72-hour reading the result was negative. Eight months later a retest was performed using hexamethylenetetramine and formaldehyde. At this test a negative result was obtained for formaldehyde. As a negative control, hexamethylenetetramine tested negative in 9 colleagues. Positive controls for formaldehyde were performed using formaldehyde-sensitised patients. (Hayakawa et al. 1988 – quoted from CIR 1992, and Loeper & Berzins 1995).

Hexamethylenetetramine has been reported as allergenic not only in occupational use but also in contact with finished rubber objects (Fregert 1981 – quoted from Loeper & Berzins 1995).

Patients sensitised by external exposure to formaldehyde have experienced dermatitis when exposed dermally to hexamethylenetetramine in rubber dress shields or when ingesting it as a drug (Fisher 1978, Sulzberger 1940 – both quoted from CIR 1992).
Positive patch test reactions have occurred in persons who were sensitised to formaldehyde when they were tested with hexamethylenetetramine (Fisher 1986 – quoted from Loeper and Berzins 1995).
Contact dermatitis was also observed when ethylenediamine sensitive persons were patch tested with hexamethylenetetramine (Balato et al. 1986 – quoted from Loeper & Berzins 1995).

See also the study performed by Merget et al. (1999) described in section 3.2.1 Inhalation.

26.2.4 Other routes

No data have been found.

26.3 Toxicity to reproduction

In a surveillance study conducted between 1985 and 1992 of Michigan Medicaid recipients involving 229,101 completed pregnancies, 209 newborns had been exposed to hexamethylenetetramine during the first trimester. Eight (3.8 %) major birth defects were observed. Nine major birth defects were expected. (Briggs et al. 1994 – quoted from HSDB 2000).

No congenital abnormalities were observed in the children of 3 women who had taken hexamethylenetetramine as well as 5 other drugs (choleinic sodium, phenolphthalein, papaverine HCL, methylhomatropine, and menthol) during the first two weeks of pregnancy (Siffel & Czeizel 1995).

26.4 Mutagenic and genotoxic effects

Urine samples were collected from 72 men, 44 of whom smoked, who worked in a tire plant where hexamethylenetetramine was a workplace pollutant. Twenty-three clerks, 16 of whom smoked, were used as controls. Urine concentrates equivalent to 10 ml of urine were tested for mutagenicity in the Ames test with metabolic activation in *Salmonella typhimurium* strains TA 98, TA100, and TA1535, and in a microtitre fluctuation test. A smoking related increase in mutagenicity was observed in strain TA98. Hexamethylenetetramine was not mutagenic in this study. (Crebelli et al. 1984, 1985 – quoted from CIR 1992 and Loeper & Berzins 1995).

26.5 Carcinogenic effects

An increase in intestinal, lung, bladder, and skin cancer as well as leukaemia has been reported among 13570 men who had worked for at least 5 years at one company in rubber making, especially in areas where the rubber ingredients were mixed and compounded. Hexamethylenetetramine was used as an accelerator in the process in this plant. However, the mixture consisted of several antioxidants and other accelerators with suspected carcinogenic properties. (Monson & Fine 1978 – quoted from IUCLID 2000 and Loeper & Berzins 1995).

The mortality from cancer was increased in a cohort of 632 Danish moulders, mainly because of excess deaths from bladder cancer. Hexamethylenetetramine is added as a catalyst to some synthetic resin moulds. However, the workers have been exposed to a mixture of chemicals including carbon monoxide, nitrogen oxides, hydrogen cyanide, ammonia, amines, aldehydes, phenols, benzene, benzoic acid, toluene, cresols, methane, ethylene, acetylene, and various polycyclic aromatic hydrocarbons (of which some are carcinogenic). (Hansen 1991).

27 Animal toxicity

27.1 Single dose toxicity

27.1.1 Inhalation

No data have been found.

27.1.2 Oral intake

The oral LD_{50}-values reported for hexamethylenetetramine range from 9200 to higher than 20000 mg/kg b.w. for rats (3 values reported) and 1853 mg/kg b.w. for mice (CIR 1992, IUCLID 2000, JECFA 1974, Loeper & Berzins 1995).

27.1.3 Dermal contact

No data have been found.

27.1.4 Other routes

The LD_{50}-values reported for hexamethylenetetramine range from 215 mg/kg b.w. for mice injected subcutaneously and from 9200 to higher than 10000 mg/kg b.w. for rats injected intravenously. In mice, toxicity symptoms observed after intraperitoneal and subcutaneous administrations were trembling, weakness of the hind quarters, and terminal convulsions. (CIR 1992, IUCLID 2000, JECFA 1974, Loeper & Berzins 1995).

27.2 Repeated dose toxicity

27.2.1 Inhalation

No data have been found.

27.2.2 Oral intake

Groups of 27-102 of each sex of three different strains of mice (CTM, C3Hf/Dp and SWR/Dp) and groups of 48 of each sex of Wistar rats received 0 or 1.0 % hexamethylenetetramine in their drinking water. The calculated daily intake equal to 1 % was 2500 mg/kg b.w. for mice and 1500-2500 mg/kg b.w. for rats according to Loeper & Berzins 1995. The mice were treated for 60 weeks and the rats for 104 weeks. One group of 50 CTM mice per sex was given 0.5 % hexamethylenetetramine for 60 weeks, and another group of 29-50 of each sex received 5 % hexamethylenetetramine for 30 weeks. One group of 12 rats of each sex were given 5 % hexamethylenetetramine for 2 weeks. After the termination of treatment, the animals were observed for the remainder of their lifetimes. Necropsy was performed on all animals, and tissues and lesions taken at necropsy were evaluated microscopically. A yellow discoloration of the furs of treated rats (but not of treated mice) was observed. The SWR mice that received 1 %

hexamethylenetetramine and the CTM mice that received 5 % hexamethylenetetramine had a slight retardation of growth. A slight reduction in survival was observed for the CTM mice dosed with 5 % hexamethylenetetramine. The same dose caused 50 % mortality in the rats. Rats from the 5 % group that did not die recovered rapidly and did not have any lasting ill effects. (Della Porta et al. 1968 – quoted from CIR 1992, JECFA 1974 and Loeper & Berzins 1995).

Groups of 5 or 15 BD II rats of each sex were administered 400 mg per day (equivalent to 1000 mg/kg b.w. per day assuming a rat weight of 400 g) of hexamethylenetetramine by gavage for 90 or 333 days. The only substance related effect was a yellow discolouration of the fur. No macroscopic changes in organ histology or in body weights could be detected. (Brendel 1964 – quoted from CIR 1992, IUCLID 2000, JECFA 1974 and Loeper & Berzins 1995).

Groups of 16 Wistar rats of each sex were fed a standard diet containing either 0 or 0.16 % (equal to an average intake of 0 or 100 mg/kg b.w. per day according to CIR 1992) of hexamethylenetetramine for life. A yellow staining of the hairs of the perineum was observed in one male and three females in the test group. The average life span was 6-9 % longer for controls than for the dosed animals. This difference was associated with slightly lower terminal body weights in the test group, especially for males. No significant differences in body weight, voluntary muscular activity, and relative organ weights of the liver, kidneys, adrenal glands or gonads were observed between the control and treatment group. The majority of deaths in controls as well as dosed rats were attributed to pneumonia. (Natvig et al. 1971 – quoted from CIR 1992and JECFA 1974).

The yellow discolouration of the fur of rats exposed to hexamethylenetetramine orally has been explained as a result of a reaction between formaldehyde present in the urine from treated rats and kynurenine which is a normal constituent of rat hair (Kewitz & Welsch 1966 – quoted from CIR 1992 and IUCLID 2000).

Groups of 2-3 cats of each sex were fed hexamethylenetetramine in the diet at doses of 0 or 50000 ppm (equal to 1250 mg/kg b.w. per day) for 2 years. One female in the dosed group died of a pyrogenic infection of the nasal cavity and paranasal sinuses in the twenty-third month. No differences between control and dosed cats were found in feed consumption, weight gain, appearance, or histology of tissues. (Kewitz 1966 – quoted from CIR 1992 and JECFA 1974).

27.2.3 Dermal contact

Groups of 6 male rabbits had 0 or 2 ml of a 0.20 % solution of hexamethylenetetramine in distilled water applied to the skin for 5 days a week for 6 weeks. The application was not under occlusive patches. General behaviour, hair growth, and weight gain of the rabbits was the same in the control and the test group. No skin irritation was observed. (COLIPA 1989 – quoted from CIR 1992).

Six male rabbits had 0.5 ml of a 0.20 % solution of hexamethylenetetramine in distilled water topically applied to both intact and abraded sites on the

flanks for 24 hours under occlusive patches. The rabbits were observed for 72 hours. Slight skin irritation was observed. (COLIPA 1989 – quoted from CIR 1992).

Mild irritation was seen when a 5 % solution of a mixture (ingredients not specified) containing 40 % hexamethylenetetramine was placed on the skin of guinea pigs (DuPont Company 1976 - quoted from Trochimowicz et al. 1993).

27.2.3.1 Sensitisation

The sensitisation potential of hexamethylenetetramine was studied in groups of 5 Dunkin-Hartley albino guinea pigs per sex by performing a guinea pig maximisation test of Magnusson and Kligman. One group was a control group and another group was induced and challenged with hexamethylenetetramine in distilled water at a concentration of 0.20 %. No erythema or oedema was observed in treated animals. (COLIPA 1989 – quoted from CIR 1992).

The sensitisation potential of hexamethylenetetramine was studied in 20 guinea pigs by performing a guinea pig maximisation test. The guinea pigs were induced by intradermal application of 0.1 ml of a 30 % solution of hexamethylenetetramine on the first day followed by epidermal application on day 8 of 0.5 g, which were covered for 48 hours. The guinea pigs were challenged on day 22 by epidermal application of 0.2 ml of a 50 % solution of hexamethylenetetramine, which was covered for 24 hours. Reactions were scored on day 24 and 25. Seventeen of the guinea pigs reacted positive with erythema and swelling. (Degussa AG 1985 – quoted from IUCLID 2000).

The sensitisation potential of AH26 (a root canal filling material containing 25 % hexamethylenetetramine, 10 % silver powder, 60 % bismuth oxide and 5 % titanium dioxide) was studied in groups of 10 female Dunkin-Hartley albino guinea pigs by performing a guinea pig maximisation test of Magnusson and Kligman. One group was a control group and another group was induced intracutaneously with AH26 in saline at a powder/saline ratio of 1.75/1 and percutaneously with AH26 in petrolatum. The animals were challenged with AH26 in petrolatum, 1/10 (w/w). Nine of ten animals were sensitised to AH26. (Kallus et al. 1983 – quoted from CIR 1992 and Loeper & Berzins 1995).

27.2.4 Other routes

27.2.4.1 Intramuscular injection

Groups of 5 BD II rats of each sex were administered 200 mg per day (equivalent to 500 mg/kg b.w. per day assuming a rat weight of 400 g) of hexamethylenetetramine by intramuscular injection for 90 days. The only substance related effect was a yellow discolouration of the fur. No macroscopic changes in organ histology or in body weights could be detected. (Brendel 1964 – quoted from CIR 1992, IUCLID 2000, JECFA 1974 and Loeper & Berzins 1995).

27.2.4.2 Ocular irritation

Six male rabbits had 0.1 ml of a 0.20 % solution of hexamethylenetetramine in distilled water applied once to the conjunctival sac of the eyes. The solution was not rinsed from the eyes. Conjunctival irritancy, iris alterations, or corneal lesions were not observed following dosing. (COLIPA 1989 – quoted from CIR 1992).

Nine New Zealand White albino rabbits had 100 mg of mascara containing 0.1 % hexamethylenetetramine applied to the conjunctival sac of one eye. The eyes of 3 rabbits of each sex were not rinsed after application of the test material while the eyes of 3 male rabbits were rinsed with deionised water 30 seconds after application. The treated eyes of all nine animals were examined 1, 2, 3, 4, and 7 days after application. The mascara was judged as mildly irritant for unrinsed eyes and non-irritant for rinsed eyes. (Stillmeadow, Inc., 1980 – quoted from CIR 1992).

27.3 Toxicity to reproduction

27.3.1 Inhalation

No data have been found.

27.3.2 Oral intake

Groups of 9-11 female Beagle dogs were fed 0, 600 or 1250 ppm (equal to about 0, 15 or 31 mg/kg b.w. per day based on an average body weight of 12 kg according to JECFA 1974) hexamethylenetetramine on days 4 to 56 after mating. Pregnancy rate, weight gain during pregnancy, length of gestation and litter size was not affected by treatment. In the 1250 ppm group, the percentage of stillborn pups was slightly increased, and the weight gain and the survival to weaning of the pups were slightly impaired. No congenital malformations were observed in any of the 264 live-born and 20 stillborn pups. Some of the pups were kept for observation and a few of them were being used for breeding. After 2 years, all were normal in behaviour, motility, and muscular coordination. Neither the bitches nor the pups had any abnormalities or reproductive disorders. (Hurni & Ohder 1973 – quoted from CIR 1992, JECFA 1974 and Loeper & Berzins 1995).

Groups of 16 Wistar rats of each sex were fed a standard diet containing either 0 or 0.16 % (equal to an average intake of 0 or 100 mg/kg b.w. per day according to CIR 1992) of hexamethylenetetramine for life. After 3 months of administration, the males and females of the control and test groups were mated. No significant difference was observed in fertility. In both groups, 16 male and 16 female offspring were chosen and fed the same diet as their parents from the time of weaning to natural death. No difference was observed in the relative organ or body weights or in voluntary muscular activity between test and control animals. A yellow staining of the hairs of the perineum was observed in some of the dosed rats. (Natvig et al. 1971 – quoted from CIR 1992, JECFA 1974 and Loeper & Berzins 1995).

Groups of 12 female and 6 male Wistar rats were given drinking water containing either 0 or 1 % (equal to about 0 or 2000 mg/kg b.w. per day according to Loeper & Berzins 1995) of hexamethylenetetramine starting two

weeks before mating. The females were treated during both pregnancy and lactation. No difference were observed between the treated and control groups in regard to fertility, litter size, and malformations. From the pups that were born, 24 of each sex were given 0 or 1 % hexamethylenetetramine in the drinking water until 20 weeks of age. Up to week 9 of age for males and week 20 of age for females, the body weights of the treated animals were significantly lower than the controls. At necropsy, no differences were observed in respect to organ weight, and gross and microscopic changes. (Della Porta et al. 1966, 1970 – quoted from CIR 1992, JECFA 1974 and Loeper & Berzins 1995).

Groups of 80, 80, or 245 rats were given 0, 5, or 50 mg/kg b.w. per day of hexamethylenetetramine in their drinking water in a five generation study that lasted 3.5 years. At half-yearly intervals starting at 1.5 years, animals (including pregnant dams) were selected from each group to be used for study of lesions. From that time on, no lesions due to hexamethylenetetramine were found in test animals, foetuses, or placenta. No further details were given. (Malorny 1966 – quoted from CIR 1992 and JECFA 1974).

Groups of 10 rats of each sex were fed 0, 400, 800, or 1600 ppm (equivalent to 0, 20, 40, or 80 mg/kg b.w. per day) of hexamethylenetetramine for 2 years. The ten pairs were mated at the age of 20, 28, and 35 weeks. No differences were observed in growth rate, survival, reproduction, offspring viability, or lesions between treated and control groups. No further details were given. (Berglund 1966 – quoted from CIR 1992 and JECFA 1974).

Groups of 1-4 mongrel dogs of each sex were fed 0 or 1250-1875 ppm (equal to about 0 or 94-141 mg/kg b.w. per day according to IUCLID 2000) of hexamethylenetetramine for 32 months. Groups of 2-3 pups of each sex from litters of these dogs were fed 0 or 1250 ppm of hexamethylenetetramine for 22 months. Subsequently, the test animals were fed control diet and the controls were fed the 1250 ppm diet for 1 year. No differences were observed in feed consumption, growth, reproduction, litter number, or litter weight between treated and control groups. Of the 30 litters in the dosed group, approximately 20 had a few stillborn and cannibalised pups, and 5 pups were born with abnormalities. Of the 16 litters in the control group, 1 had stillborn pups, and no pups had malformations. No further details were given. (Kewitz 1966 – quoted from CIR 1992, IUCLID 2000 and JECFA 1974).

27.3.3 Dermal contact

No data have been found.

27.3.4 Other routes

No data have been found.

27.4 Mutagenic and genotoxic effects

27.4.1 *In vitro* studies

In four studies, hexamethylenetetramine was not mutagenic when tested in 2-5 strains (TA98, TA100, TA1535, TA1537 and TA1538) of *Salmonella*

typhimurium in Ames tests with and without metabolic activation systems (Crebelli et al. 1984, 1985, Orstavik & Hongslo 1984, Andrews et al. 1980 – all quoted from CIR 1992).
In one study, hexamethylenetetramine was reported to be mutagenic towards strain TA98 and TA100 (but not towards strain TA1535, TA1537 and TA1538) without metabolic activation systems (Shimuzu et al. 1985 – quoted from Loeper & Berzins 1995).
After *in vitro* nitrosation hexamethylenetetramine has been reported to be mutagenic toward strain TA98 and TA100 with and without metabolic activation systems (Crebelli et al. 1984, Andrews et al. 1980 – both quoted from CIR 1992).

Hexamethylenetetramine induced a DNA-damaging effect in the recombination assay using the spores of *Bacillus subtilis* strains H17 and M45 when tested in the absence of a metabolic activation system. The DNA-damaging effect was decreased in the presence of a metabolic activation system. (Ueno & Ishizaki 1984 – quoted from Loeper & Berzins 1995).

Hexamethylenetetramine inhibited the growth of a mutant of *Escherichia coli*, which is deficient in DNA polymerase. The inhibition of cultures was concluded to be dependent on the degradation of hexamethylenetetramine to formaldehyde. (Fluck et al. 1976, Gillner 1987 – both quoted from Loeper & Berzins 1995).

The number of transformations was dose-dependently and significantly increased when hexamethylenetetramine was tested in the Styles´ cell transformation assay using baby hamster kidney BHK-21/cl.13 cells in the concentration range of 1 to 10000 µg/ml. The transformation activity was observed at a non-toxic or very weak toxic concentration and it was not dependent on a metabolic activation system. As a comparison, an equal number of transformations and equivalent toxicity was observed with 20 µg formaldehyde/ml and 1000 µg hexamethylenetetramine/ml. (Plesner & Hansen 1983 – quoted from CIR 1992 and Loeper & Berzins 1995).

Hexamethylenetetramine was negative in the mouse lymphoma assay with L5178Y TK+/- cells (Dooley et al. 1985 – quoted from IUCLID 2000).

Hexamethylenetetramine was negative for chromosomal aberrations in human leucocytes but positive in HeLa cells (Roehrborn & Vogel 1967, Balderman & Roehrborn 1967 – both quoted from IUCLID 2000).

27.4.2 *In vivo* studies

Hexamethylenetetramine caused mutations in larval spermatocytes of *Drosophila melanogaster* at concentrations, which were higher than those used in medical therapy (concentrations were not stated but for medical therapy doses of 1 g twice daily is recommended according to Lægemiddelkataloget (2001)). The causative agent of the mutagenic effect was formic acid, which was present as an impurity in formaldehyde. (Auerbach 1951, 1977, Stumm-Tegethoff 1964, Nafei & Auerbach 1964 – all quoted from CIR 1992, JECFA 1974 and Loeper & Berzins 1995).

Hexamethylenetetramine induced dominant lethal mutations in C3H mice which were administered the compound intraperitoneally or orally at a dose

of 25000 mg/kg b.w. Doses of 800 to 10000 mg/kg b.w. did not cause dominant lethal mutations. (Balderman et al. 1967, Röhrborn & Vogel 1967 – both quoted from Loeper & Berzins 1995).

In a micronucleus assay in C3H mouse, no clastogenic activity was observed in bone marrow after oral administration of approximately 69, 206 or 618 mg/kg b.w. of hexamethylenetetramine for 1 or 5 days. Bone marrow samples were collected 6, 12, and 24 hours after application of the acute dose and 6 hours after the last exposure in the 5-day study. (Vujosevic et al. 1986 – quoted from IUCLID 2000).

27.5 Carcinogenic effects

27.5.1 Inhalation

No data have been found.

27.5.2 Oral intake

Groups of 7-15 Wistar rats of each sex in three successive generations received 1% hexamethylenetetramine (equal to about 2000 mg/kg b.w. per day according to Loeper & Berzins 1995) in the drinking water up to the age of 40 weeks in the F1 and F2 generations and up to 20 weeks in the F3 generation. Another group of rats received 2 % hexamethylenetetramine in the drinking water, and 16 offspring of each sex were treated for 59 weeks with the same dose. A group of 48 rats of each sex served as a control group. All groups were observed for 2 years after exposure. No evidence of carcinogenicity was found in any of the hexamethylenetetramine treated groups of rats. (Della Porta et al. 1970 – quoted from CIR 1992, JECFA 1974 and Loeper & Berzins 1995).

Groups of 27-102 of each sex of three different strains of mice (CTM, C3Hf/Dp and SWR/Dp) and groups of 48 of each sex of Wistar rats received 0 or 1.0 % hexamethylenetetramine in their drinking water. The calculated daily intake equal to 1 % was 2500 mg/kg b.w. for mice and 1500-2500 mg/kg b.w. for rats according to Loeper & Berzins 1995. The mice were treated for 60 weeks and the rats for 104 weeks. One group of 50 CTM mice per sex was given 0.5 % hexamethylenetetramine for 60 weeks, and another group of 29-50 of each sex received 5 % hexamethylenetetramine for 30 weeks. One group of 12 rats of each sex were given 5 % hexamethylenetetramine for 2 weeks. After the termination of treatment, the animals were observed for the remainder of their lifetimes. Necropsy was performed on all animals, and tissues and lesions taken at necropsy were evaluated microscopically. No evidence of substance related carcinogenicity was found in any of the hexamethylenetetramine treated groups of animals. (Della Porta et al. 1968 – quoted from CIR 1992, JECFA 1974 and Loeper & Berzins 1995).

A total of 80, 80, or 245 rats were given 0, 5, or 50 mg/kg b.w. per day of hexamethylenetetramine in their drinking water in a five generation study that lasted 3.5 years. At half-yearly intervals starting at 1.5 years, animals (including pregnant dams) were selected from each group to be used for pathological studies. From that time on, no changes due to hexamethylenetetramine were found in test animals, foetuses, or placenta.

Tumours were observed in three of 48 animals in the high dose group. No further details were given. (Malorny 1966 – quoted from CIR 1992 and JECFA 1974).

Groups of 30 NMRI/Han albino mice of each sex were fed 0 or 1 % of hexamethylenetetramine (equivalent to 1500 mg/kg b.w. per day) in the feed for 2 years. Twenty neoplasms were found in the dosed group and 11 in the control group. With the exception of one control male and two dosed males, all neoplasms occurred in females. Most of the malignant tumours were subcutaneous carcinomas and adenocarcinomas. The author concluded that the possibility of an increased tumour incidence effect by hexamethylenetetramine could not be ruled out. A further study was performed using groups of 50 female mice, which were administered hexamethylenetetramine at concentrations of 0, 0.1, 0.5, or 1 % in the diet. After 31 weeks, no difference in neoplasm incidence was observed between the groups. (Kewitz 1966 – quoted from CIR 1992 and JECFA 1974).

27.5.3 Dermal contact

Groups of 13 mice were treated daily by cutaneous application of chloroform or 10 % hexamethylenetetramine in chloroform for 300 days. No malignant tumours were found in any group. (Kewitz 1966 – quoted from CIR and JECFA 1974).

39-44 CTM mice of each sex and 20 Wistar rats of each sex were injected subcutaneously 5 times every other day with 5 g/kg b.w. of a 30 % solution of hexamethylenetetramine in water. The animals were observed for 100-104 weeks. Necropsy was performed on all animals, and tissues and lesions taken at necropsy were evaluated microscopically. No evidence of substance related carcinogenicity was found in any of the hexamethylenetetramine treated groups of animals. (Della Porta et al. 1968 – quoted from CIR 1992, JECFA 1974 and Loeper & Berzins 1995).

Groups of 7-15 albino rats of each sex were injected subcutaneously with 1 ml of a 40 % solution of hexamethylenetetramine or with sodium chloride or sucrose (negative controls) weekly for 1½ year. In the dosed rats, 1 injection site spindle-cell sarcoma, 1 distal spindle-cell sarcoma, 1 alveolar mammary carcinoma, 1 fibrosarcoma, and 2 benign tumours were seen. In the control group, 1 blastoma was found in a sodium chloride injected animal. (Kewitz 1966 – quoted from JECFA 1974).

27.5.4 Other routes

No data have been found.

28 Regulations

28.1 Ambient air

-

28.2 Drinking water

-

28.3 Food

JECFA has set an ADI (acceptable daily intake) of 0-0.15 mg/kg b.w. for hexamethylenetetramine (JECFA 1974).

Hexamethylenetetramine may be used as a preservative (E239) in Provolone cheese in some countries.
However, on the Danish market it may not be used as a food additive (Positivlisten 2000).

28.4 Cosmetics

Hexamethylenetetramine may legally be used as a preservative in cosmetic products in Europe at a maximum concentration of 0.15%. It may also be used in other concentrations for other purposes in cosmetics. (MM 2000).

28.5 Drugs

Hexamethylenetetramine may be used as a drug for urinary infections (Lægemiddelkataloget 2001).

28.6 Soil

-

28.7 Occupational Exposure Limits

The occupational exposure limits in Norway, Sweden and Poland varies between 3 and 5 mg/m^3 (RTECS 2000).

28.8 Classification

Hexamethylenetetramine is classified for flammability (F;R11 – highly flammable) and for sensitising properties (R42/43 – may cause sensitisation by inhalation and skin contact). (MM 2002).

28.9 IARC

-

28.10 US-EPA

-

29 Summary

29.1 Description

Hexamethylenetetramine occurs as hygroscopic and colourless crystals or as a white crystalline powder with no or mild ammonia odour. It has a low vapour pressure (0.004 mmHg) and a high water solubility (449 g/l).

29.2 Toxicokinetics

Hexamethylenetetramine is rapidly absorbed in humans following oral administration. It is distributed to various tissues and is detectable in e.g. amniotic fluid and milk. In slightly acidic environments such as in sweat, gastric fluid or urine, hexamethylenetetramine partially degrades to formaldehyde and ammonia. Upon contact with the skin, hexamethylenetetramine has also been reported to produce formic acid. In the gastric fluid, about 10-30 % of an orally administered dose of hexamethylenetetramine is degraded. The half-life for a formulation of hexamethylenetetramine in humans is about 4 hours. In the same study, more than 80 % of the oral dose of hexamethylenetetramine was excreted unchanged in the urine during the first 24 hours following administration.

29.3 Human toxicity

29.3.1 Single dose toxicity

Inflammation of the bladder and an increased concentration of nitrogen in the blood have been reported following an accidental ingestion of an overdose of hexamethylenetetramine-mandelate.

29.3.2 Repeated dose toxicity

Pulmonary or nasal symptoms (e.g. wheezy breathing, chest tightness, shortness of breath, couching, sneezing, rhinorrhoea, obstruction) have been reported in workers exposed to hexamethylenetetramine in a production plant, in the lacquer and plastics industries, in a tire manufacturing plant and in a foundry. In all of the workplaces, the workers were exposed to other chemicals (e.g. formaldehyde, ammonia, resorcinol, phenol, furfuryl alcohol, cyanides, epoxy resins, curatives) as well. Lung function measurements in one of the studies revealed significant reductions in expiratory flow rates at low lung volumes. In another study, an intracutaneous skin test with hexamethylenetetramine gave positive reactions in all workers and a provocative inhalation test with an aerosol of a lacquer product revealed allergic reactions from either the lungs, the nose or the skin.

Hexamethylenetetramine has been reported to cause allergic eczema and irritation of the skin and eyes in e.g. workers in the rubber, lacquer and plastics industry but also in people in contact with finished rubber objects. No signs of sensitisation were observed in a maximization test, which was performed in 25 adults with mascara, which contained 0.1 %

hexamethylenetetramine. However, several patients have tested positive in allergic patch tests to 2 % hexamethylenetetramine. In the same studies three to four times as many patients tested positive to 1-2 % formaldehyde. Positive patch test reactions for hexamethylenetetramine have occurred in persons who were sensitised to formaldehyde or ethylenediamine. Case reports also exist of workers who had positive patch tests to hexamethylenetetramine but who tested negative to formaldehyde.

Adverse effects have been reported in less than 3.5 % of patients receiving hexamethylenetetramine and its salts orally as a drug. The most frequent adverse effect is gastrointestinal disturbances. Rarely, hypersensitivity reactions have occurred. Other adverse effects reported rarely are headache, dyspnoea, generalized oedema, tinnitus, muscle cramps, dysuria, and microscopic or gross haematuria.

29.3.3 Toxicity to reproduction

Only the expected number of congenital abnormalities was observed in more than 200 newborns that had been exposed to hexamethylenetetramine during the first trimester.

29.3.4 Mutagenic and genotoxic effects

Hexamethylenetetramine was not mutagenic in Ames test and in a microtitre fluctuation test with urine concentrates from 72 men who worked in a tire plant where hexamethylenetetramine was a workplace pollutant.

29.3.5 Carcinogenic effects

An increase in certain types of cancer has been reported among workers in a rubber making company and among Danish moulders. Both groups of workers have been exposed to hexamethylenetetramine but also to a mixture of other chemicals including some with suspected and/or confirmed carcinogenic properties.

29.4 Animal toxicity

29.4.1 Single dose toxicity

The oral LD_{50}-values reported for hexamethylenetetramine range from 9200 to higher than 20000 mg/kg b.w. for rats and 1853 mg/kg b.w. for mice.

29.4.2 Repeated dose toxicity

No adverse effects were noted in mice (CTM and C3Hf/Dp), rats (Wistar and BD II) and cats dosed with up to 2500, 1500 or 1250 mg/kg b.w., respectively, of hexamethylenetetramine in their drinking water, in their feed or by gavage for 13-104 weeks. A yellow discoloration of the fur of treated rats (but not of treated mice and cats) was observed. The discolouration has been explained as a result of a reaction between formaldehyde present in the urine from treated rats and kynurenine, which is a normal constituent of rat hair. SWR/Dp mice that received 2500 mg/kg b.w. and CTM mice that received 12500 mg/kg b.w. had a slight retardation of growth. A slight reduction in survival was also observed for CTM mice dosed with 12500 mg/kg b.w. A dose of 7500 mg/kg b.w. caused 50 % mortality in the rats.

No to mild skin irritation was observed in rabbits and guinea pigs exposed dermally to hexamethylenetetramine in concentrations of 0.2-2 %. A 0.2 % solution of hexamethylenetetramine was non-irritant to the rabbit eye while a mascara containing 0.1 % hexamethylenetetramine was mildly irritant for unrinsed rabbit eyes.

Hexamethylenetetramine caused skin sensitisation in 17/20 of guinea pigs induced with a 30 % solution and challenged with a 50 % solution in a maximisation test. No skin sensitisation was observed in another maximisation test where hexamethylenetetramine was used in a concentration of 0.2 %.

29.4.3 Toxicity to reproduction

No substance-related reproductive or developmental effects were seen in 4 studies where rats were fed or given hexamethylenetetramine in their drinking water in doses up to about 2000 mg/kg b.w. However, a significant lower body weight was found in pups, which were born of dams treated during pregnancy and lactation with 2000 mg/kg b.w. of hexamethylenetetramine and, which in addition were treated with the same dose of the chemical for the first 20 weeks of age.

In dogs fed about 15 mg/kg b.w. of hexamethylenetetramine, no substance-related reproductive or developmental effects were noted. In dogs fed about 31 mg/kg b.w. of hexamethylenetetramine, the percentage of stillborn pups was slightly increased, and the weight gain and the survival to weaning of the pups were slightly impaired. In another study where dogs were fed about 94 mg/kg b.w. of hexamethylenetetramine, a few pups were born with abnormalities.

29.4.4 Mutagenic and genotoxic effects

In most studies, hexamethylenetetramine was not mutagenic in Ames test with and without metabolic activation systems. It was negative in a mouse lymphoma assay and for chromosomal aberrations in human leucocytes. Hexamethylenetetramine was positive for chromosomal aberrations in HeLa cells, for DNA-damage in a recombination assay with spores of *Bacillus subtilis*, for inhibition of growth of a mutant of *Escherichia coli*, and for an increased number of transformations in the Styles´ cell transformation assay with baby hamster kidney cells. In the cell transformation assay an equal number of transformations and equivalent toxicity was observed with a 50 times smaller dose of formaldehyde.

Hexamethylenetetramine was negative *in vivo* for clastogenic activity in the bone marrow in a micronucleus assay in C3H mouse. It was positive for dominant lethal mutations in C3H mice orally administered a very high dose but negative at doses below 10000 mg/kg b.w. Hexamethylenetetramine caused mutations in larval spermatocytes of *Drosophila melanogaster* at concentrations, which were higher than those used in medical therapy.

29.4.5 Carcinogenic effects

No evidence of substance-related carcinogenicity was found in 4 different studies where mice or rats were fed or given hexamethylenetetramine in their drinking water in doses up to about 2500 mg/kg b.w. for up to 2 years or in 2 studies where mice or rats were exposed dermally to hexamethylenetetramine in a concentration of up to 30 % for up to 2 years. In rats injected subcutaneously with a 40 % solution of hexamethylenetetramine for 1½ year, more tumours were found than in the control group.

30 Evaluation

The critical effect of hexamethylenetetramine is the sensitisation it may cause following exposure by inhalation or skin contact. Skin sensitisation has been observed in a guinea pig maximisation test as well as in humans in several patch tests. Several workers with mixed exposures, including exposure to hexamethylenetetramine, have reported allergic eczema and/or asthma-like symptoms. In one study, reductions in expiratory flow rates at low lung volumes was reported. In another study, intracutaneous skin tests with hexamethylenetetramine gave positive reactions in all workers and a provocative inhalation test with an aerosol of a lacquer product showed allergic reactions from either the lungs, the nose or the skin.
It is a cause of concern that no inhalation studies with hexamethylenetetramine have been performed in animals since it because of the mixed exposures is unclear from the human studies whether the symptoms from the lungs are caused by hexamethylenetetramine.

No systemic effects following inhalation of hexamethylenetetramine have been reported in humans. No toxicokinetic inhalation studies have been performed. However, due to the high water solubility of hexamethylenetetramine it is most likely absorbed following inhalation.

A low order of acute oral toxicity has been observed in experimental animals.

Irritation of the skin and eyes has been reported in workers with mixed exposures, including exposure to hexamethylenetetramine, as well as in laboratory animals.

Only few adverse effects (of which the most frequent is gastrointestinal disturbances) have been reported in patients receiving hexamethylenetetramine and its salts orally as a drug.
In laboratory animals exposed orally to high doses of hexamethylenetetramine, only slight growth retardation and survival was observed.

No substance-related reproductive or developmental effects were observed in rats exposed to high doses (up to 2000 mg/kg b.w.) of hexamethylenetetramine in their drinking water. Dogs seemed more sensitive since some developmental effects (increased percentage of stillborn pups, decreased weight gain and survival to weaning of pups) were observed in dogs fed about 31 mg/kg b.w. of hexamethylenetetramine. However, at 15 mg/kg b.w. no developmental effects were noted.
In humans, only the expected number of congenital abnormalities was observed in more than 200 newborns that had been exposed to hexamethylenetetramine during the first trimester.

Both positive and negative results were obtained in assays for mutagenicity and genotoxicity of hexamethylenetetramine. However, in the *in vivo* assays, positive results were only observed when the administered doses were very high.

Oral and dermal carcinogenicity studies in rats and mice exposed to high doses of hexamethylenetetramine revealed no evidence of substance-related tumours.

Some of the effects observed following exposure to hexamethylenetetramine may be due to its degradation products, e.g. formaldehyde, ammonia, and formic acid, which may be formed in slightly acidic environments. Formaldehyde is known to be a strong sensitising agent to the skin and is, according to the EU classification criteria, classified as such (R43).

31 References

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Appendix 5: 1-Methyl-1,2-ethanediyl dioleate

32 General description

32.1 Identity

Molecular formula: $C_{39}H_{72}O_4$

Structural formula:

CH_3-$(CH_2)_7$-CH:CH-$(CH_2)_7$-$COOCH_2$-(CH-CH_3)-COO-$(CH_2)_7$-CH:CH-$(CH_2)_7$-CH_3

Molecular weight: 605.0

CAS-no.: 105-62-4

Synonyms:
1,2-Bisoleoyloxypropane
1,2-Dioleoylooxypropane
1-Methyl-1,2-ethanediyl 9-octadecenoate
9-Octadecenoic acid, 1-methyl-1,2-ethanediyl ester
Octadec-9-enoic acid, 1-methyl-2-octadec-9-enoyloxyethyl ester
9-Octadecenoic acid, 1,3-propanediyl ester
Oleic acid, propylene ester
1,2-Propanediol dioleate
1,2-Propylene glycol dioleate
Propylene glycol dioleate

32.2 Physical / chemical properties

Description: 1-Methyl-1,2-ethanediyl dioleate is a liquid. It is a diester of propylene glycol and the fatty acid, oleic acid.

Melting point: -

Boiling point: -

Density: -

Vapour pressure: -

Concentration of saturated vapours: -

Conversion factor: 1 ppm = 25.2 mg/m^3 (at 20°C and 760 mmHg)
1 mg/m^3 = 0.040 ppm

Solubility: -

References: IUCLID (2000), Johnson (1999), TSCA (2000).

33 Toxicokinetics

No data have been found.

34 Human toxicity

Vapour of heated 1-methyl-1,2-ethanediyl dioleate can cause irritation when inhaled (Unichema International – quoted from IUCLID 2000).

No other data have been found.

35 Animal toxicity

No data have been found.

36 Regulations

Propylene glycol esters have been used as emulsifiers in foods and pharmaceuticals. The Federal Drug Administration (FDA) in the US has determined that propylene glycol mono- and diesters of edible fats or fatty acids or oleic acid can be used safely in food in amounts not in excess of that reasonably required to produce their intended effect. The esters can also be used in food contact materials. (Rosen 1978 – quoted from Johnson 1999).

1-Methyl-1,2-ethanediyl dioleate is used in cosmetics (Johnson 1999). It is not mentioned in the statutory order on cosmetics, which means that it legally can be used in cosmetics in Europe without any limitations except as a colouring agent, a preservative, or a UV-filter (MM 2000).

37 Summary

1-Methyl-1,2-ethanediyl dioleate is a diester of propylene glycol and the fatty acid, oleic acid. It is a liquid that is used e.g., in foods and pharmaceuticals as an emulsifier and in cosmetics.

Vapour of heated 1-methyl-1,2-ethanediyl dioleate can cause irritation in humans when inhaled.

38 Evaluation

No toxicological studies regarding effects following exposure to 1-methyl-1,2-ethanediyl dioleate have been found.

Only one reference to 1-methyl-1,2-ethanediyl dioleate has been found in which is was stated that vapour of heated 1-methyl-1,2-ethanediyl dioleate can cause irritation in humans when inhaled. This study is of limited value because of the lack of information on exposure levels and duration. In addition, the irritation might be caused by degradation products of 1-methyl-1,2-ethanediyl dioleate formed by the heating of the substance.

No useful toxicological data regarding 1-methyl-1,2-ethanediyl dioleate are therefore available for evaluation.

39 References

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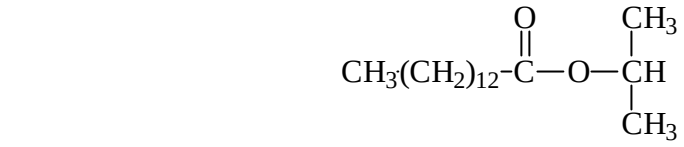
Appendix 6: Isopropyl myristate

40 General description

40.1 Identity

Molecular formula: $C_{17}H_{34}O_2$

Structural formula:



Molecular weight: 270.5

CAS-no.: 110-27-0

Synonyms: Isopropyl tetradecanoate
Myristic acid, isopropyl ester
Tetradecanoic acid, isopropyl ester
Tetradecanoic acid 1-methylethyl ester

40.2 Physical / chemical properties

Description: Colourless and practically odourless liquid with a low viscosity, good penetration and spreading properties, and a non-oily feel.

The commercial product usually contains small amounts of esters of palmitic and other saturated fatty acids.

Melting point: Approximately 0-3°C

Boiling point: Decomposes at 208°C

Density: 0.85 g/ml (at 20°C)

Vapour pressure: 9.35×10^{-5} mmHg (1.25×10^{-2} Pa) (at 25°C)

Concentration of saturated vapours: 0.12 ppm (= 1.3 mg/m^3)(at 25°C and 760 mmHg)

Conversion factor: 1 ppm = 11.2 mg/m^3 (at 20°C and 760 mmHg)
1 mg/m^3 = 8.9×10^{-2} ppm

Solubility: Practically insoluble in water, glycerol, sorbitan, and propylene glycol. Soluble in castor oil, cottonseed oil, acetone, chloroform, ethyl acetate, ethanol, toluene, and mineral oil.

References: BIBRA (1988), ChemFinder (2001), CIR (1982), HSDB (2000), IUCLID (2000), Merck Index (1996).

41 Toxicokinetics

41.1 Absorption, distribution and elimination

Four monkeys were exposed for 5 seconds to the aerosols of an antiperspirant spray containing C^{14}-labelled isopropyl myristate (the dose was not stated). Labelled material was mainly found in the lungs. Very little systemic absorption (0.25 %) occurred. About 85 % of the absorbed dose was eliminated in 24 hours, mainly as exhaled carbon dioxide. (Finkelstein & Wulf 1974 – quoted from BIBRA 1988, CIR 1982 and IUCLID 2000).

Whole body auto-radiography of hairless mice showed no visible penetration into the skin or organs of isopropyl myristate, whereas microautoradiography of angora rabbits and guinea pigs showed local penetration following dermal application. Whole body auto-radiography also revealed that isopropyl myristate injected subcutaneously into mice was distributed into almost all organs. (Suzuki et al. 1978 – quoted from IUCLID 2000).

41.2 Mode of action

No information has been found.

41.3 Influence on skin permeability of other chemicals

Isopropyl myristate enhanced the *in vitro* penetration rate of the nitrosamine N-nitrosodiethanolamine through human abdominal skin at 200 times the rate for water and 344 times the rate for propylene glycol (Bronaugh et al. 1981 – quoted from BIBRA 1988 and IUCLID 2000). It also increased the rate of benzyl alcohol penetration (Barry et al. 1985 – quoted from BIBRA 1988). Betamethasone 17-benzoate penetration of intact human forearm skin was increased by isopropyl myristate (Pepler et al. 1971 – quoted from CIR 1982). In an *in vivo* study, dexamethasone penetrated the stripped forearm skin (but not the intact skin) of nine humans seven times better when in gelled isopropyl myristate as compared with petrolatum (Dempski et al. 1969 – quoted from BIBRA 1988 and CIR 1982).

The dermal absorption of ethanol and butanol through hairless mouse skin *in vitro* was 35 or 3 times, respectively, greater when isopropyl myristate was used as a vehicle compared to water (Garcia et al. 1980 – quoted from BIBRA 1988 and IUCLID 2000).

42 Human toxicity

42.1 Single dose toxicity

42.1.1 Inhalation

No relevant data have been found.

42.1.2 Oral intake

No data have been found.

42.1.3 Dermal contact

See section 3.2 Repeated dose toxicity.

No evidence of phototoxicity was observed in 10 volunteers when a bath oil containing 43% isopropyl myristate was applied dermally at 4.25 mg/cm^2 under an occlusive patch for 24 hours. The application site was exposed to ultraviolet irradiation (UV-range A) from a solar simulator after 6 and 24 hours. (CTFA 1978 – quoted from BIBRA 1988 and CIR 1982).

42.1.4 Other routes

No data have been found.

42.2 Repeated dose toxicity

42.2.1 Inhalation

No data have been found.

42.2.2 Oral intake

No data have been found.

42.2.3 Dermal contact

42.2.3.1 Local effects

In studies with single or repeated dermal administration of undiluted isopropyl myristate, no or minimal skin irritation was seen. The studies lasted up to 21 days and were performed on groups of 5-200 subjects and included both covered and uncovered application. (Anon 1953, Avon 1971, 1975, Campbell & Bruce 1981, Hill top research 1976, Motoyoshi et al. 1979 – all quoted from BIBRA 1988, CIR 1982 and/or IUCLID 2000). In one study the skin was scratched (scarified) beforehand (Frosch & Kligman 1976 – quoted from BIBRA 1988).

When applied in petrolatum under cover for 48 hours to the backs of 50 subjects, the highest non-irritant concentration of isopropyl myristate was

10%. No details were given of effects seen at higher concentrations. (Meneghini et al. 1971 – quoted from BIBRA 1988 and IUCLID 2000).

42.2.3.2 Skin sensitisation

Negative results were obtained in patch tests with 5% isopropyl myristate in petrolatum on 290 patients with eczema (Meneghini et al. 1971 – quoted from BIBRA 1988), and with 30% isopropyl myristate in petrolatum in an unspecified number of patients at a dermatological clinic (Hjort & Trolle-Lassen 1963 – quoted from BIBRA 1988).

Several experimental studies with healthy volunteers have failed to detect any skin sensitising potential (Anon 1953, CTFA 1976, 1978a, b, Kligman 1974 – all quoted from BIBRA 1988, CIR 1982, and/or IUCLID 2000).
In one such study, flannel impregnated with 170 mg undiluted isopropyl myristate under cellophane was allowed to remain on the backs of 200 volunteers for 5 days, and in another study, material saturated with 1.28-1.70 g undiluted isopropyl myristate was applied for 24 hours every other day for 15 applications to 50 volunteers. In these two studies a 48-hour challenge application was given 3 weeks later. (Anon 1953 – quoted from BIBRA 1988 and IUCLID 2000).
In a maximization test on 25 volunteers, pre-treatment of the forearm with an irritant (sodium lauryl sulphate) was followed by five 48-hour applications of 20% isopropyl myristate in petrolatum with 24 hours between each application, and a challenge application was given 10 days later (Kligman 1974 – quoted from BIBRA 1988, CIR 1982, and IUCLID 2000).
Other studies involving repeated contact with various cosmetic preparations containing 15-58% isopropyl myristate also gave negative skin sensitisation results in groups of 25-320 volunteers (CTFA 1976, 1978a, b – quoted from BIBRA 1988 and CIR 1982).

No photo-allergic reactions were induced in 25 volunteers when a bath oil containing 43% isopropyl myristate was applied dermally at 4.25 mg/cm^2 under an occlusive patch for 24 hours, twice weekly for 3 weeks. Each application was followed by three exposures to ultraviolet irradiation (UV-range A) from a solar simulator sufficient to produce slight redness, and a challenge patch followed by irradiation was applied 10 days later. (CTFA 1978 – quoted from BIBRA 1988, CIR 1982 and IUCLID 2000).

A woman developed a mild dermatitis after using a feminine hygiene spray containing isopropyl myristate at an unspecified concentration for 6 months. On closed patch testing she had a strong positive reaction to undiluted or 10% isopropyl myristate. (Fisher 1973 – quoted from BIBRA 1988 and CIR 1982).

Three of 41 hospital workers with hand eczema had positive skin-prick test reactions to 20% isopropyl myristate. It was not stated what isopropyl myristate was diluted in. None of 55 patch-tested with the same concentration under cover for 48 hours showed a positive response. It is unclear whether these 55 included any of the 41 tested by skin-prick. (Nielsson 1985 – quoted from BIBRA 1988).

42.2.4 Other routes

No data have been found.

42.3 Toxicity to reproduction

No data have been found.

42.4 Mutagenic and genotoxic effects

No data have been found.

42.5 Carcinogenic effects

No data have been found.

43 Animal toxicity

43.1 Single dose toxicity

43.1.1 Inhalation

Twenty rats were exposed for 6.5 seconds/minute for an hour to an aerosol antiperspirant containing 16-20% isopropyl myristate at a nominal concentration of 33-41 mg/l. During exposure all rats exhibited lethargy, and slight muscle and eye discharge. No deaths occurred, and no evidence of systemic toxicity was found at necropsy or during the 14-day post-exposure observation period. (Hazleton Laboratories 1978 – quoted from BIBRA 1988 and CIR 1982).

Groups of 6 rats served as controls or were exposed for four 15-minute periods each separated by 5-minute fresh air periods to an aerosol antiperspirant containing 4.7% isopropyl myristate at a nominal concentration of 9.7 mg/l. No deaths occurred and no significant differences were found between control and exposed rats during exposure or at necropsy. (CTFA 1974 – quoted from BIBRA 1988 and CIR 1982).

43.1.2 Oral intake

The oral LD_{50}-value of undiluted isopropyl myristate has been reported to be higher than 13700 mg/kg b.w. for rats (4 studies) and from 42400 to higher than 85000 mg/kg b.w. for mice (2 studies) (Anon 1953, Avon 1971, Inolex 1975, Kolmar Research Center 1972, Lebarco Laboratories 1973, Platcow & Voss 1954 – all quoted from BIBRA 1988 and CIR 1982).

43.1.3 Dermal contact

43.1.3.1 Acute toxicity

The dermal LD_{50}-value of undiluted isopropyl myristate has been reported to be higher than 5000 mg/kg b.w. for rabbits (1 study) (Moreno 1974 – quoted from BIBRA 1988 and CIR 1982).

In rabbits exposed dermally to a single dose of 7600 mg/kg b.w. of isopropyl myristate experienced no overt toxic symptoms or significant haematological changes occurred. Urinary analysis revealed no evidence of metabolic disturbance or kidney damage. (Anon 1953 – quoted from BIBRA 1988).

No deaths or abnormalities at autopsy were reported in a study where 6 guinea pigs were dosed dermally (with an antiperspirant) with about 1000 mg/kg b.w. of isopropyl myristate (CTFA 1972 – quoted from BIBRA 1988 and CIR 1982).

43.1.3.2 Skin irritation

The Draize primary skin irritation test or a slight modification of it was used in rabbits to evaluate undiluted isopropyl myristate in 5 studies and four product formulations containing the ester in 5 studies. Isopropyl myristate caused no to minimal irritation. (Avon 1974, 1976, 1977, Bio/dynamics 1978, CFTA 1972, 1978, Consumer Product Testing 1978, Inolex 1975, Leberco Laboratories 1973, MB Research Laboratories 1974 – all quoted from CIR 1982).

Isopropyl myristate was slightly irritating in one of the rabbit tests, which was performed according to OECD-guideline 404 and following the principles of GLP (ECETOC 1995 – quoted from Bagley et al. 1996 and IUCLID 2000).

43.1.4 Other routes

43.1.4.1 Eye irritation

The Draize rabbit eye irritation test or a modification of it was used to evaluate undiluted isopropyl myristate in more than 10 separate studies and four product formulations containing the ester in 4 studies. No or minimal irritation generally followed a single application of the undiluted material or the product formulations (Avon 1972a, b, c, 1976, 1977, Bio/dynamics 1978, CFTA 1972, 1978, Guillot et al. 1977, Kolmar Research Center 1967, Leberco Laboratories 1973, Platcow & Voss 1954, Weil & Scala 1971 – all quoted from BIBRA 1988 and/or CIR 1982; Ohno et al. 1999), although in one study slight corneal opacity was reported in a minority of rabbits (Guillot et al. 1982 – quoted from BIBRA 1988).

43.2 Repeated dose toxicity

43.2.1 Inhalation

Two groups of twenty guinea pigs were exposed for an hour three times a day, seven days a week for 4 (half of the animals) or 13 (the other half of the animals) weeks to an aerosol antiperspirant containing 16-20% isopropyl myristate. Test groups were exposed to mean isopropyl myristate concentrations of 10-13 or 36-45 mg/m^3. A control group of 40 guinea pigs were exposed to air. One female guinea pig died for unknown causes. Both absolute and relative lung weights increased in the exposed animals but no histological changes were found. The organs examined were not specified. (Bio/dynamics 1979 – quoted from BIBRA 1988, CIR 1982 and IUCLID 2000).

Four groups of nine cynomolgus monkeys were exposed for an hour three times a day, seven days a week for 13 weeks to an aerosol antiperspirant containing 16-20% isopropyl myristate. Test groups were exposed to isopropyl myristate concentrations of about 0.95, 1.5, 6.0 or 6.7 mg/m^3. A control group of 9 monkeys were exposed to air. During the study, the treated monkeys wheezed and coughed and two of the monkeys exposed to about 1.5 mg/m^3 of isopropyl myristate developed nosebleeds. Lung function tests after 6 and 13 weeks were normal, as were the results of haematology, blood chemistry and urinalysis. No gross lesions were seen at necropsy, and organ weights were unaffected by treatment. Histological examinations revealed a dose-related accumulation of macrophages within the alveolar and

bronchiolar walls of the lungs of treated monkeys. (Hazleton Laboratories 1978 – quoted from BIBRA 1988, CIR 1982 and IUCLID 2000).

Groups of 12 female CD rats and of 12 female hamsters were exposed by whole body inhalation to aerosols of a diluted complex fragrance mixture at 0 or 50 mg/m^3 (aerodynamic mean diameter = 1.4 µm) for 4 hours per day, 5 days per week for 13 weeks. The fragrance mixture consisted of approximately 200 ingredients with close to one-half of the ingredients present at a level of 1% or more. The animals were exposed to isopropyl myristate at a maximal concentration of 0.16 mg/m^3. No toxicological significant effects were observed on survival, behaviour, body weights, organ weights, haematology, clinical chemistry, gross pathology, or histopathology. (Fukayama et al. 1999).

43.2.2 Oral intake

Groups of 50 rats were fed 2.5, 5, or 10% of isopropyl myristate (equal to about 2000, 3700 or 7900 mg/kg b.w. per day) in their diet for up to 16 weeks. Histopathological examinations revealed no damage to any organs or tissues at any dose. At the highest dose, the liver weight and the blood levels of two liver enzymes were increased. In addition the males had increased spleen, small intestine and kidney weights, and in both sexes the proportion of neutrophilic leucocytes was increased. At the mid-dose only transient changes in some of the organ weights (spleen, liver, small intestine) were observed. (Gaunt et al. 1972 – quoted from BIBRA 1988 and IUCLID 2000).

Groups of Wistar rats were given 0, 100, 500 or 1000 mg/kg b.w. per day of isopropyl myristate by gavage once a day, 5 days per week for 28 days. No mortality, symptoms of intoxication, or substance-related injury of organs were recorded. Histologically, a reversible hyperplasia of the forestomach mucosa was observed in all test groups including the negative control group indicating local irritating effects of the olive oil carrier. (Henkel KgaA – quoted from IUCLID 2000).

43.2.3 Dermal contact

43.2.3.1 Systemic toxicity

Rabbits had dermal applications of 5100 mg/kg b.w. of isopropyl myristate daily for 20 days. Skin inflammation, liver damage (mild cloudy swelling), and leucocytosis were observed. No changes were found in the kidneys, testes, or spinal cord. (Anon 1953 – quoted from BIBRA 1988 and IUCLID 2000).

Groups of 10 rabbits were dosed for five days a week for 4 weeks on abraded skin of their back with either distilled water or 320-400 mg/kg b.w. of isopropyl myristate from an aerosol antiperspirant containing 16-20% of the chemical. None of the animals died and no changes considered to be related to the product were seen in general behaviour, body weights, or haematological studies. See also section 4.2.3.2 *Local effects* regarding local dermal effects. (IRDC 1978 – quoted from BIBRA 1988, CIR 1982, and IUCLID 2000).

Groups of 6 rabbits either remained untreated or were dosed for five days a week for a total of 21 dermal applications with 730-800 mg/kg b.w. of isopropyl myristate from an aerosol antiperspirant containing 43-47% of the chemical. One of the dosed animals died from causes thought to be unrelated to treatment. No significant pathological changes were discovered at necropsy other than local skin damage – see also section 4.2.3.2 *Local effects* for more details. (CFTA 1972 – quoted from BIBRA 1988 and CIR 1982).

43.2.3.2 Local effects

A 0.5 ml (425 mg) sample of undiluted isopropyl myristate was applied for three consecutive days to a 2 $inch^2$ area of clipped skin on a total of 42 rabbits (7 studies). Isopropyl myristate was moderately to severely irritating under the conditions of the study as evidenced by the occurrence of oedema, severe erythema, drying, cracking, and scaling. No further details were given. (Avon 1970, 1971a, b, c, 1972a, b, c – all quoted from CIR 1982).

New Zealand white rabbits or Albino Spartan mice had daily dermal applications of undiluted isopropyl myristate for up to 14 or 28 days, respectively. Erythema, lichenification and fissure formation occurred at the application site. Microscopically, the treated skin of exposed animals showed acanthosis, parakeratosis, hyperkeratosis, focal erosion, and focal haemorrhage. In mice, the skin lesions tended to regress during continued treatment while the lesions in rabbits regressed slowly after cessation of treatment. No further details were given. (Fitzgerald et al. 1968 – quoted from CIR 1982 and IUCLID 2000).

Groups of 10 rabbits were dosed for five days a week for 4 weeks on their abraded back with either distilled water or 320-400 mg/kg b.w. of isopropyl myristate from an aerosol antiperspirant containing 16-20% of the chemical. Moderate to severe erythema, desquamation, slight coriaceousness, moderate fissuring, and atonia occurred at the application site. Microscopically, the treated skin of all exposed rabbits showed marked to severe acanthosis and hyperkeratosis with varying degrees of parakeratosis and mixed inflammatory cell infiltration. No further details were given. (IRDC 1978 – quoted from CIR 1982).

Groups of 6 rabbits either remained untreated or were dosed for five days a week for a total of 21 dermal applications with 730-800 mg/kg b.w. of isopropyl myristate from an aerosol antiperspirant containing 43-47% of the chemical. Signs of product-related changes in the skin included erythema, oedema, drying, cracking, and fissuring. No further details were given. (CFTA 1972 – quoted from CIR 1982).

White albino rabbits, which had 1, 5 or 100% of isopropyl myristate in propylene glycol applied to the ears twice daily 5 days a week for 2 weeks developed significant comedones formation even at the lowest concentration (Fulton et al. 1976 – quoted from BIBRA 1988 and IUCLID 2000).

43.2.3.3 Skin sensitisation

In 2 guinea pig sensitisation tests with 2 or 10 animals, respectively, isopropyl myristate suspended at 0.1% in physiologic saline showed no evidence of a

sensitisation potential (Inolex 1975, Platcow & Voss 1954 – both quoted from CIR 1982).

Isopropyl myristate was found to be weakly positive for skin sensitisation in the local lymph node assay (LLNA) in mice. In this assay, groups of 5 female CBA/J mice were treated once a day for 3 consecutive days on the dorsum of both ears with 25 µl of 25, 50 or 100% of isopropyl myristate in acetone/olive oil (4:1) or with vehicle alone. Five days after the initial treatment, all mice were injected intravenously via the tail vein with marked methylthymidine. The incorporation of methylthymidine in the cells of the draining auricular lymph nodes was determined. It is generally taken as a measure for the lymphocyte proliferation provoked by sensitising chemicals during the induction phase. The authors are suggesting that the positive result might be a false-positive response since a) isopropyl myristate is not expected based on structure to be a skin sensitiser, b) it has been reported that some irritants can produce a positive response in LLNA and isopropyl myristate is known to be a skin irritant at high concentrations, c) and isopropyl myristate was only weakly positive at the highest concentration tested. (Ryan et al. 2000).

43.2.4 Other routes

43.2.4.1 Eye irritation

Applications of isopropyl myristate to the eyes of rabbits daily for 3 consecutive days apparently caused no irritation (Avon 1970, 1971a – all quoted from BIBRA 1988 and CIR 1982) or slight irritation that had vanished after 7 days (Avon 1971b – quoted from BIBRA 1988 and CIR 1982).

43.3 Toxicity to reproduction

Twenty Swiss albino mice (housed one male and one female per cage) had 0.1 ml of 1% isopropyl myristate (equivalent to 42.5-56.6 mg/kg b.w.) in acetone applied to the skin once weekly for 18 months. No abnormalities were found in litters, which were examined grossly. (Giles & Byron 1968 – quoted from BIBRA 1988 and IUCLID 2000).

43.4 Mutagenic and genotoxic effects

Isopropyl myristate tested negative in an Ames test with 5 strains of *Salmonella typhimurium* (TA 98, TA 100, TA 1535, TA 1537 and TA 1538) both with and without metabolic activation (Blevins & Tayler 1982 – quoted from BIBRA 1988 and IUCLID 2000).

43.5 Carcinogenic effects

In a lifetime study, groups of 50 female Swiss mice were given skin applications from the age of 7 weeks on their backs of 0.02 ml of 10, 50 or 100% isopropyl myristate (equivalent to 85, 425 or 850 mg/kg b.w.) in acetone twice a week. Control groups consisted of 135 untreated animals (negative), 50 treated with acetone (negative), and 50 treated with 7,12-dimethylbenzanthrancene (positive). Two benign skin tumours remote from the site of application developed in exposed mice. Internal tumours (including lymphomas, lung adenomas, liver haemangiomas and thymomas) were also found. However, no significant differences were observed between

exposed or negative control animals in the incidence of skin or internal tumours. (Stenbäck & Shubik 1974 – quoted from BIBRA 1988, CIR 1982 and IUCLID 2000).

No skin tumours were found when 0.1 ml of a 1% solution of isopropyl myristate (equivalent to 42.5-56.6 mg/kg b.w.) in acetone was applied once a week to the clipped skin of 20 Swiss albino mice for 18 weeks. Acetone was used as a negative control and 9,10-dimethyl-1,2-benzanthracene as a positive control. Five mice escaped and 4 died before the end of the study in the exposed group. (Giles & Byron 1968 – quoted from BIBRA 1988, CIR 1982 and IUCLID 2000).

In a lifetime study, groups of 5 New Zealand white rabbits were given skin applications on the interior left ear from the age of 8 weeks of 0.02 ml of 10, 50 or 100% isopropyl myristate (equivalent to 0.85, 4.25 or 8.5 mg/kg b.w.) in acetone twice a week. No tumours of the skin or internal organs developed. (Stenbäck 1977 – quoted from BIBRA 1988 and IUCLID 2000).

A 50% solution of isopropyl myristate (in ethanol according to BIBRA and IUCLID but in isopropyl alcohol according to CIR) significantly accelerated the carcinogenic activity of 0.15% benzo[a]pyrene (known skin carcinogen) on the skin of mice (Horton et al. 1966 – quoted from BIBRA 1988, CIR 1982 and IUCLID 2000).

44 Regulations

44.1 Ambient air

-

44.2 Drinking water

-

44.3 Food

JECFA has concluded that isopropyl myristate will not present safety concerns at the current level of intake as a flavouring agent. Isopropyl myristate has not been allocated an ADI owing to lack of data. (JECFA 2000).

44.4 Cosmetics

A major use of isopropyl myristate is in cosmetics and topical medicinal preparations (HSDB 2000). It is used as a solubilising, spreading, and penetrating agent in anhydrous skin lubricating lotions with high lanolin content (CIR 1982).

Isopropyl myristate is not mentioned in the statutory order on cosmetics, which means that it legally can be used in cosmetics in Europe without any limitations except as a colouring agent, a preservative, or a UV-filter (MM 2000).

44.5 Soil

-

44.6 Occupational Exposure Limits

-

44.7 Classification

-

44.8 IARC

-

44.9 US-EPA

-

45 Summary

45.1 Description

Isopropyl myristate is a colourless and practically odourless liquid with a low vapour pressure (9.35×10^{-5} mmHg). It is practically insoluble in water.

45.2 Toxicokinetics

Very little systemic absorption (0.25 %) of isopropyl myristate occurred when four monkeys were exposed for 5 seconds to the aerosols of an antiperspirant spray containing the chemical. Following dermal application, isopropyl myristate showed local penetration in rabbits and guinea pigs but no visible penetration into the skin or organs in hairless mice.

A study with subcutaneous injection in mice indicates that if absorbed, isopropyl myristate will be distributed into almost all organs.

Isopropyl myristate has been shown to enhance the penetration rate of several other chemicals through human skin. It also has been shown to enhance the *in vitro* dermal absorption of ethanol and butanol through hairless mouse skin.

45.3 Human toxicity

In studies with single or repeated dermal administration of undiluted isopropyl myristate, no or minimal skin irritation was seen. When applied in petrolatum under cover for 48 hours to the backs of 50 subjects, the highest non-irritant concentration of isopropyl myristate was 10%.

No evidence of phototoxicity or photo-allergic reactions was observed in two studies with human volunteers exposed dermally to isopropyl myristate and UV-light.

Several experimental studies with healthy volunteers have failed to detect any skin sensitising potential of isopropyl myristate. That was the case in e.g. a maximization test on 25 volunteers, where a pre-treatment of the forearm with an irritant was followed by five 48-hour applications of 20% isopropyl myristate in petrolatum with 24 hours between each application, and a challenge application was given 10 days later. However, one case report exists of strong positive patch test reaction to isopropyl myristate in a woman that for 6 months had used a feminine hygiene spray containing the chemical. A case report also exist of three hospital workers with hand eczema who had positive skin-prick test reactions to 20% isopropyl myristate.

No data have been found for reproductive and developmental effects, for mutagenic and genotoxic effects, or for carcinogenic effects.

45.4 Animal toxicity

45.4.1 Single dose toxicity

The acute toxicity of undiluted isopropyl myristate following oral or dermal application is low (oral LD_{50}-value >13.7 g/kg b.w. (rats), 42.4 - > 85 g/kg b.w. (mice); dermal LD_{50}-value > 5 g/kg b.w. (rabbits)).
No deaths or evidence of systemic toxicity occurred in rats exposed to aerosol antiperspirants containing 5-20% isopropyl myristate at nominal concentrations of 10-41 mg/l for 6.5 seconds/minute for an hour or for four 15-minute periods separated by 5 minutes.

Undiluted isopropyl myristate or product formulations containing it caused no to minimal irritation of rabbit skin and eyes in several studies performed according to the Draize primary skin irritation test, the Draize rabbit eye irritation test or slight modifications of them.

45.4.2 Repeated dose toxicity

Lung weights increased (but no histological changes were found) in guinea pigs exposed for an hour three times a day, seven days a week for 4 or 13 weeks to isopropyl myristate (in an aerosol antiperspirant) in concentrations from 10 mg/m^3.
In monkeys exposed for 13 weeks to isopropyl myristate (in an aerosol antiperspirant) in concentrations between 0.95 and 6.7 mg/m^3, histological examinations revealed a dose-related accumulation of macrophages within the alveolar and bronchiolar walls of the lungs. During the study, the treated monkeys wheezed and coughed. However, lung function tests were normal, as were the results of haematology, blood chemistry and urinalysis. No gross lesions were seen at necropsy, and organ weights were unaffected by treatment.
No toxicological significant effects were observed in rats and hamsters exposed to isopropyl myristate aerosols (in a complex fragrance mixture) at a maximal concentration of 0.16 mg/m^3 for 4 hours per day for 13 weeks.

No mortality, symptoms of intoxication, or substance-related injury of organs were recorded in rats gavaged with isopropyl myristate in doses up to 1000 mg/kg b.w. per day for 28 days or in rats fed isopropyl myristate in doses up to 2000 mg/kg b.w. per day for up to 16 weeks. Transient changes in some of the organ weights were observed in rats fed 3700 mg/kg b.w., and the blood levels of two liver enzymes and the proportion of neutrophilic leucocytes were increased in rats fed 7900 mg/kg b.w. per day of isopropyl myristate.

No systemic toxicity was observed in rabbits dosed dermally with up to 800 mg/kg b.w. of isopropyl myristate for about 20 days. Skin inflammation, mild cloudy swelling of the liver and leucocytosis were observed in rabbits dosed dermally with 5100 mg/kg b.w. of isopropyl myristate for the same period.

Isopropyl myristate was moderately to severely irritating (erythema, oedema, drying, cracking, scaling and fissuring) to rabbit and mice skin in studies lasting from 3 to 28 days. The animals were exposed dermally to 16-100% of isopropyl myristate. Microscopically, the treated skin of exposed animals showed acanthosis, parakeratosis, hyperkeratosis, and mixed inflammatory cell infiltration. In mice, the skin lesions tended to regress during continued

treatment while the lesions in rabbits regressed slowly after cessation of treatment.

Comedones were observed in rabbits, which had 1% or more of isopropyl myristate in propylene glycol applied to the ears twice daily for 2 weeks.

Applications of isopropyl myristate to the eyes of rabbits daily for 3 consecutive days apparently caused no irritation or slight irritation that had vanished after 7 days.

In 2 guinea pig sensitisation tests, isopropyl myristate suspended at 0.1% in physiologic saline showed no evidence of a sensitisation potential. However, isopropyl myristate was found to be weakly positive for skin sensitisation in the local lymph node assay in mice. The authors suggested that the positive result might be a false-positive response.

45.4.3 Toxicity to reproduction

No abnormalities were found in litters (examined grossly) of mice, which had 0.1 ml of 1% isopropyl myristate in acetone applied to the skin once weekly for 18 months.

45.4.4 Mutagenic and genotoxic effects

Isopropyl myristate tested negative in an Ames test with 5 strains of *Salmonella typhimurium* both with and without metabolic activation.

45.4.5 Carcinogenic effects

No significant differences were observed in the incidence of skin or internal tumours between negative control animals (mice and rabbits) and animals, which were given dermal applications of undiluted (or diluted) isopropyl myristate twice a week in lifetime (or shorter) studies.

A 50% solution of isopropyl myristate in ethanol (or isopropyl alcohol?) significantly accelerated the carcinogenic activity of 0.15% of the known skin carcinogen, benzo[a]pyrene, on the skin of mice.

46 Evaluation

The critical effect of isopropyl myristate is the local effects (mainly irritation) it might cause. In animals, undiluted isopropyl myristate was moderately to severely irritating to the skin following repeated exposure and at most slightly irritating to the eyes. However, in the majority of studies with human volunteers no or minimal skin irritation has been observed following repeated dermal administration of undiluted isopropyl myristate. In one human study the highest non-irritant concentration of isopropyl myristate was 10%. After application to rabbit skin comedones formation was observed. The wheezing and coughing of monkeys exposed by inhalation to a formulation containing isopropyl myristate is probably a result of respiratory tract irritation since the monkeys had normal lung function tests and the only histological observation was a dose-related accumulation of macrophages within the lungs. In addition, results of haematology, blood chemistry and urinalysis were normal as were organ weights. The only observed effect in guinea pigs exposed by inhalation to a formulation containing isopropyl myristate was increased lung weights but no histological changes were found. It should be noted, that in the inhalation studies, isopropyl myristate has only been tested as part of a formulation and not as the pure substance.

Experimental studies with healthy volunteers and the guinea pig sensitisation test have failed to show any potential for skin sensitisation. The local lymph node assay in mice was weakly positive for skin sensitisation. One human case report of a positive patch test to isopropyl myristate indicates that the chemical might sensitise humans via the skin. A few human case reports of positive skin-prick tests to isopropyl myristate also exist.

Isopropyl myristate was of low acute toxicity to laboratory animals by the oral and dermal routes. Low systemic toxicity was observed in animals exposed by inhalation, dermally, or orally to isopropyl myristate. Only when applied in high doses in the oral or dermal studies, repeated administration produced some changes in organ weights, increased blood levels of two liver enzymes, and/or leucocytosis. Only few studies exist on the absorption of isopropyl myristate. Following inhalation, the absorption was very low (less than 1%) in monkeys exposed for 5 seconds. Autoradiography of animals exposed dermally to isopropyl myristate only showed local penetration but no penetration into the organs. The low systemic toxicity could therefore possibly be explained in a low absorption of isopropyl myristate.

Only one developmental study and one mutagenic study have been performed. None of them caused any concern. However, the value of the developmental study is limited because of the relatively low dose of isopropyl myristate and the dermal route of application.

In three carcinogenic studies with dermal application of isopropyl myristate, no significant differences in the incidence of skin or internal tumours was observed between control and exposed animals. However, the chemical did accelerate the carcinogenic activity of a known skin carcinogen, benzo[a]pyrene.

It is a cause of concern that isopropyl myristate has the ability to enhance the dermal absorption of other chemicals since it, as an inert ingredient in pesticide formulations, might alter the absorption of the active substance or of other of the inert ingredients and thus possibly alter the toxicity of these chemicals.

47 References

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Appendix 7: Sodium ligninsulphonate

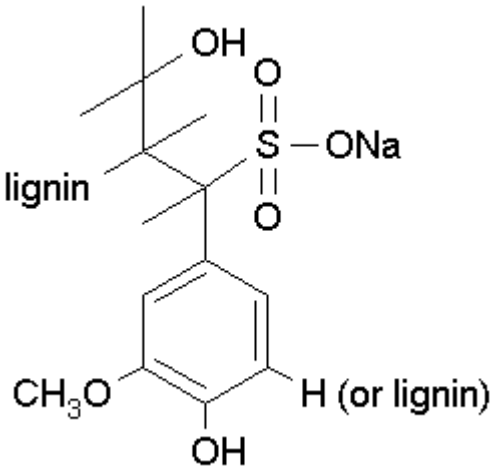
48 General description

Sodium ligninsulphonate is a by-product of the sulphite pulping process for paper. Commercial formulations are mixtures of sodium ligninsulphonates and wood sugars.

48.1 Identity

Molecular formula: -

Structural formula:



Molecular weight: 250-100000

CAS-no.: 8061-51-6

Synonyms: Lignosulphonic acid, sodium salt
Sodium lignosulphonate
Sodium lignosulphonic acid
Sulphonated lignin sodium salt

48.2 Physical / chemical properties

Description: Sodium ligninsulphonate is a light tan to dark brown nonhygroscopic powder with no pronounced odour.

Melting point: No definite melting point.

Boiling point: Decompose above 200 °C.

Density: -

Vapour pressure: -

Concentration of

saturated vapours:	-
Solubility:	Sodium ligninsulphonate forms colloidal solutions or dispersions in water. It is practically insoluble in all organic solvents. The pH of a 1:100 solution is approximately between 3 and 8.
References:	Aldrich Chemical Co., Inc. (2001), ChemFinder (2001), Committee on Food Chemicals Codex (1996), HSDB (2000), RTECS (2000), Ward & Tankersley (1980).

49 Toxicokinetics

No data have been found. Systemic effects following oral administration indicate that sodium ligninsulphonate is absorbed following administration by this route. However, because of the size of the molecule and its ionisation in solution, the absorption is probably limited.

50 Human toxicity

No data have been found.

51 Animal toxicity

51.1 Single dose toxicity

51.1.1 Inhalation

The LC_{50}-value reported for rats is greater than 480 mg/m^3 (RTECS 2000).

Sodium ligninsulphonate may be irritating to mucous membranes and upper respiratory tract (Aldrich Chemical Co., Inc. 2001).

51.1.2 Oral intake

The oral LD_{50}-value reported for mice is 6 g/kg (RTECS 2000) and for rats greater than 40 g/kg (Luscombe & Nicholls 1973).

51.1.3 Dermal contact

Sodium ligninsulphonate may cause eye and skin irritation (Aldrich Chemical Co., Inc. 2001).

51.1.4 Other routes

The intraperitoneal LD_{50}-value reported for rats is 260 mg/kg (RTECS 2000).

Sodium ligninsulphonate has caused drowsiness and muscle weakness; species, route of exposure, and dose levels were not stated (Aldrich Chemical Co., Inc. 2001).

An anticoagulant effect of sodium ligninsulphonate has been demonstrated in mongrel dogs; route of exposure and dose levels were not stated. The anticoagulant activity of sulphonated lignins was related to their molecular weight with higher molecular weight fractions possessing greater anticoagulant activity than the lower molecular weight fractions. (Loomis & Beyer 1953 – quoted from Luscombe & Nicholls 1973 and Samson & Hollis 1992).

A European patent exists for a method of making a sodium ligninsulphonate with anti-thrombotic activity. Low doses (7.5-15 mg/kg b.w.) of this sodium ligninsulphonate administered by intravenous injection significantly decreased the incidence of thrombus formation and also the amount of thrombus formed in a White New Zealand rabbit thrombosis model despite the lack of anti-coagulant effect (as measured by lack of significant effect on prothrombin time, activated partial thromboplastin time, thrombin time, reptilase time, fibrinogen level and platelet count). At higher dosages (intravenous injection of 0.19 mg/ml blood), sodium ligninsulphonate caused significant changes in the blood coagulation with prolonged bleeding time in greyhound dogs. Within the first 15 minutes behavioural changes (increased

salivation, unsteadiness and anxiety) were noted in the dogs. (Samson & Hollis 1992).

51.2 Repeated dose toxicity

51.2.1 Inhalation

No data have been found.

51.2.2 Oral intake

Groups of 20 male and 20 female Wistar rats were given AHR-2438B in their drinking water at levels of 0, 0.25, 2.5, 25 or 100 g/l (according to the authors equal to 0, 17, 168, 2830 or 10020 mg/kg b.w. per day in males and 0, 26, 283, 2420 or 9990 mg/kg b.w. per day in females) for 16 weeks. AHR-2438B is a purified sodium salt of a ligninsulphonate with an average molecular weight of 5000. No adverse effects on growth, organ weights, haematology, biochemistry, urine analysis and histopathology were observed at the three lowest dose levels. At the highest dose level, animals of both sexes showed skin lesions at the base of the tail. The lesions seemed to occur as a result of local irritation caused by adhering sticking faecal matter containing large quantities of the test material. Anaemia and raised leucocyte levels in the high dose animals was suggested to be a result of the presence of the tail lesions. In addition the high dose animals showed an increase in the absolute and relative weights of the liver, kidney and spleen, and male rats had a decreased weight gain. Histological changes were consistent with reticulo-endothelial activation in the liver and with vacuolar degeneration of the proximal convoluted tubules in the kidney. No ulcerative lesions were found in any section of the gastrointestinal tract. Blood coagulation times were unchanged throughout the period of dosing. (Luscombe & Nicholls 1973).

Groups of 5 male albino guinea pigs received as drinking fluid an aqueous solution of sodium ligninsulphonate at concentrations of 0, 10, 20, 50 or 100 g/l (according to the authors equal to 0, 1740, 2720, 1570 or 7780 mg/kg b.w. per day) for 1-5 weeks. At the end of the experiment, the stomach, duodenum and colon were examined histologically for ulcerations. Two animals in the 50 g/l dose group died after a week. All of the animals administered the two highest doses lost weight and some of them developed mild diarrhoea and had blood and mucus in the faeces. Animals receiving the two lowest doses gained less weight than controls but otherwise appeared clinically undisturbed. No ulcerations were found in the duodenum in any animal. However, ulcers of the upper part of the colon were found in 1-4 of the animals in each dosed group. The ulcers in the colon were more numerous (up to 100 or more) in the animals, which received the smaller concentrations of sodium ligninsulphonate. In the animals given higher concentrations, the ulcers numbered from 2 to 10. Ulcers of the upper two-thirds of the stomach developed in 4 animals in each of the two highest dosed groups. (Watt & Marcus 1976).

Groups of 8 male albino guinea pigs received as drinking fluid either water or a 1% aqueous solution of sodium ligninsulphonate (equivalent to 1700 mg/kg b.w. per day) for 2-6 weeks. None of the dosed animals developed diarrhoea. However, half of the dosed animals developed ulcers in the upper part of the colon. These animals had blood in the faeces and a lower weight gain than

the rest of the group. One of the guinea pigs with ulcers died on the 13th day of the experiment. (Marcus & Watt 1974).

Groups of 10 male Sprague-Dawley rats were fed either a control diet or a diet containing 3% of seven selected ligninsulphonate fractions for 14 days. In general, the diets containing ligninsulphonate were less digestible and caused a slightly lower pH value in the caecum and colon than the control diet. The caecum weighed more in rats fed ligninsulphonate than in control rats. Some of the ligninsulphonate fractions affected the short-chain fatty acid concentration and the concentration of two beneficial gastrointestinal tract bacteria. (Flickinger et al. 1998).

51.2.3 Dermal contact

A United States patent exist for a method of treating viral infections caused by Herpes simplex, types I and II, by topically applying a ligninsulphonate to the infected tissue. The inventors have examined different commercially available ligninsulphonates as well as fractions thereof. In general, the higher molecular weight fractions with the sugars removed are the most effective antiviral agents. The ligninsulphonates was shown to be effective against Herpes simplex virus in infected human cell lines in culture as well as in vaginal infections in female mice and guinea pigs. (Ward & Tankersley 1980).

51.2.4 Other routes

Female mongrel dogs with total gastric fistula were administered 500 mg of sodium ligninsulphonate (AHR-2438B) dissolved in 10 ml water twice during a two-hour period directly into the stomach through a cannula. Pepsin digestion was analysed using coagulated egg albumin as the substrate. AHR-2438B was an effective inhibitor of pepsin proteolysis. (Alphin et al 1971).

51.3 Toxicity to reproduction

No data have been found regarding reproductive and developmental effects following exposure by inhalation, oral administration, or dermal contact.

Sodium ligninsulphonate (as well as other surfactants) were tested for estrogenic activity in a recombinant yeast screen. The natural oestrogen 17β-oestradiol as well as other known chemicals with estrogenic activity was used as positive controls. Sodium ligninsulphonate did not possess estrogenic activity. (Routledge & Sumpter 1996).

51.4 Mutagenic and genotoxic effects

No data have been found.

51.5 Carcinogenic effects

No data have been found.

52 Regulations

No data have been found.

53 Summary

53.1 Description

Sodium ligninsulphonate is a light tan to dark brown nonhygroscopic powder with no pronounced odour. Commercial formulations are mixtures of sodium ligninsulphonates with different molecular weights and wood sugars.

53.2 Toxicokinetics

Systemic effects following oral administration indicate that sodium ligninsulphonate is absorbed following administration by this route.

53.3 Human toxicity

No data have been found.

53.4 Animal toxicity

53.4.1 Single dose toxicity

LD_{50}-values reported for oral administration of sodium ligninsulphonate ranged from 6 g/kg to greater than 40 g/kg for mice and rats, respectively, and the LC_{50}-value for inhalatory administration to rats was reported to be greater than 480 mg/m^3.

Sodium ligninsulphonate may irritate eyes, skin, and the upper respiratory tract. It has caused drowsiness and muscle weakness.

At injections of 7.5-15 mg/kg b.w., sodium ligninsulphonate significantly decreased the incidence and amount of thrombus formation in a rabbit model despite a lack of anti-coagulant effect. At higher dosages, an anticoagulant effect of sodium ligninsulphonate was demonstrated in dogs. Within the first 15 minutes behavioural changes were noted in the dogs.

53.4.2 Repeated dose toxicity

Guinea pigs exposed to sodium ligninsulphonate in the drinking water in doses at or above 1700 mg/kg b.w. per day for up to 6 weeks developed ulcers in the upper part of the colon. At higher doses, stomach ulcers as well as weight loss, diarrhoea and deaths also occurred.

In a dog model, sodium ligninsulphonate was an effective inhibitor of pepsin proteolysis.

Rats exposed to sodium ligninsulphonate in the drinking water in doses at about 10000 mg/kg b.w. per day for 16 weeks had histological changes of the liver and kidneys, and an increased weight of the same organs as well as the spleen. In addition, these rats had skin lesions at the base of the tail, anaemia

and raised leucocyte levels. Sodium ligninsulphonate caused no adverse effects at a dose level up to about 2500 mg/kg b.w. per day.

Ligninsulphonates may modify digestive physiology and gastrointestinal tract characteristics of rats.

In a United States patent, ligninsulphonates applied topically was shown to be effective against Herpes simplex virus in infected human cell lines in culture as well as in vaginal infections in female mice and guinea pigs.

53.4.3 Toxicity to reproduction

No data have been found regarding reproductive and developmental effects following exposure by inhalation, oral administration, or dermal contact.

Sodium ligninsulphonate did not possess estrogenic activity in a recombinant yeast screen.

53.4.4 Mutagenic and genotoxic effects

No data have been found.

53.4.5 Carcinogenic effects

No data have been found.

54 Evaluation

Based on the available knowledge, the critical effect of sodium ligninsulphonate in pesticide formulations is probably the irritative effects that it may cause on the eyes, the skin, and the upper respiratory tract. However, there is no information about the dose levels causing irritation, or whether the irritation was caused by the sodium ligninsulphonate powder or by the chemical in a solution.

Following oral intake of relatively high doses, sodium ligninsulphonate may affect the gastrointestinal tract. Pepsin proteolysis may be inhibited but sodium ligninsulphonate has also been shown to cause ulcers of the colon and stomach in guinea pigs, but not in rats at high doses. Guinea pigs are known to be highly susceptible to ulcerative disease of the colon (Marcus et al. 1974). This may explain the difference in effect on the gastrointestinal tract in rats and guinea pigs. At high doses, the liver and kidney of rats had histological changes, and sodium ligninsulphonate in the faeces irritated the skin at the base of the tail causing lesions, anaemia and an increased leucocyte level.

Following injections, sodium ligninsulphonate inhibited the blood coagulation in dogs and decreased the incidence and amount of thrombus formation in rabbits despite a lack of anti-coagulant effect. In rats, dosed orally with sodium ligninsulphonate, no anti-coagulant effect was noted. The differences in effect on blood coagulation in the different studies could have many explanations. The studies were performed with different species of which some may be more sensitive than others. In addition, different routes of administration were used. The bioavailability of sodium ligninsulphonate is most likely much higher following injection than following oral administration. However, no toxicokinetic data exist for sodium ligninsulphonate to be used for confirmation of this.

No carcinogenic or other chronic studies of sodium ligninsulphonate were found. Therefore it is not known whether sodium ligninsulphonate may cause cancer or other effects following chronic exposure.

Sodium ligninsulphonate did not possess estrogenic activity in a recombinant yeast screen. No data have been found regarding reproductive and developmental effects following exposure by inhalation, oral administration, or dermal contact.

In conclusion, it has been reported that sodium ligninsulphonate may irritate the eyes, skin and upper respiratory tract; however, there is no information about the dose levels causing irritation. Relatively high doses of sodium ligninsulphonate have to be ingested in order to cause toxic effects in short-term studies. In addition, oral intake and injections are not relevant routes of exposure for sodium lignosulphonate in pesticide formulations.
Based on the available data, the main concern regarding exposure to sodium ligninsulphonate in pesticide formulations is the irritation that it may cause. However, it should be noted that no data regarding effects following long-

term exposure as well as of reproductive and developmental effects are available.

55 References

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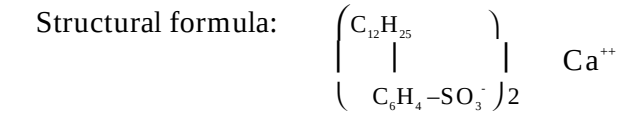
Appendix 8: Calciumdodecylbenzenesulphonate (CaDBS)

56 General description

56.1 Identity

Calciumdodecylbenzenesulfonate, CaDBS, is an anionic surfactant used as dispersing agent for pesticide formulation. It is often produced and used in a mixture of linear alkylbenzene sulfonates, LAS. Only very few data were found specifically on CaDBS.

Molecular formula:	$(C_{18}H_{29}SO_3^-)_2 \cdot Ca^{++}$
Structural formula:	
Molecular weight:	691.14
CAS-no.:	26264-06-2
Synonyms:	Calcium bis(dodecylbenzenesulfonate) Dodecyl-benzenesulfonic acid, calcium salt

56.2 Physical / chemical properties

Description:	The substance is reported to be a yellowish-brown liquid with a solvent odour or a light to white granular solid.
Melting point:	-
Boiling point:	-
Density:	-
Vapour pressure:	-
Concentration of saturated vapours:	-
Conversion factor:	1 ppm = 28.74 mg/m^3 (at 20°C and 760 mmHg) 1 mg/m^3 = 0.035 ppm
Solubility:	Water: yes
References:	RTECS (2001), HSDB (2001).

57 Toxicokinetics

Only one study was found on calciumdodecylbenzenesulfonate (CaDBS).

2.1 Absorption, distribution, metabolism and elimination

After oral administration to Wistar rats of 2 mg radiolabelled CaDBS, the radiolabel was detected in plasma after 15 minutes, reaching the maximum of 0.8 µg/g CaDBS at 2 hours, and then decreasing with time, the mean biological half-live being calculated to 10.9 hours.
Four hours after administration, the concentration of the radiolabel was high in the digestive tract, large intestine and in the urinary bladder (22.56, 43.24 and 34.89 µg/g salt, respectively). Concentrations were also high in the liver, kidney, testis, spleen and lung.
At 168 hours after administration, 51 % of the radioactivity was excreted in faeces and 50 % in urine. The 2 metabolites in urine and 2 of the 4 faecal metabolites were identified by thin layer chromatography to probably be sulfophenyl butanoic and sulfophenyl pentanoic acids. (Sunakawa et al. 1979 – quoted from WHO 1996).
The urinary metabolites point at CaDBS being degraded by ω-oxidation followed by β-oxidation of LAS (Michael 1968 – quoted from WHO 1996).

57.1 Mode of action

No data on CaDBS were found in the literature.

58 Human toxicity

No data were found in the literature on CaDBS.

59 Animal toxicity

Only data on acute oral toxicity were found on CaDBS.

59.1 Single dose toxicity

59.1.1 Oral intake

A rat LD_{50} of 4 g/kg is listed in RTECS. However, no details are given. (Yakkyoku, 1950 – quoted from RTECS 2001).

An LD_{50}-value in mouse of 3680 mg/kg is listed in RTECS. However, no details are given (Yakkyoku 1950 – quoted from RTECS 2001).

60 Regulations

60.1 Ambient air

Denmark (C-value): -

60.2 Drinking water

Denmark: -

60.3 Soil

Denmark: -

60.4 Occupational Exposure Limits

Denmark: -

ACGIH: -

Germany: -

60.5 EU Classification

-

60.6 IARC

-

60.7 US-EPA

-

61 Summary

Information on calciumdodecylbenzenesulfonate (CaDBS) is very scarce. Only one metabolism study and one report of acute oral toxicity in rats and mice were found in the literature.

61.1 Description

Calciumdodecylbenzenesulfonate is a yellowish liquid with a solvent odour or a white granular solid.

61.2 Toxicokinetics

CaDBS is readily absorbed from the gastrointestinal tract. No accumulation is seen in any organ. CaDBS is excreted equally via urine and faeces. From thin layer chromatography, the two urinary and two of the four faecal metabolites were identified to probably be sulfophenylbutanoic acid and sulfophenylpentanoic acid. The urinary metabolites point at CaDBS being degraded by ω-oxidation followed by β-oxidation of linear alkylbezene sulfonate, LAS.

61.3 Human toxicity

No data were available on CaDBS.

61.4 Animal toxicity

Low acute oral toxicity is reported from rats and mice with LD_{50} – values of 4000 mg/kg in rats and 3680 mg/kg in mice.

62 Evaluation

The database on calciumdodecylbenzenesulfonate is very limited. An evaluation of the toxicology of the substance is not possible on this basis.

The few available data show that the calciumdodecylbenzenesulfonate is readily absorbed from the gastroinstestinal tract and excreted equally through urine and faeces with no accumulation in the tissues.

Calciumdodecylbenzenesulfonate is of low acute oral toxicity.

No further data are available on the substance.

The toxicology of calciumdodecylbenzenesulfonate is expected to be related to the anion part of the substance, the identity of the salt being of secondary importance. A larger database is available on sodiumdodecylbenzenesulfonate and on linear alkylbenzene sulfonates (LAS) in general. Analogy considerations with LAS indicate that the critical effects of calciumdodecylbenzenesulfonate are probably skin, eye and respiratory tract irritancy. However, differences in chain length in the compounds studied may bias such structure-activity comparisons.

63 References

HSDB (2001). Calciumdodecylbenzenesulfonate. In: Hazardous Substances Data Base.

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Appendix 9: Ethylene glycol (EG)

64 General description

64.1 Identity

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

64.2 Physical / chemical properties

Description:	Ethylene glycol is a clear, colourless, slightly viscous, hygroscopic liquid with a sweet taste.
Melting point:	-13 °C
Boiling point:	197.56 °C (at 760 mmHg)
Density:	1.113 g/ml (at 20 °C)
Vapour pressure:	0.06 mmHg (8 Pa) (at 20 °C)
Concentration of saturated vapours:	79 ppm (204 mg/m^3) (at 20 °C and 760 mmHg) (calculated)
Conversion factor:	1 ppm = 2.58 mg/m^3 (at 20 °C and 760 mmHg) 1 mg/m^3 = 0.388 ppm
Solubility:	Water: Miscible. Miscible with lower aliphatic alcohols, glycerol, acetic acid, acetone and similar ketones, aldehydes and pyridine. Practically insoluble in benzene, chlorinated hydrocarbons, petroleum ether and oils.
$\log P_{octanol/water}$:	-1.93 to -1.36 (at 25 °C).

References: IUCLID (2000), ATSDR (1997), BUA (1991), ACGIH (2001).

65 Toxicokinetics

65.1 Absorption, distribution, and excretion

65.1.1 Inhalation

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

65.1.2 Oral intake

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

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

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

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

and was no longer detectable for the low dose group (10 mg/kg) by 12 hours post-dosing and by 24 hours for the 150 and 500 mg/kg dose groups. The estimated half-time for elimination of ethylene glycol from blood was less than 2 hours for all dose levels. Blood levels of glycolic acid increased to a peak at 3 hours post-dosing, except at the lowest dose level (10 mg/kg) where this metabolite could not be detected in the blood. The blood levels decreased by 24 hours post-dosing to undetectable levels. The concentrations of oxalic acid in the blood varied between undetectable and about 2 times the limit of quantification over the 24-hour collection period.
There were no substantial differences in the urinary elimination profiles between the pregnant and non-pregnant dose groups, at comparable dose levels. Urinary elimination of ethylene glycol and its metabolites demonstrated dose-dependency, with the high dose groups (2500 mg/kg) eliminating almost 70% of the administered dose in urine, compared with about 16% in the low dose groups (10 mg/kg). The shift in urinary elimination was mainly due to increased urinary glycolic acid and ethylene glycol, and not to increased elimination of oxalic acid.

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

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

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

65.1.3 Dermal contact

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

^{14}C-ethylene glycol (in acetone vehicle) was applied to the surface of three different fresh human skin samples at a dose of 8 µg/cm^2. After 24 hours of exposure, 18.3% of the applied dose was recovered in the receptor fluid (absorbed through the skin), 8.3% in the skin, and 12.5% in the skin surface wash (total accountability was approximately 39%). Individual differences existed for the three samples; average absorption was 26.6% relative to the 8 µg/cm^2 applied dose for a 24-hour exposure duration. This represented a flux of approximately 0.09 µg/cm^2/hour for ethylene glycol. The maximum flux observed was 0.25 µg/cm^2/hour. (Driver et al. 1993).

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

Following administration of 100 mg/kg undiluted radiolabeled ethylene glycol to mice using an occlusive bandage, 99.5% of the dose was recovered, with tissues and excreta accounting for 76.5%. Most was recovered as volatile organic radioactivity (25-39%) or as radioactive carbon dioxide (8-12%). Urine and faeces each accounted for another 4.9% of the dose. Tissue recoveries were less than 1% of the dose, while the residual carcass contained about 10-18% of the dose. Following application of 1000 mg/kg undiluted ethylene glycol or as an 50% aqueous solution, the total recovery was 89% of the dose with 84% in tissues and excreta, and approximately 7% in faeces, cage wash water, and carcass. According to the authors, mice absorbed 85-100% of the administered dermal dose. (Frantz et al. 1991 – quoted from ATSDR 1997).

65.2 Metabolism

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

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

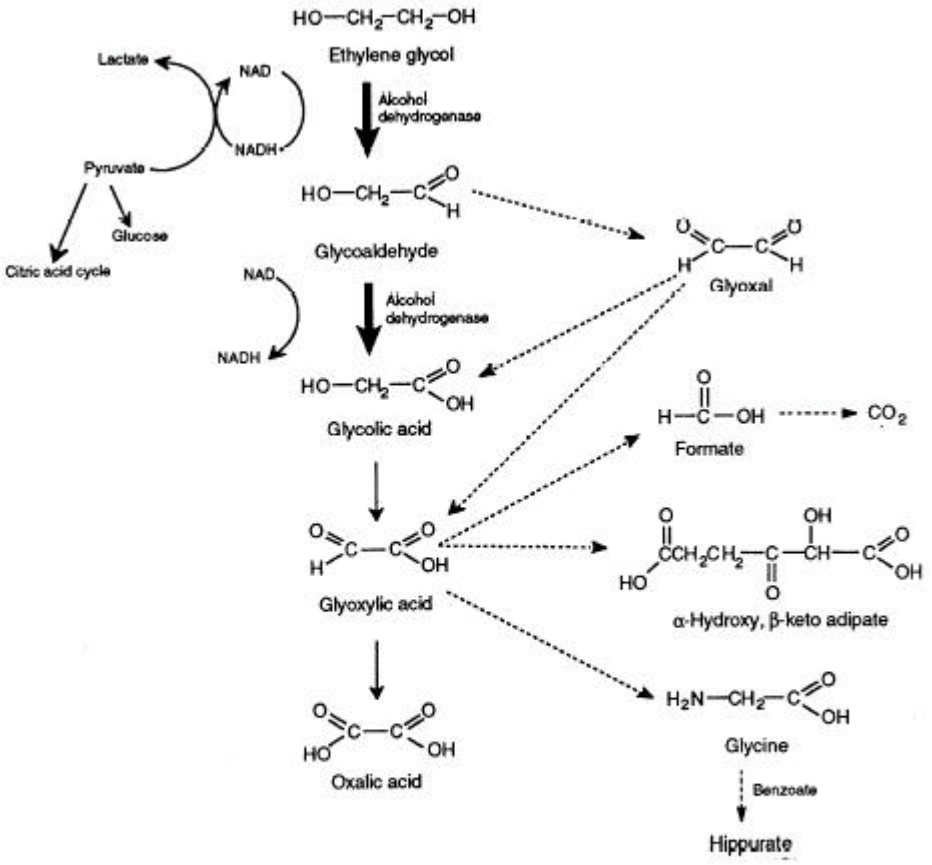
65.3 Mode of action

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

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

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

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



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

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

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

The third stage is known as the "renal failure" stage and occurs 24 to 72 hours after ingestion. This stage ischaracterised by profound acidosis caused, according to LaKind et al. (1999), primarily by the accumulation of the metabolites glycolic acid and glyoxylic acid.
Recent studies of cases of human ethylene glycol poisoning have demonstrated that the major determinant of the metabolic acidosis is the degree of glycolic acid accumulation and glycolate accumulation correlates with the increase in anion gap or decrease in arterial bicarbonate concentrations in humans, as well as in animals. Several studies have suggested accumulation of glyoxylate, although at much lower levels (60 µM) than that of glycolate (15 mM). Glycolate is less toxic *in vitro* than either glycolaldehyde or glyoxylate. Whether the levels of glyoxylate that have been measured in human cases can contribute to the clinical features cannot yet be determined. (Jacobsen & McMartin 1997).
Calcium oxalate precipitation within the renal tubules has long been accepted as an important pathogenic factor for the development of renal toxicity;

however, the mechanism of the renal toxicity is not yet known and there is no evidence directly linking oxalate precipitation with development of renal tubular necrosis. Furthermore, the renal damage can occur at levels of exposure where no or few crystals of oxalate are detected. Therefore, the renal toxicity has also been suggested to occur from a metabolite-induced cytotoxicity. (Jacobsen & McMartin 1997, LaKind et al. 1999).
Glycolic acid and glyoxylic acid react with bicarbonate causing a decrease in the pH in body fluids, particularly in blood. An elevated anion gap develops, and the serum osmolal gap across cells increases, resulting in renal oedema that compromises intrarenal blood flow and promotes renal failure. Blood phosphorous (inorganic) levels are increased due to uncoupling of oxidative phosphorylation, and, as a result, blood calcium levels are lowered. Acidosis results in oliguric or anuric renal failure with renal changes. (LaKind et al. 1999).

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

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

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

66 Human toxicity

66.1 Single and repeated dose toxicity

66.1.1 Inhalation

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

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

66.1.2 Oral intake

There are numerous case reports in the literature of poisoning in humans due to accidental or intentional ingestion of ethylene glycol. In the United States, 6 to 60 deaths/year have been attributed to ethylene glycol ingestion. (LaKind

et al. 1999, Jacobsen & McMartin 1997, ACGIH 2001, BUA 1991, ATSDR 1997).

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

The oral dose of ethylene glycol required to cause death in humans is not well defined in the literature (ATSDR 1997).
The minimal lethal dose has been estimated, based on poisonings from accidental or intentional ingestion, at about 100 ml (about 111 g corresponding to about 1.6 g/kg b.w. for an adult) (LaKind et al. 1999, ACGIH 2001, BUA 1991, Cavender & Sowinski 1994, ATSDR 1997, NTP 1993).

However, early diagnosis and appropriate therapeutic measures can significantly reduce mortality, so that even doses of 970 ml (about 1080 g corresponding to 15 g/kg b.w. for an adult) have been survived (Gaultier et al. 1976 – quoted from BUA 1991).

66.1.3 Dermal contact

The potential for subchronic human exposure to ethylene glycol (as well as other substances) to induce effects in motor-servicing workers who performed various tasks that brought them into contact with motor vehicle antifreeze has been studied by Laitinen et al. (1995 – quoted from LaKind et al. 1999). Individual exposures were intermittent, but the workers had been performing their jobs for up to 23 years. Exposures resulted in enhanced urinary excretion of ethylene glycol by workers compared with office worker controls, as well as enhanced ammonia excretion (typical of chronic acidosis associated with ethylene glycol exposure). Because measured airborne levels of ethylene glycol were below the detection limits, the authors concluded that exposure occurred primarily via dermal contact with ethylene glycol during extended contact with the antifreeze.

66.1.4 Skin irritation

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

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

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

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

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

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

66.1.5 Eye irritation

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

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

66.1.6 Skin sensitisation

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

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

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

66.2 Toxicity to reproduction

No data have been found.

66.3 Mutagenic and genotoxic effects

No data have been found.

66.4 Carcinogenic effects

No data have been found.

67 Animal toxicity

67.1 Single dose toxicity

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

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

67.1.1 Inhalation

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

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

67.1.2 Oral intake

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

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

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

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

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

67.1.3 Dermal contact

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

67.1.4 Skin irritation

Guillot et al. (1982) have evaluated skin irritation in rabbits by determination of the primary cutaneous irritation index under patch-test and by determination of the cumulative irritation index after repeated exposure (6 weeks, MMII) according to official French methods but with some complements or modifications.
In the primary cutaneous irritation test (the experimental procedure is quoted from BUA 1991), 0.5 ml of neat ethylene glycol was applied to undamaged and scarified skin of 6 rabbits and covered with an occlusive dressing. The duration of exposure was 23 hours and assessments of the effects were made one hour and 48 hours after removal of the dressing. Ethylene glycol showed a primary irritation index of 0.08 (an index of up to 0.5 is considered non-irritant and an index of 0.5-2.0 as slightly irritant).
In the cumulative cutaneous irritation test (the experimental procedure is quoted from IUCLID 2000), three rabbits were treated daily with 2 ml of neat ethylene glycol or with a 10% aqueous solution for 6 weeks. Ethylene glycol undiluted showed a mean maximum irritation index of 0.47 interpreted as 'well tolerated' and the 10% solution an index of 0 interpreted as 'very well tolerated (the maximum index that could be scored is not stated; the highest index scored was 1.83 for polypropylene glycol and interpreted as 'relatively well tolerated).
Ethylene glycol was tested for skin irritative properties according to the Draize test in female New Zealand rabbits. Skin response was evaluated 24 and 72 hours after treatment. Ethylene glycol had a low potential for skin irritation (score 0.4, with a score of <2.0 meaning mild or no irritation). (Clark et al. 1979).

67.1.5 Eye irritation

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

Rats, guinea pigs, rabbits, dogs, and monkeys were exposed to a concentration of 57 mg/m^3 of ethylene glycol (vapour) 8 hours a day, 5 days per week for 6 weeks; none of the animals showed any signs of ocular irritation. In rabbits exposed similarly to 10 mg/m^3, mild conjunctivitis was noted in one eye of each of 2 rabbits during the 4^{th} and 5^{th} weeks, which persisted until the end of the exposure; each of these rabbits also developed a small lesion over the irritated eye. These signs were, according to the authors,

probably brought on by accidental trauma, which may have been aggravated by the exposure. See also part 4.2.1. (Coon et al. 1970).

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

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

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

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

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

67.1.6 Sensitisation

No data have been found.

67.2 Repeated dose toxicity

67.2.1 Inhalation

67.2.1.1 Rats

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

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

67.2.1.2 Mice

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

BUA 1991, ACGIH 2001, Cavender & Sowinski 1994 and from IUCLID 2000).

67.2.1.3 Guinea pigs

Guinea pigs (15 male and female animals per group, no information about distribution between sexes) were exposed to ethylene glycol (vapour) continuously at a concentration of 12 ± 2 mg/m^3 for 90 days (continuous study), or at concentrations of 10 ± 1 or 57 ± 14 mg/m^3 for 8 hours a day, 5 days per week for 6 weeks (repeated study). The control group consisted of 73 animals. (Coon et al. 1970).
In the continuous study, 3 animals died during exposure. All haematological data were within normal limits. Observations during necropsy revealed normal organs and tissues. Histopathological examination showed inflammatory changes in the lungs of exposed animals and to a lesser degree in controls. Occasional foci of inflammatory cells were seen in kidneys from several animals, but this was not interpreted, by the authors, as being specific chemically induced changes.
In the repeated study, there were no deaths at both exposure levels and all haematological values were within normal limits. At 10 mg/m^3, histopathological examination revealed hepatic fatty changes in 2 of 8 animals and focal necrosis in the liver in 1 of 8 animals; focal necrosis of the liver was also seen in 1 of 3 control guinea pigs. At 57 mg/m^3, histopathological examinations revealed non-specific inflammatory changes in the lungs and occasionally the hearts of exposed animals. The livers of 1 of 8 animals revealed areas of focal necrosis; this was not considered, by the authors, to be chemically induced. None of the animals showed any signs of ocular or nasal irritation.

67.2.1.4 Rabbits

Male New Zealand albino rabbits (3 animals per group) were exposed to ethylene glycol (vapour) continuously at a concentration of 12 ± 2 mg/m^3 for 90 days (continuous study), or at concentrations of 10 ± 1 or 57 ± 14 mg/m^3 for 8 hours a day, 5 days per week for 6 weeks (repeated study). The control group consisted of 12 animals. (Coon et al. 1970).
In the continuous study, 1 rabbit died during exposure. All haematological data were within normal limits. Observations during necropsy revealed normal organs and tissues. Histopathological examination showed inflammatory changes in the lungs of exposed animals and to a lesser degree in controls. One rabbit had hamartomatosis (a benign tumour-like nodule) in liver bile ducts, but this was not interpreted, by the authors, as being a specific chemically induced change. Moderate to severe eye irritation was observed and erythema, oedema, and discharge began after 3 days of exposure, the oedema being severe enough to result in virtual closure of the eyes.
In the repeated study, there were no deaths at both exposure levels and all haematological values were within normal limits. At 10 mg/m^3, mild conjunctivitis was noted in one eye of each of two rabbits during the 4th and 5th weeks, which persisted to the end of the exposure; each of these rabbits also developed a small lesion over the irritated eye. These signs were, according to the authors, probably brought on by accidental trauma, which may have been aggravated by the exposure. At 57 mg/m^3, histopathological examinations revealed non-specific inflammatory changes in the lungs and

occasionally the hearts of exposed animals. None of the animals showed any signs of ocular or nasal irritation.

67.2.1.5 Dogs

Male Beagle dogs (2 animals per group) were exposed to ethylene glycol (vapour) continuously at a concentration of 12 ± 2 mg/m^3 for 90 days (continuous study), or at concentrations of 10 ± 1 or 57 ± 14 mg/m^3 for 8 hours a day, 5 days per week for 6 weeks (repeated study). The control group consisted of 12 animals. (Coon et al. 1970).
In the continuous study, all haematological data were within normal limits. Observations during necropsy revealed normal organs and tissues. Histopathological examination showed inflammatory changes in the lungs of exposed animals and to a lesser degree in controls.
In the repeated study, there were no deaths at both exposure levels and all haematological values were within normal limits. At 10 mg/m^3, histopathological examination revealed mild congestion in the spleens of both exposed animals. At 57 mg/m^3, histopathological examinations revealed non-specific inflammatory changes in the lungs and occasionally the hearts of exposed animals. None of the animals showed any signs of ocular or nasal irritation.

67.2.1.6 Monkeys

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

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

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

67.2.2 Oral intake

67.2.2.1 Rats

Sprague-Dawley rats (10 males and females per group) received ethylene glycol in their drinking water for 10 or 90 days. In the 10-day study, the concentrations were 0, 0.5, 1.0, 2.0, or 4.0%. Based on a water consumption of 100 ml/kg b.w. per day, these concentrations correspond to 0, 554, 1108, 2216, or 4432 mg/kg b.w./day. In the 90-day study, females received the same concentrations in the drinking water whereas males received 0, 0.25 (227 mg/kg b.w./day), 0.5, 1.0, or 2%. (Robinson et al. 1990 – quoted from BUA 1991 and from IUCLID 2000).
In the 10-day study, high-dose (4%) male rats showed a strong reduction in body weight and in heart, liver, spleen and thymus weights. High-dose female rats had changes in blood parameters (decreased haemoglobin and haematocrit and markedly reduced numbers of erythrocytes and leucocytes) and decreased thymus weights. In male animals at the 2 and 4% dose levels, histopathological examination of the kidneys revealed a dose-dependent increase in the incidence and severity of kidney damage, which involved dilation, degeneration and necrosis of the renal tubules, acute inflammation and intratubular deposits of proteinaceous material and calcium oxalate crystals. In females, the effects on kidneys were limited to tubular dilation and intratubular deposition of proteinaceous material at the highest dose level. A NOAEL of 1% ethylene glycol in the drinking water (1108 mg/kg b.w./day) for 10 days of exposure can be considered.
In the 90-day study, 8 female and 2 male animals from the high-dose group (4% and 2%, respectively) died. Body weight gain was markedly reduced in high-dose (2%) males. In females, the leucocyte count was significantly reduced at 0.5, 2. and 4%. In male animals at the 1 and 2% dose levels and in female animals at the 2 and 4% dose levels, histopathological examination of the kidneys showed a dose-dependent increase in the incidence and severity of kidney damage, which involved dilation, degeneration and inflammation of the renal tubules, and renal pelvis. A NOAEL of 0.5% ethylene glycol in the drinking water (554 mg/kg b.w./day) for 90 days of exposure can be considered based on the renal effects in male animals.

In a 13-week study, ethylene glycol was administered in the feed to Fischer 344/N rats (10 males and 10 females) at dose levels of 0, 0.32, 0.63, 1.25, 2.5, or 5.0% (equivalent to 0, 160, 315, 625, 1250, or 2500 mg/kg b.w./day assuming than an adult rat consumes 50 g feed/kg b.w./day). Four high-dose male rats died. The body weight gain for male rats in the 2.5 and 5% dose groups was depressed by more than 10% when compared to the control group. The relative kidney weight was significantly increased in both male and female rats in the two highest dose groups (2.5 and 5%) and the relative thymus weight was significantly decreased in high-dose (5%) male rats. Serum urea nitrogen and serum creatinine levels were significantly elevated in male animals at the two highest dose levels (2.5 and 5%) and kidney lesions were observed in all male rats at these dose levels. Damaged kidneys contained calcium oxalate crystals mainly located within tubular lumens in the renal cortex, but were also occasionally found in tubules in the medulla. Crystals were also observed in the urinary bladder, the urethral lumen, and in the brain of some high-dose male rats (5%). Severe toxic nephrosis (distension and dilation of renal tubules, necrosis and regeneration of tubule epithelium, thickening of basement membranes, and fibrosis) was diagnosed

in the 5% male dose group and moderate toxic nephrosis in the 2.5% male dose group. Toxic lesions in the kidneys of female rats, which were multifocal and tended to be subcapsular, were only observed in the high-dose group (5%); no crystals were observed. Based upon the renal effects observed in male rats, a NOAEL of 1.25% (equivalent to 625 mg/kg b.w./day) can be considered. According to the author, this dose level corresponds to 0.6 to 1.0 g/kg b.w./day. (Melnick 1984).

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

Fischer 344 rats (130 males and females per group) were fed diets (0.1, 0.5, or 2.5%) yielding approximate dosages of 40, 200, or 1000 mg/kg b.w. per day of ethylene glycol for 24 months. Two untreated control groups were included. (DePass et al. 1986a).
High-dose (2.5%) male rats had a significant increase in mortality rate from the 9th through the 16th month of the study and the last high-dose male rat died after 474 days of the study. Other significant findings in high-dose male rats only included: increased water intake; decreased body weight gain; decreased red blood cell count, haematocrit, and haemoglobin concentration; increased neutrophilic leukocyte count; increased serum levels of creatinine and urea; increased urine volume; increased absolute and relative kidney weights after 6 and 12 months of treatment; and decreased absolute and relative liver weights after 12 months of treatment. In female rats of the highest dosage group, kidney weights were increased after 6 and 18 months of treatment, but not after 12 months. Calcium oxalate crystals were found in urine samples from all but one male of the high-dose animals after 12 months of treatment; at 18 and 24 months, all but one of the high-dose female rats had calcium oxalate crystals.
At the 6-month sacrifice, the incidence of renal lesions (tubular hyperplasia, tubular dilation, peritubular nephritis, and calcium oxalate crystalluria) was significantly increased in the high-dose males (2.5%) and calcium oxalate crystals were present in the urinary bladder in two high-dose male rats; these conditions were absent in the other male dose groups and in females.
At the 12-month sacrifice, all high-dose males (2.5%) had chronic nephritis (multiple severe histopathological changes including tubular dilation and proteinosis, glomerular shrinkage, tubular cell hyperplasia, and chronic interstitial nephritis) with calcium oxalate crystalluria and 50% had oxalate crystals in the urinary bladder; these findings were not present in males at the lower dose levels or in females.
By the time of the 18-month sacrifice, all of the high-dose males (2.5%) had died or were sacrificed moribund. In most of these animals, oxalate nephrosis was the primary cause of death. Kidneys from these animals had tubular obstruction by large crystals with secondary tubular dilation and

degeneration. Calculi were sometimes found within the renal pelvic space, urethras, and urinary bladder, often with an associated hydronephrosis. Extrarenal lesions included cellular hyperplasia of the parathyroid glands and a significant increase in the incidence of splenic haemosiderosis.
Among the females sacrificed at 2 years, the incidences of haemosiderosis of the mesenteric lymph node (2.5%) and mild fatty metamorphosis of the liver (0.5 and 2.5%) were significantly increased. No biologically significant lesions were observed in the male rats sacrificed at 2 years.
Based on the effects observed in the kidneys, a NOAEL of 200 mg/kg b.w. per day is considered. For females, a NOAEL of 40 mg/kg b.w. per day can be considered based on the effects observed in the liver of high-dose females.

Sprague-Dawley rats (16 male and female animals per group) received ethylene glycol in their diet at concentrations of 0, 0.1, 0.2, 0.5, 1.0, or 4.0% (equivalent to 0, 50, 100, 250, 500 or 2000 mg/kg b.w./day assuming than an adult rat consumes 50 g feed/kg b.w./day). An increased mortality rate was observed in males of the two highest dose groups (1 and 4%) and in females of the highest dose group (4%). A significant decrease in growth was observed in male rats from week 16 at 4% and after week 70 at 1%, and in female rats after about one year at 4%. A significantly increased water consumption was noted in male rats at 1 and 4% and in female rats at 4%, and in these dose groups protein was found in the urine. The mean terminal kidney, lung and liver weights were lower (whether the reduction is significant is not stated in the publication) in male animals at 0.1% and above compared to controls. Calculi and crystal deposition in the kidneys were observed in male rats from 0.5% (only 1/16 animals at 0.5%) and in female rats at 4% and from 1%, respectively. Morphological changes in the kidneys also included degeneration of the tubular epithelium, manifested mainly as cytoplasmic vacuolisation; however, no details are provided at which dose levels these morphological changes were observed. According to the author, it appears probable that the NOEL is no higher than 0.2% (equivalent to 100 mg/kg b.w./day) ethylene glycol in the diet but may be less. (Blood 1965).

67.2.2.2 Mice

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

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

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

67.2.2.3 Monkeys

Rhesus monkeys (2 males and 1 female) were given ethylene glycol in their diet at 0.2% (males) or 0.5% (female) for 3 years. No calculi or abnormal calcium deposits were demonstrated by X-ray examination and no other effects on organs and tissues were observed at the histopathological examination. (Blood et al. 1962).

67.2.3 Dermal contact

No data have been found.

67.3 Toxicity to reproduction

67.3.1 Inhalation

CD rats and CD-1 mice (25 animals per group) were exposed to a respirable ethylene glycol aerosol (mass median aerodynamic diameter (MMAD) 2.3 µm) on gestational days (GD) 6 to 15, 6 hours a day, by whole-body exposures (target concentrations: 0, 150, 1000, or 2500 mg/m^3). Rats were sacrificed on gestation day 21, and mice on gestation day 18. (Tyl et al. 1995a).
The total concentrations of ethylene glycol (aerosol plus vapour) were 79, 89, or 84% of target concentrations, respectively (119 ± 13, 888 ± 149, or 2090 ± 244 mg/m^3). The vapour phase was 82% of the total concentration for the 150 mg/m^3 group; for the higher aerosol concentrations, the vapour phase was 19-20% of the total concentration.

All rat dams survived to scheduled termination. Food and water consumption, maternal body weights and weight gain, and maternal organ weights (other than liver) were unaffected by exposure. A significant increase in absolute and relative liver weight was observed at 2500 mg/m^3. Gestational parameters (pre- and post-implantation loss, live foetuses/litter, sex ratio, and foetal body weight/litter) were unaffected by exposure. There was no significant increase in the incidence of any individual malformation, in the incidence of pooled external, visceral, or skeletal malformations, or in the incidence of total malformations by foetus or by litter. There were no significant increases in the incidence in any foetal external or visceral variations. There was some evidence of treatment-related reductions in ossification of the foetal skeleton, including an increase in the incidence of poorly ossified humerus (forelimb) and zygomatic arch (face) at the highest exposure level (2500 mg/m^3) and an increase in the incidence of poorly ossified metatarsals and proximal phalanges of the hindlimb at 1000 mg/m^3 but not at 2500 mg/m^3. According to the authors, the NOAEL was 1000 mg/m^3 for maternal and 150 mg/m^3 for developmental toxicity.
All mouse dams survived to scheduled termination. One female at 2500 mg/m^3 had a totally resorbed litter at termination; all other pregnant animals had one or more live foetuses at sacrifice. Clinical signs included only wet fur for all ethylene glycol exposed mice. Reduced body weight and body weight gain were observed at 1000 and 2500 mg/m^3 both during and after the exposure period. Gravid uterine weight was also reduced at the two highest exposure levels so that body weight corrected for gravid uterine weight was unaffected by treatment. Liver and kidney weights were unaffected by treatment. The following gestational parameters were affected: the number of viable implantations per litter was reduced at 2500 mg/m^3, the number of nonviable implantations per litter was increased at 1000 and 2500 mg/m^3, the number of early resorptions was increased (not significantly) at 2500 mg/m^3; the sex ratio was reduced at 1000 mg/m^3 but not at 2500 mg/m^3, and the foetal body weights per litter (male, female, and total) were reduced at 1000 and 2500 mg/m^3. There was a significant increase in the incidence of a number of external, visceral, and skeletal malformations at 1000 and 2500 mg/m^3, as well as in the incidence of pooled external, visceral, and skeletal malformations, and in the incidence of total malformations. Malformations were found in the head (exencephaly), face (cleft palate, foreshortened and abnormal face, and abnormal facial bones), and skeleton (vertebral fusions, and fused, forked, and missing ribs). The incidences of many foetal variations were also increased at the two highest dose levels, but only a few at the lowest dose level (150 mg/m^3). According to the authors, the NOAEL was 150 mg/m^3 for maternal and at or below 150 mg/m^3 for developmental toxicity.

Ethylene glycol was teratogenic to mice by whole-body exposure to aerosol (1000 and 2500 mg/m^3), see the study described above. According to the authors, the results were confounded by possible exposure from ingestion after grooming and/or from percutaneous absorption. Therefore, CD-1 mice (30 animals per group) were exposed to ethylene glycol aerosol (MMAD 2.6 ± 1.7 µm) on gestational days 6 to 15, 6 hours a day, by nose-only (target concentrations: 0, 500, 1000, or 2500 mg/m^3) or whole-body exposures (target concentrations: 0 or 2100 mg/m^3). Control environments were water aerosol (4200 mg/m^3 for nose-only and 2700 mg/m^3 for whole-body). On gestation day 18, the dams were sacrificed. (Tyl et al. 1995b).

In either exposure regimen, body weights, body weight gain (absolute or corrected for the weight of the gravid uterus), and liver weights (absolute or relative) were unaffected by treatment. Microscopic examination of maternal kidneys indicated no treatment-related incidence or severity of renal lesions. There were no treatment-related differences among groups in the number of corpora lutea per dam, the number of total or viable implantations per litter, or on foetal sex ratio. There was no significant increase in the incidence of any individual external or visceral malformation or variations, or of all external or visceral malformations or in total variations.
In the nose-only experiment, maternal clinical signs associated with the animals struggling while in restraint were observed. Maternal kidney weights were increased at concentrations of 1000 (absolute) and 2500 (absolute and relative) mg/m^3 and there was a trend toward reduced gravid uterine weight at these concentrations as well. The percentage of live foetuses was reduced slightly (but not significantly) at the two highest concentrations. At the highest concentration (2500 mg/m^3), the foetal body weights per litter were significantly reduced and the incidences of one skeletal malformation (fused ribs) and 18 skeletal variations were increased. According to the authors, exposure of CD-1 mice to a respirable ethylene glycol aerosol during organogenesis by nose-only inhalation resulted in minimal maternal toxicity at 1000 and 2500 mg/m^3 and developmental toxicity at 2500 mg/m^3; the NOAEL was 500 mg/m^3 for maternal and 1000 mg/m^3 for developmental toxicity.
In the whole-body dose group (2100 mg/m^3), the gravid uterine weight, the percentage of live foetuses (due to an increase in late resorptions), and the foetal body weights per litter were significantly reduced. There was an increase in the incidence of a number of skeletal malformations, including fused thoracic arches, extra thoracic arches, fused lumbar arches and centra, fused ribs, and extra ribs between existing ribs, as well as in the incidence of pooled skeletal malformations and a total of 63 skeletal variations exhibited significantly increased incidences.

67.3.2 Oral intake

67.3.2.1 Rats

To assess the possible effects of ethylene glycol on reproductive performance, a three-generation reproduction study was performed in Fischer 344 rats. Ethylene glycol was administered in the diet at approximate doses of 40, 200, or 1000 mg/kg per day (weekly calculated doses ranged from 40-50, 200-300, or 1000-1300 mg/kg/day for males and from 40-60, 200-300, or 900-1200 for females). Two untreated diet control groups were included. At approximately 100 days of age, 10 males were mated to 20 females in each dose group. Necropsies and microscopic examinations of several organs and tissues were performed (5 animals of each sex from each dose level) on the F_2 parents and on the F_3 weanlings. There was no treatment-related effect on body weight gain or diet consumption, nor was there any mortality among parental rats. No treatment-related effect was observed for any of the reproductive indices (fertility index, gestation index, gestation survival index, survival indices, and days from first mating to litter) for all three generations, or on neonatal body weight at days 4, 14, or 21 postpartum. The histopathological examinations revealed no treatment-related findings in the F_2 parents and in the F_3 weanlings, including kidney damage. The NOAEL for reproductive toxicity was 1000 mg/kg b.w./day. (DePass et al. 1986b).

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

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

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

Price et al. (1988 – quoted from BUA 1991 and from IUCLID 2000) also have administered ethylene glycol by gavage to CD rats from gestation day 6 to 20 at doses of 0, 250, 1250, or 2250 mg/kg b.w./day. The offspring were reared by untreated dams and examined in respect to postnatal growth and survival rate, bodily development, the onset of sexual maturity, locomotive activity, and performance in a complex test of learning ability. At the two highest dose levels, there was a significant increase in the duration of gestation; microscopic examination revealed an increase in kidney damage.

At the highest dose level, maternal body weight gain was reduced. In offspring from dams exposed at the highest dose level, foetal mortality was increased, there was a reduction in live litter size and in the weight of the neonates, and there was a significant increase in the number of malformations (particularly in the form of hydrocephaly and abnormalities of the axial skeleton). Prenatal exposure to ethylene glycol had no adverse effect on postnatal learning behaviour.

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

67.3.2.2 Mice

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

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

In order to determine a NOAEL for developmental toxicity of ethylene glycol administered orally, CD-1 mice received 0, 50, 150, 500, or 1500 mg/kg b.w. per day by gavage on gestation days 6 to 15. There were no apparent treatment-related effects in dams. Effects observed in foetuses at 1500 mg/kg/day included reduced body weights; fused ribs and arches; poor ossification in thoracic and lumbar centra; and increased occurrence of an extra 14^{th} rib. At 500 mg/kg/day, slight reductions in foetal body weight and increased incidences of extra ribs were observed. According to the authors,

the NOAEL for developmental toxicity was 150 mg/kg b.w./day. (Neeper-Bradley et al. 1995 – abstract quoted from TOXLINE 1995-1998).

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

67.3.2.3 Rabbits

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

67.3.3 Dermal contact

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

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

67.4 Mutagenic and genotoxic effects

67.4.1 *In vitro* studies

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

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

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

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

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

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

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

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

A negative result was also obtained for chromosome aberrations when ethylene glycol was tested in Chinese Hamster Ovary cells with and without metabolic activation (Galloway et al. 1985,1987 – quoted from NTP 1993, Ballantyne 1985, Slesinski et al. 1986 – both quoted from IUCLID 2000 and from BUA 1991). When tested in another assay at concentrations from 10 to 100 mg/ml, ethylene glycol did not produce an increase in the incidence of chromosome aberrations; according to IUCLID, the result of the assay was ambiguous (Union Carbide Corporation 1985 – quoted from IUCLID 2000).

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

67.4.2 *In vivo* studies

Ethylene glycol has been tested for mutagenic effects in the micronucleus assay in Swiss mice by oral administration of 2.5, 3.125, 6.25, or 12.5 ml/kg b.w. (corresponding to 2.8, 3.5, 7.0, or 13.9 g/kg b.w.; 4, 4, 4, or 2 animals in the dose groups, respectively) and by intraperitoneal injection of 1.25, 2.5, or 6.25 ml/kg b.w. (corresponding to 1.4, 2,8, or 7.0 g/kg b.w.; 5, 5, or 1 animal in the dose groups, respectively). A control group of 19 animals was included. Animals in the high-dose oral group (13.9 g/kg b.w.) showed toxic effects. At all, but the lowest oral dose level (2.8 g/kg b.w.), there was an increase in the numbers of micronuclei in polychromatic erythrocytes when compared to the control. According to IUCLID, a weak positive but not dose dependent result was obtained. (Conan et al. 1979).

When tested in mice for chromosome aberrations following an intraperitoneal injection of 2.5 ml/kg b.w., a negative result was obtained (Conan et al. 1979 – quoted from BUA 1991).

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

67.5 Carcinogenic effects

67.5.1.1 Rats

Fischer 344 rats (130 males and females per group) were fed diets (0.1, 0.5, or 2.5%) yielding approximate dosages of 40, 200, or 1000 mg/kg b.w. per day of ethylene glycol for 24 months. Two untreated control groups were included. The only tumour type for which there was a significant difference was fibroadenoma of the mammary gland in females at the lowest dose level (0.1%). According to the authors, this finding was most probably unrelated to

ethylene glycol treatment because of the absence of an effect at the two higher dose levels. Non-neoplastic findings are described in 4.2.2.1. (DePass et al. 1986a).

In Sprague-Dawley rats (16 male and female animals per group), which received ethylene glycol in their diet at concentrations of 0, 0.1, 0.2, 0.5, 1.0, or 4.0% (equivalent to 0, 50, 100, 250, 500 or 2000 mg/kg b.w./day) for 2 years, mammary tumours developed in many female rats towards the end of the experiment. According to the author, the distribution of the tumours in both males and females was such that it was impossible to correlate incidence with treatment. Few tumours were identified in organs within the body and none was associated with the occurrence of calculi. (Blood 1965).

67.5.1.2 Mice

In B6C3F1 mice (60 animals of each sex per group), which were fed diets containing ethylene glycol for 103 weeks (male mice: approximately 0, 1500, 3000, or 6000 mg/kg b.w./day; female mice: approximately 0, 3000, 6000, or 12000 mg/kg b.w./day), no treatment-related neoplasms were observed at the 15-month interim evaluations or at the end of the 2-year studies. Non-neoplastic findings are described in 4.2.2.2. (NTP 1993).

CD-1 mice (80 males and females per group) were fed diets (0.1-0.05, 0.7-0.24, or 0.35-1.27%) yielding approximate dosages of 40, 200, or 1000 mg/kg b.w. per day of ethylene glycol for 24 months. Two untreated control groups were included. The only tumour type for which there was any evidence of a possible increased incidence was lymphosarcoma in females. The time-adjusted incidence was significantly increased according to one of three trend tests. Comparisons among groups for differences in tumour proportions indicated that the observed results could have occurred by chance alone. Non-neoplastic findings are described in 4.2.2.2. (DePass et al. 1986).

68 Regulations

68.1 Ambient air

Denmark (C-value): -

68.2 Drinking water

Denmark: -

68.3 Soil

Denmark: -

68.4 Occupational Exposure Limits

Denmark: 10 ppm (26 mg/m^3), notation H (At 2002).
Atomised 10 mg/m^3 (At 2002).

ACGIH: 39 ppm (100 mg/m^3) (ACGIH 2001)

68.5 Classification

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

68.6 IARC

-

68.7 US-EPA

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

69 Summary

69.1 Description

Ethylene glycol (EG) is a clear, colourless, slightly viscous, hygroscopic liquid with a sweet taste. It is miscible with water and has a low vapour pressure (0.06 mmHg at 20 °C).

69.2 Toxicokinetics

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

The metabolism of EG occurs in the liver and kidney. The initial step is a conversion of the parent compound to glycolaldehyde, which is further oxidised to glycolic acid. Glycolic acid is then converted to glyoxylic acid, which is converted either to carbon dioxide or to oxalic acid. Generally, metabolism begins immediately after administration of EG, and excretion of most of the parent compound and metabolites is complete 12 to 48 hours after dosing. The major excretory end products are carbon dioxide in exhaled air, and glycolate and unchanged EG in the urine.

69.3 Human toxicity

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

Several deaths due to accidental or intentional ingestion of EG have been reported; the minimal lethal oral dose for humans has been estimated to be about 1.6 g/kg b.w. (adults). The clinical signs which follow acute poisoning after ingestion of EG can be divided into three (possibly four) stages: 1) effects on the central nervous system, which occur 30 minutes to 12 hours after ingestion; 2) effects on the cardiopulmonary system occurring 12 to 72 hours after ingestion, this stage may also be characterised by severe metabolic acidosis; 3) effects on the kidneys, which occur 24 to 72 hours after ingestion, this stage is characterised by profound metabolic acidosis; and 4)

degenerative effects on the central nervous system occurring 6 or more days after ingestion, symptoms which are uncommon.

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

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

69.4 Animal toxicity

69.4.1 Single dose toxicity

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

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

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

No data on sensitisation in experimental animals have been found.

69.4.2 Repeated dose toxicity

Rats, guinea pigs, rabbits, dogs and monkeys were exposed to EG (vapour) either continuously at a concentration of 12 mg/m^3 for 90 days (continuous study), or at concentrations of 10 or 57 mg/m^3 for 8 hours a day, 5 days per week for 6 weeks (repeated study). In the continuous study, one rat, one rabbit and 3 guinea pigs died during exposure as well as four control rats;

moderate to severe eye irritation was observed in rats and rabbits; and histopathological examination showed inflammatory changes in the lungs of exposed animals and to a lesser degree in controls. In the repeated study, at 10 mg/m^3, histopathological examination revealed mild congestion in the spleens of both dogs, hepatic fatty changes in 2/8 guinea pigs and in 1/8 rats, and focal necrosis in the liver of 1/8 guinea pigs and of 1/8 rats; focal necrosis of the liver was also seen in 1 of 3 control guinea pigs; at 57 mg/m^3, histopathological examinations revealed non-specific inflammatory changes in the lungs and occasionally the hearts of exposed animals.

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

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

69.4.3 Toxicity to reproduction

When rats were exposed to a respirable EG aerosol by whole-body exposures (6 hours a day, gestational days 6 to 15), there was some evidence of treatment-related reductions in ossification of the foetal skeleton at 2500 mg/m^3 and an increase in the incidence of poorly ossified metatarsals and proximal phalanges of the hindlimb at 1000 mg/m^3; the only maternal effect observed was a significant increase in liver weight (absolute and relative) at 2500 mg/m^3. The NOAEL was 1000 mg/m^3 for maternal and 150 mg/m^3 for developmental toxicity.
In mice exposed similarly as the rats, several gestational parameters were affected from 1000 mg/m^3 and there was a significant increase in the incidence of a number of external, visceral, and skeletal malformations. The incidences of many foetal variations were also increased from 1000 mg/m^3, but only a few at the lowest dose level (150 mg/m^3). Reduced maternal body weight and body weight gain and reduced gravid uterine weight were observed from 1000 mg/m^3. The NOAEL was 150 mg/m^3 for maternal and at or below 150 mg/m^3 for developmental toxicity.
In a subsequent nose-only study (aerosol, 6 hours a day, gestational days 6 to 15) in mice, foetal body weights per litter were significantly reduced and the incidences of one skeletal malformation (fused ribs) and 18 skeletal variations were increased at 2500 mg/m^3; maternal kidney weights were increased from 1000 mg/m^3. The NOAEL was 500 mg/m^3 for maternal and 1000 mg/m^3 for developmental toxicity.

In a three-generation dietary reproduction study in rats, no treatment-related effects were observed in the F_2 parents and in the F_3 weanlings, including kidney damage; the NOAEL for reproductive toxicity was 1000 mg/kg b.w./day (the highest dose level in the study). In a continuous breeding study in mice, EG was administered in the drinking water for 14 weeks; the NOAEL for reproductive effects was 0.5% (corresponding to an average dose of 840 mg/kg b.w./day) with foetotoxic effects, including malformations, being observed at the higher concentration of 1%.

In Fischer 344 rats, the NOAEL for developmental toxicity as well as for maternal toxicity (only body weights of the dams were examined) was 1000 mg/kg b.w./day (the highest dose level in the study) when EG was administered in the diet from gestation day 6 to 15. When EG was administered by gavage (from gestation day 6 to 15) to CD rats, the NOAEL for developmental toxicity was 500 mg/kg b.w./day with effects (reduced body weights; duplicated or missing ribs, centra, and arches; and poor ossification) being observed at 1000 mg/kg b.w./day; maternal effects (increased relative liver weight) was observed as well at this dose level. Similarly, another gavage study in CD rats showed that administration of EG (from 1250 mg/kg b.w./day, the lowest dose level in the study) during organogenesis produced severe dose-related developmental toxicity, including malformations, at dose levels where no serious maternal effects (reduced maternal body weight gain at 1250 mg/kg b.w./day) were observed. However, in a third gavage study, where EG was administered to CD rats from gestation day 6 to 20, no toxicity were observed in offspring at dose levels up to 1250 mg/kg b.w./day; maternal effects at this dose level included kidney damage. In Wistar rats, a NOAEL of 638 mg/kg b.w./day for developmental toxicity (foetotoxicity as well as malformations) was observed

following administration of EG by a stomach tube from gestation day 6 to 15; no information was given with regard to maternal effects.
When EG was administered by gavage (from gestation day 6 to 15) to CD-1 mice, the NOAEL for developmental toxicity was 150 mg/kg b.w./day with effects (slight reductions in foetal body weight and increased incidences of extra ribs) being observed at 500 mg/kg b.w./day; no maternal effects were observed at any dose level (up to 1500 mg/kg b.w./day). Another gavage study in CD-1 mice showed that administration of EG (from 750 mg/kg b.w./day, the lowest dose level in the study) during organogenesis produced severe dose-related developmental toxicity, including malformations, a dose level where no maternal effects were observed.
In New Zealand White (NZW) rabbits, the NOAEL for maternal toxicity was 1000 mg/kg b.w./day and the NOAEL for developmental toxicity was 2000 mg/kg b.w./day (the highest dose level in the study), when EG was administered by gavage on gestational day 6 through 19.

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

69.4.4 Mutagenic and genotoxic effects

EG has shown negative results in the following *in vitro* test systems: in the Ames test (several tests in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; TA1538), in the SOS chromotest in *Escherichia coli* PQ37, in the DNA damage and repair assay in *Escherichia coli* (WP2, WP2uvrA, WP67, CM611, WP100, W3100polA+, p3478polA-), for gene conversion in *Saccharomyces cerevisiae*, for aneuploidy induction in the fungus *Neurospora crassa*, in a cytogenetic assay in Chinese Hamster Ovary (CHO) cells, for gene mutation in the HGPRT assay in CHO cells, in the mouse lymphoma assay, for sister chromatid exchanges and chromosome aberrations in CHO cells, and for unscheduled DNA synthesis in rat hepatocytes. In one HGPRT assay and in one test for chromosome aberration, both in CHO cells, the result of the assay was reported as being ambiguous. Most of the assays were performed both with and without metabolic activation.

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

69.4.5 Carcinogenic effects

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

administered diets yielding dosages of up to approximately 1000 mg/kg b.w./day for 24 months.

70 Evaluation

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

There are numerous case reports in the literature of poisoning in humans due to accidental or intentional ingestion of EG; the minimal lethal oral dose for humans has been estimated to be about 1600 mg/kg b.w. for adults. The clinical symptoms of acute EG poisoning in humans can be divided into three (possibly four) fairly distinct stages (effects on the CNS, cardiopulmonary effects, renal effects, and delayed neurological effects); toxicity in stage 2 and 3 is characterised by severe metabolic acidosis. The severity of these stages and the advance from one stage to the next depends greatly on the amount of EG absorbed.
The acute toxicity of EG in experimental animals closely mirrors the acute effects seen in humans; one exception is the fourth clinical stage as no studies have been found that reported this stage in animals. EG is of low acute toxicity in experimental animals, except the cat, with reported oral LD_{50}-values ranging from >2000 to 15400 mg/kg b.w.; a minimal lethal dose of 3800 mg/kg b.w. has been reported for rats, of around 7000 mg/kg b.w. for dogs and, of 1000 mg/kg b.w. for cats. Comparing the lethal oral dose in humans to the minimum lethal dose in experimental animals, EG appears to be two to five times more acutely toxic to humans and cats, on a body weight basis, than to rats and dogs. EG is classified for acute toxic effects following oral administration according to the EU classification criteria.
The very limited data on acute inhalation and dermal toxicity in experimental animals also indicate a low acute toxicity by these routes with a reported LC_{50}-value (one hour) of 10.9 g/m^3 in rats and dermal LD_{50}-values of around 10600 mg/kg b.w. for the rabbit.

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

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

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

Repeated oral administration of EG to rats results primarily in toxic effects in the kidneys. Male rats are far more sensitive to the renal effects of EG than are female rats as renal effects occur in male rats at dose levels from about 200-250 mg/kg b.w./day (16-week and 2-year feeding studies) but in female rats at dose levels above 1000 mg/kg b.w./day. Mice appear to be relatively resistant to EG induced kidney damage when compared to rat as incidence and severity of nephropathy were not affected in either sex at dietary levels (2-year feeding study) of up to 6000 mg/kg b.w./day in males and of up to 12000 mg/kg b.w./day in females.
The incidence and severity of the renal effects appear to depend on the dose level as well as on the exposure duration. A NOAEL for renal effects (in male rats) of about 550 mg/kg b.w./day can be considered from a 90-day drinking water study (Robinson et al. 1990) and of about 625 mg/kg b.w./day from a 13-week feeding study (Melnick 1984) whereas 2-year feeding studies have revealed NOAELs of 100 (Blood 1965) and 200 mg/kg b.w./day (DePass et al. 1986a), respectively. This difference in the NOAELs could be a result of different sensitivity in the rat strains used in the two studies (Blood: Sprague-Dawley; DePass et al.: Fischer 344/N), but no data are available to further elucidate this aspect. However, the validity of the study by DePass et al. is

considered to be better than that of the study by Blood, e.g., 130 animals of each sex per group compared to 16 animals of each sex per group; more detailed descriptions of results including incidence and severity of renal effects in the various dose groups. The lowest NOAEL (about 70 mg/kg b.w./day) for damage to the kidneys in male Wistar rats has been observed in a 16-week dietary study (Gaunt et al. 1974 – quoted from BUA 1991 and IUCLID 2000); however, BUA and IUCLID do not give any details about the kidney damage (type, incidence, severity) and the study report is not public available. Overall, a NOAEL for renal effects in male rats, the most sensitive species, of 200 mg/kg b.w./day is considered taken into account the reliability of the various studies as discussed above. According to NTP (1993), the study by DePass et al. (1986a) was considered adequate to evaluate the chronic toxicity of EG in F344 rats; therefore, NTP only has conducted a 2-year study in mice.
According to DePass et al. (1986), the greater susceptibility of male rats to EG induced renal toxicity may be the result of more efficient conversion of EG to toxic metabolites including oxalate in male rats, as well as to the more rapid progression of spontaneous nephropathy in the male. High-dose females had significant amounts of urinary oxalate crystals at 12, 18, and 24 months, so conversion of EG to oxalate clearly occurred in females also. The critical factor responsible for the more severe nephrotoxicity in male rats was probably the greater incidence and severity of spontaneous nephropathy in the male.

No data on toxicity to reproduction in humans have been found.
Dietary exposure of male and female F344 rats to EG at dose levels up to 1000 mg/kg b.w./day (the highest dose level in the study) for three generations produced no effects on fertility, fecundity, or reproductive performance. When EG was administered to CD-1 mice in the drinking water for 14 weeks (continuous breeding study), reduced fertility and fecundity, and foetotoxic effects, including malformations were observed at about 1640 mg/kg b.w./day; the NOAEL was about 840 mg/kg b.w./day. Administration of EG via the gastrointestinal route (gavage) at high concentrations has resulted in developmental toxicity, including teratogenicity in rats and mice. Developmental toxicity was observed in CD rats in two gavage studies at dose levels from about 1000 mg/kg b.w./day (gavage) and in Wistar rats from about 860 mg/kg b.w./day whereas one gavage study did not show any developmental toxicity in CD rats at 1250 mg/kg b.w./day. When EG was administered to F344 rats in the diet, no developmental effects were observed at dose levels up to 1000 mg/kg b.w./day (the highest dose level in the study). No serious maternal effects were noted at the dose levels resulting in developmental toxicity. Mice appear to be far more sensitive to the developmental effect exerted by EG with severe developmental toxicity, including malformations, being observed in a gavage study at dose levels from 750 mg/kg b.w./day (the lowest dose level in the study), a dose level where no maternal effects were observed. In another gavage study, lower dose levels were administered and a NOAEL for developmental toxicity of 150 mg/kg b.w./day can be considered from this study. One gavage study in rabbits indicates that this species is fairly resistant to the developmental effects exerted by EG as no developmental effects were observed at dose levels up to 2000 mg/kg b.w./day (the highest dose level in the study). Following dermal application of EG to CD-1 mice, no developmental effects were observed at dose levels up to about 3550 mg/kg b.w./day (the highest dose level in the study).

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

Most of the mutagenicity and genotoxicity tests available indicate that EG is not a mutagenic or genotoxic substance although some positive results have been reported. In the micronucleus assay in mice, the increased numbers of micronuclei was observed following administration (oral, intraperitoneal injection) of very high doses (2.8 to 13.9 g/kg b.w.) and thus, the result is not considered as being reliable. Overall, EG is considered not to be a mutagenic or genotoxic substance.
No evidence of a carcinogenic effect of EG was observed at dietary concentrations of up to approximately to 2000 mg/kg b.w./day for 2 years in rats or of up to approximately 12000 mg/kg b.w./day for 2 years in mice.
No data on mutagenic and genotoxic effects, or carcinogenic effects of EG in humans have been found.

The toxicity of EG is primarily a result of the effects of its metabolites although the effects on the central nervous system observed shortly after acute ingestion is partly attributed to unmetabolised EG.
The metabolic acidosis is usually attributed to the acidic metabolites of EG and recent studies of cases of human EG poisoning (discussed in Jacobsen & McMartin 1997) have demonstrated that the major determinant of the metabolic acidosis is the degree of glycolic acid accumulation as glycolate accumulation correlates with the increase in anion gap or decrease in arterial bicarbonate concentrations observed in poisoned humans, as well as in animals.
The mechanism of the renal toxicity is not yet known. Calcium oxalate precipitation within the renal tubules has long been accepted as an important pathogenic factor for the development of renal toxicity; however, there is no evidence directly linking oxalate precipitation with development of renal tubular necrosis and renal damage can occur at exposure levels where no or few oxalate crystals are detected. The renal toxicity has also been suggested to occur from a metabolite-induced cytotoxicity, such as from glycolaldehyde or glyoxylate, which are both highly toxic *in vitro*, or via the metabolic

acidosis resulting from accumulation of glycolic acid. As a result of the decrease in the pH in body fluids, an elevated anion gap develops and the serum osmolal gap across cells increases, resulting in renal oedema that compromises intrarenal blood flow and promotes renal failure. These suggestions are in concordance with the data published by Pottenger et al. (2001) showing that oxalate was a very minor metabolite in rats in both blood and urine at oral (gavage) dose levels up to 2500 mg/kg b.w. and the data discussed in Jacobsen & McMartin (1997) showing that glycolate accumulation correlates with the increase in anion gap or decrease in arterial bicarbonate concentrations observed in poisoned humans, as well as in animals.
Although the mechanism(s) behind the developmental effects is not yet known, a link between maternal metabolic acidosis and developmental toxicity has been suggested with glycolic acid being the predominant toxic metabolite.

70.1.1 Conclusion

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

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

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

Mice appear to be far more sensitive to the developmental toxicity exerted by EG than are rats and rabbits. No data on reproductive toxicity in humans are available. Therefore, humans are considered to be as sensitive as mice to the developmental effects of EG. In a nose-only inhalation study, the NOAEC for developmental effects, including teratogenicity, in mice was 1000 mg/m^3 while the NOAEC was at or below 150 mg/m^3 in a whole-body inhalation

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

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

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Appendix 10: Propylene glycol (PG)

72 General description

72.1 Identity

Molecular formula:	$C_3H_8O_2$
Structural formula:	CH_3-CHOH-CH_2OH
Molecular weight:	76
CAS-no.:	57-55-6
Synonyms:	Propanediol Monopropylene glycol Propane-1,2-diol alpha-Propylene glycol Sirlene 1,2-Propanediol 1,2-Dihydroxypropane Methylethylene glycol Trimethyl glycol 1,2-Propylene glycol

72.2 Physico / chemical properties

Description:	Propylene glycol (PG) is a colourless viscous liquid. It is soluble in water and hygroscopic.
Melting point:	-59 °C
Boiling point:	189 °C
Density:	1.036 g/ml (at 20°C)
Vapour pressure:	0.07 mmHg (9.3 Pa) (at 20°C)
Concentration of saturated vapours:	291 mg/m^3 (calculated)
Conversion factor:	1 ppm = 3.16 mg/m^3 (at 20°C and 760 mmHg) 1 mg/m^3 = 0.316 ppm
Solubility:	Water: ≈100 g/l (at 21°C). Also soluble in alcohol.
Partition coefficient Log K_{ow} :	-0.92
References:	ATSDR (1997), ChemFinder (2002), LaKind et al. (1999).

73 Toxicokinetics

73.1 Inhalation

No data were found on toxicokinetics of PG via the inhalation route.

73.2 Oral intake

Data indicated that absorption from the gastrointestinal tract is fairly rapid. The maximum plasma concentration of PG in humans was reached within 1 hour after oral exposure (ATSDR 1997).

In dogs, gavage doses of 2, 8 and 12 ml PG/kg water (corresponding to 1036, 4144 and 6216 mg PG/kg b.w.) lead to maximum blood concentrations within 30 minutes for the low dose, and after 2-4 hours for the mid- and high doses (Lehman et al. 1937 – quoted from Mortensen 1993).

In humans, the maximum plasma concentration of PG was reached within 1 hour after oral exposure. No details on the dose were given. (Yu et al. 1985 – quoted from ATSDR, 1997).

The major route of metabolism for PG is by liver alcohol dehydrogenase to lactaldehyde, then to lactic acid and pyruvic acid. An alternative route of metabolism is via phosphorylated glycol. The two metabolites, lactic and pyruvic acids, enter the energy-generating process (i.e. tricarboxylic acid cycle and/or gluconeogenic pathway). Pyruvic acid can also be metabolised further to carbon dioxide and water. (Mortensen 1993, LaKind 1999).

Metabolism of PG in humans was non-linear, based on a saturable clearance. Elimination half-life following oral exposure was about 3.8 hours. The total body clearance in humans was about 100 ml/kg b.w./hour (corresponding to 103.6 g/kg b.w./hour) (Yu et al. 1985 - quoted from ATSDR 1997 and Mortensen 1993).
In another investigation, 20-25% of orally administered PG was eliminated via the urine in 10 hours. (Hanzlik et al. 1939 – quoted from Mortensen 1993).

Dose-dependent elimination was seen in rats, with saturation of the pathways at doses above 5880 mg/kg b.w. and a maximum elimination rate of 0.63 g PG/kg b.w./hour (Morshed et al. 1988 - quoted from ATSDR 1997).

In cats, PG has been shown to be metabolised to d- and l-lactate. In rats, approximately 33% of an oral dose PG was excreted as the unchanged substance in urine, while dogs excreted 45% unchanged. (Van Winkle 1941, Lehman & Newman 1937 - quoted from Mortensen 1993).

73.3 Dermal contact

Absorption through damaged skin was studied in 45 patients with second and third degree burns over more than 20% of their total body surface. The

sulfadiazine preprations applied dermally over a period of 3-7 contained PG, which was detected in the serum of 24 patients at average levels of 0.08 mg/ml with a range of 0-1.3 mg/ml. PG was detected in the urine of 40 patients at average levels of 1.3 mg/ml, with a range of 0-23.0 mg/ml. (Kulick et al. 1985 – quoted from ATSDR 1997).

73.4 Mode of action

No data were found.

74 Human toxicity

74.1 Single dose toxicity

Acute effects include primarily CNS depression and lactic acidosis form extremely high doses.

74.1.1 Inhalation

Ninety-three asthma-patients showed no adverse clinical effects from 15 minutes inhalation through a mask of an aerosol consisting of 0.005% isoproterenol-hydrochloride in a 40 % PG isotonic saline solution (Cohen & Crandall 1964).

74.1.2 Oral intake

A 15-month infant was given a vitamin C preparation containing 22.5 ml PG (equivalent to 23.8 g) as vehicle. The child experienced hypoglycaemia, irregular heart rate, sinus arythmia, tachypnea, tachycardia and perspiration. The effects disappeared upon cessation of treatment. (Martin & Finberg 1970 - quoted from LaKind et al. 1999).

No effects on the basal metabolic rate were demonstrated in a study of 3 human subjects administered 50 ml (51800 mg) PG (Hanzlik et al. 1939 - quoted from LaKind et al. 1999).

74.1.3 Dermal contact

An severely burnt 8-month infant (large surface of the skin with second and third-degree burns was treated topically over 70 hours with silver sulfadiazine containing 76.7 mg PG/g. The PG dose was calculated to 630 g/kg b.w. The treatment resulted in a peak serum PG level of 1059 mg/dl and caused acute metabolic acidosis and cardio-respiratory arrest, which may be due to the high serum PG levels. (Fligner et al. 1985 - quoted from LaKind et al. 1999).

74.1.4 Irritation and sensitisation

Cases of contact dermatitis have been reported from therapeutic and cosmetic products containing PG as a vehicle. Numerous patch test studies have been designed to determine the incidence and nature of PG skin reactions. Most of the positive reactions were found to be primary irritation, but several investigators were unable to conclude whether reactions to PG exposure were dermal irritation or true allergy.

In a Draize test, 204 subjects were treated with 12 % and 89 subjects wit 60% PG in petrolatum over a 3-5 week period. No allergic response was seen at challenge with 72 hour contact to 12 % PG. (Marzulli & Maibach 1974 – quoted from Mortensen 1993).

In a modified Draize sensitisation study, 13 of 203 subjects showed a significant response to PG, by none of these subjects reacted to provocative use testing with 100 % PG. The authors concluded that PG was at lest a minimal irritant. (Trancik & Maibach 1982 - quoted from LaKind et al. 1999).

Frosch & Kligman (1977 - quoted from LaKind et al. 1999) classified 100% PG as a moderate skin irritant.

In another test, undiluted PG was applied to 1556 subjects for 20-24 hours. Skin reactions were seen in 12.5% of subjects, 70% of which were considered irritative and 30 % allergic, depending on onset time and area involved. Twelve out of 42 subjects with skin reaction also showed reaction were then tested with 10% PG in water. (Hannuksela et al. 1975 - quoted from Mortensen 1993).

Andersen (1980) pointed at the difficulty to evaluate a positive reaction and its clinical relevance, as false positive reactions occur commonly, and impurities may play a role. The incidence of PG allergy in the population was considered to be very low in relation to the extended use in consumer products.

74.2 Repeated dose toxicity

74.2.1 Inhalation

Exposure of children and adults to up to 94 mg PG/m^3 (mean of 69 mg PG/m^3) over weeks to months did not result in any adverse effects. (Harris & Stokes Jr, 1943 - quoted from A&H 1983).

No other data were found.

74.2.2 Oral intake

A child was given 2-4 ml of a vitamin D preparation containing 98% PG, twice a day for up to 13 months. The treatment corresponds to an exposure of 114-228 mg PG/kg b.w. day. After the 13 months, the child began to experience repeated seizures followed by a period of unconsciousness within 2 to 4 hours after the second daily dose. The symptoms disappeared when the treatment ceased. (Arulanantham & Genel 1978 - quoted from LaKind et al. 1999).

74.2.3 Dermal contact

No data were found.

74.3 Toxicity to reproduction

No data were found.

74.4 Mutagenic and genotoxic effects

No data were found.

74.5 Carcinogenic effects

No data were found.

75 Animal toxicity

75.1 Single dose toxicity

75.1.1 Inhalation

Rabbits were exposed to of 10% PG aerosol for 20 or 120 minutes. No deaths were reported. In the tracheal epithelium, the goblet cells were degenerated at both observation times, whereas ciliated cells were slightly affected at 20 min., and seriously at 120 minutes. No details of the aerosol particle size or other exposure conditions were given. (Konrádová et al. 1978 - quoted from ATSDR and LaKind et al. 1999).

Dogs were exposed for 15 minutes to a 10 or a 20% PG aerosol and haemolysis or haemodynamic effects were investigated. No effects on the blood were seen. No details of the aerosol particle size or other exposure conditions were given. (Konrádová et al. 1978 - quoted from ATSDR and LaKind et al. 1999).

75.1.2 Oral intake

Reported LD_{50}-values in rats for PG range from 21 to 33.5 g/kg b.w., and minimum lethal doses of 19.8 and 20.9 g/kg were reported for the substance. (IUCLID 2000, LaKind et al. 1999).

Rats were administered single doses of 10 ml of 10, 20, 50 or 100 % PG solutions/kg (equivalent to 1.04, 2.08, 5.2 and 10.4 g PG/kg b.w.). No deaths were reported. At the two highest doses, PG produced decreased respiration and marked CNS depression, including ataxia, ptosis (hanging eye lid), decrease of spontaneous motor activity and of body/limb tone, and of respiration. (Singh et al. 1982 - quoted from LaKind 1999).

Rats administered a single dose of 23.5 g/kg PG developed acute haemorrhagic enteritis and extensive adrenocortical haemorrhage. Widespread lymphocyte depletion was also evident. (Clark et al. 1979 - quoted from LaKind 1999).

Single oral doses of 730 or 2940 mg PG/kg b.w. were administered to adult female albino Wistar rats to examine haematological effects. PG was found to decrease packed cell volume for up to 2 days. Red blood cell count was reversibly decreased. (Saini et al. 1996 - quoted from LaKind et al. 1999).

LD_{50}-values of 22, 23.9, 24.8 and 31.9 g/kg b.w. were reported in mice for PG (LaKind et al. 1999 and IUCLID 2000). The study of Singh et al. reported above also included mice, in which similar effects as for the rats were described. (Singh et al. 1982 - quoted from LaKind 1999).

Single doses of 23310 mg PG/kg b.w. administered to mice by stomach tube caused slight microscopic changes in the kidney with nuclear pyknosis and

vacuolar degeneration of the cytoplasm. A few cortical tubules contained protein debris. (Laug et al. 1939 - quoted from LaKind et al. 1999).

In rabbits, the LD_{50}-values for PG were reported to range from 18 to 19.3 g/kg b.w. (IUCLID 2000). Dosing by stomach tube with 13.73 to 21 g/kg b.w. PG resulted in increased respiratory rate, loss of equilibrium, depression, analgesia and coma. Deaths occurred in 18 to 36 hours. (Braun & Cartland 1936 - quoted from LaKind et al. 1999).

LD_{50}-values in guinea pigs of 18.35, 18.9 and 19.6 g/kg b.w. were reported (LaKind et al. 1999).

An LD_{50}-value in dogs of 22 g/kg b.w. has been cited (IUCLID 2000).

75.1.3 Dermal contact

A LD_{50}-value in rabbits of 20800 mg/kg b.w. is reported without any details (Raw Material Data Handbook 1974 – quoted from IUCLID 2000).

75.1.4 Irritation and sensitisation

Several dermal irritation studies with PG in rabbits were conducted. Very few details were reported. The results ranged from none to mild irritation. (IUCLID 2000).

PG was reported to be not irritating to mildly irritating to rabbits eyes in a number of eye irritation tests in rabbits reported with very few details (IUCLID 2000).

No studies on sensitisation were found.

75.2 Repeated dose toxicity

75.2.1 Inhalation

Nineteen Sprague-Dawley rats/sex/dose were exposed nose-only to an aerosol containing 0, 160, 1000 or 2200 mg PG/m^3 (0, 51, 321 or 707 ppm), 6 hours/day, 5 days/week over 90 days. Treatment related nose bleeding occurred from week 2 through the study. The authors attributed the effect to dehydration of the tissues. At the two highest dose levels, the number, size and mucous content of the goblet cells in the nasal epithelium were significantly increased. Slight dose related body weight reduction was reported females. Significant but not dose-related changes in haematological parameters were seen. No toxicological effects were seen on liver, kidney, spleen or lung. (Suber et al. 1989).

Twenty-nine monkeys (Macacus Rhesus) were continuously exposed over 13 months to levels of 32-112 ppm PG (not further specified. equivalent to 101-354 mg/m^3). Thirteen animals died or were killed because of illness due to infection. No effects were seen on body weights, respiratory system, gastrointestinal tract, liver, kidney or spleen. The haemoglobin count was elevated at 112 ppm compared to controls. However, as many of the monkeys suffered from parasite infection and lung mites, the authors concluded that the haematological effects were not related to PG. (Robertson et al. 1947 - quoted from ATSDR 1997).

75.2.2 Oral intake

Inbred albino rats were administrated 2.45 % or 4.9 % PG (approximately 1225 or 2450 mg/kg b.w./day). The duration of exposure was not given in the reference. No effects of PG were reported on growth rate, food and water consumption, survival, gross and microscopic lesions in lung, heart, liver, spleen, kidney, adrenal or testis. (Morris et al. 1942 – quoted from LaKind et al. 1999).

Six male Wistar rats/group were dosed with 0 or 2942 mg PG/kg/day in the water for 10, 20 or 30 days. No death was observed. Body weights were reduced 41% at 10 days, but were then increased at 20 and 30 days. Enzyme activity in the gastrointestinal tract was increased, but no effects on the jejunal surface were seen. (Morshed et al. 1991 – quoted from ATSDR 1997).

Rats were given drinking water containing 1, 2, 5, 10, 25 or 50 % PG v/v (corresponding to approximately 1320, 2640, 6600, 13200 or 26400 mg PG/kg b.w./day) for 140 days (20 weeks). Animals of the two highest exposure groups died within 10 weeks. No adverse effects were seen up to 10% PG (13200 mg). (Clayton & Clayton 1982 – quoted from LaKind et al. 1999 and IUCLID 2000).

In a two-year feeding study in CD-1 rats, levels of 0, 6250, 12500, 25000 or 50000 ppm PG (corresponding to approximately 0, 200, 400, 900 or 1700 mg/kg b.w./day in males and 300, 500, 1000 or 2100 mg/kg b.w./day in females) were used. No significant compound-related effects were observed in any treated group when compared to controls with respect to behaviour, body weights, organ weights, haematology, or microscopic examination of the heart, lung, liver, kidney and adrenal. (Gaunt et al. 1972 - quoted from ATSDR 1997, LaKind et al. 1999 and IUCLID 2000).

Rats administered up to 1834 mg/kg b.w./day for 2 years showed very slight liver damage, but no renal pathology (Clayton & Clayton 1982 – quoted from LaKind et al. 1999).

Cats fed 1200 ppm PG in the diet for 2 weeks showed increased haptoglobin concentration (Weiss et al. 1992 – quoted from ATSDR 1997).

Cats were fed 1600 or 8000 mg PG/kg b.w./day for 3 or 5 weeks. At the high dose, the animals developed decreased activity, mental depression and slight to moderate ataxia. The high dose cats also had polyuria and polydipsia, and showed decreased erythrocyte counts Heinz body formation and hypercellularity of the bone marrow, while the level of D-lactate was 44 fold higher than controls. No effects were reported at the low dose. (Christopher et al. 1989 and 1990 – quoted from LaKind et al. 1999 and ATSDR 1997).

In a 12-week study, kittens administered 5 or 10 % PG in the diet (corresponding approximately to 1100 and 2400 mg/kg b.w./day, respectively) showed increased Heinz body formation. (Hickman et al. 1990 – quoted from LaKind et al. 1999).

Cats administered 0, 80, 443, 675, 1763 or 4239 ppm PG/kg feed for 13 weeks showed increased Heinz-body formation from 443 ppm. This effect

was dose related as the haemosiderin content in the liver and the spleen from 673 ppm PG. (DOW 1979 – quoted from IUCLID 2000).

Male and female cats were treated in their diet with 0, 6, or 12 % PG (corresponding to 0, 2100 or 3560 mg/kg b.w./day) for 13 weeks. Haematological parameters were examined with 2 weeks interval in the treatment period. Haemoglobin was significantly decreased in the low, but not the high dose group. Erythrocyte count was significantly decreased in both treated groups, but not in controls. Reticulocyte aggregate numbers were significantly increased in the 12 % group and Heinz bodies significantly increased in both treated groups. (Bauer et al. 1992 – quoted from LaKind et al. 1999).

Cats exposed in the diet to 2400 mg PG /kg b.w./day for 17 weeks showed Heinz body formation (Weiss et al. 1990 - quoted from ATSDR 1997).

Dogs were exposed to PG in the drinking water at levels of 5% PG twice a day for females and 600 ml of 10 % PG for males daily over 9 months. No functional deficits in the kidney or liver were reported and no pathological changes were found in these organs. (VanWinkle & Neuman 1941– quoted from LaKind et al. 1999).

Groups of 5 male and 5 female dogs were given 2000 or 5000 mg/kg b.w./day for 2 years. An additional "control" group were given 63500 mg dextrose/kg/day. At 2000 mg/kg b.w./day, no effects were seen on body weights or on any organs. At 5000 mg/kg b.w./day slight decrease in erythrocyte count, haemoglobin and haematocrit, which was reversible, was noted, but no effects on the bone marrow could be shown. Increased urinary output and decreased water intake were noted. The relevance of these findings was not discussed in any of the references. (Weil et al. 1971 – quoted from ATSDR 1997, LaKind et al. 1999 and IUCLID 2000).

75.2.3 Dermal contact

No effects on the kidney were seen from PG doses of 0.16 to 5.0 ml/kg b.w. applied to the shaved abdominal skin or rabbits for 30 days (Hanzlik et al. 1947 - quoted from LaKind et al. 1999).

75.3 Toxicity to reproduction

75.3.1 Inhalation

75.3.1.1 Fertility

White rats exposed continuously to a concentration of 55-112 ppm PG for 18 months showed no adverse effects on the ability to produce live young, or on survival of the offspring (Roberson et al. 1947).

No data were found on developmental effects of PG after inhalation.

75.3.2 Oral intake

75.3.2.1 Fertility

No effects on reproductive parameters were reported from administration to CD-1 mice of 5 % PG in the drinking water (equivalent to 10100 mg/kg b.w./day over 14 weeks in a continuous breeding study. The offspring were examined for reproductive parameters, but did not show any effect of PG either. (Gulati et al. - quoted from LaKind et al. 1999).

A multigeneration, continuous breeding feeding study in Swiss albino mice was performed, using concentrations of 1, 2.5 or 5% in the drinking water (corresponding to 1820, 4800 or 10100 mg/kg b.w./day) over 98 days from day 1 before mating in the parent generation (F_0). No significant effects were seen on the fertility of this generation. No effects were noted in the offspring (F_1) or the following generation (F_2) for mating or fertility indices, mean number of liver pups per litter, proportion of pups born live, or gender of pups born live. (Morrissey 1989 - quoted from LaKind et al. 1999).

No significant differences were seen on fertility rate, numbers of resorptions, average litter size or birth weight in CD1-mice treated by gavage with 10000 mg PG/kg b.w./day on gestation days 8-12. (Kavlok et al. 1987 - quoted from LaKind et al. 1999).

75.3.2.2 Developmental toxicity

Testing for developmental effects in several mammalian species using a 10-day exposure identified no effects at the highest dose tested, e.g. respectively 1600 mg/kg b.w./day for Wistar rats and CD-1 mice; 1550 mg/kg b.w./day for golden hamsters and 1230 mg/kg b.w./day for Dutch-belted rabbits. No differences in numbers of soft tissue changes or skeletal abnormalities between treated groups and control groups, and no effects on maternal or fetal survival were noted. (Food and Drug Research Laboratories Report No 1573j-1574j 1973 – quoted from LaKind et al. 1999).

In a developmental screening test, mice were administered 10000 ppm PG via gavage on days 8 to 12 of gestation. No significant differences were reported for maternal deaths, numerous reproductive endpoints, postnatal pup weight gain or abnormalities in the pups. No further details were found. (Kavlok et al. 1987 – quoted from LaKind et al. 1999).

75.3.3 Dermal exposure

No data were found.

75.4 Mutagenic and genotoxic effects

75.4.1 *In vitro* studies

PG was negative in an Ames test conducted without metabolic activation in *Salmonella typhimurium* strains TA98, TA 100, TA 1535 and TA 1537. (Pfieffer & Dunkelberg 1980 – quoted from IUCLID 2000).

PG was also negative in another Ames test in strains TA 92, TA 94, TA 98, TA 100, TA 1535 and TA 1537 conducted with metabolic activation. (Ishidate et al. 1984 – quoted from IUCLID 2000).

A third Ames test was conducted in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 at concentrations of 20-5000 µg PG/plate with and without metabolic activation. The result of this study was also negative. (BASF 1981 – quoted from IUCLID).

A chromosome aberration assay was conducted twice in human lymphocytes, using concentrations of 476, 1910 and 3810 µg PG/l, according to OECD guideline 473. PG did not cause a statistically significant increase in the proportion of metaphases containing chromosome aberrations. (EC GmbH 1990 – quoted from IUCLID 2000).

A chromosome aberration test conducted in human embryonic lung cells (WI-38) with 0.001, 0.01 and 0.1 µg PG/ml was negative (Litton Bionetics Inc. 1974 - quoted from IUCLID).

In another chromosome aberration test, Chinese hamster lung fibroblast (CHL) cells were exposed to 32 mg PG/ml without metabolic activation. The test was positive. However, when the concentration was doubled and metabolic activation added, no increase in chromosome aberrations was found (Ishidate et al. 1984, 1988 - quoted from Mortensen 1993).

A DNA damage and repair assay conducted with PG in Chinese hamster V79-cells with and without activation was negative (Swenberg et al. 1976 - quoted from IUCLID).

A cell transformation assay conducted with PG in Syrian hamster embryo cells was negative (Mutat Res 1983 - quoted from IUCLID).

75.4.2 *In vivo* studies

No chromosome aberrations in the bone marrow were seen in a cytogenetic assay in rats following administration by gavage of a single dose or 5 doses of 30, 2500 and 5000 mg PG /kg b.w. The same dosing regime was used in a dominant lethal assay which was negative. (Litton Bionetics 1974 – quoted from IUCLID 2000).

A dominant lethal assay in mice treated intraperitoneally with a single dose of 10 mg PG/kg b.w was negative (Kennedy Jr. et al. 1975 – quoted from IUCLID 2000 and A&H 1983).

75.5 Carcinogenic effects

75.5.1 Inhalation

No data were found.

75.5.2 Oral intake

No treatment-related increase in neoplasms was seen in a two-year study in rats treated with up to 2500 mg/kg b.w./day in the diet. Other effects seen in this study are reported under section 4.2.2. (Gaunt et al. 1972 – quoted from ATSDR 1997).

75.5.3 Dermal contact

No increase in tumours was observed after twice weekly application of PG to the skin of Swiss mice for 120 weeks, at doses up to 2 mg. No further details were given. (Stenbak & Shubik 1974 - quoted from ATSDR 1997).

76 Regulations

76.1 Ambient air

-

76.2 Drinking water

-

76.3 Soil

-

76.4 Occupational Exposure Limits

-

76.5 ADI

EU: 25 mg/kg b.w. (SCF 1993)

76.6 EU-Classification

The substance is not adopted on Annex I to directive 65/548/EEC (MM 2002)

76.7 IARC

-

76.8 US-EPA

-

77 Summary

77.1 Description

Propylene Glycol (PG) is a colourless viscous liquid. PG is soluble in water and hygroscopic and has a low vapour pressure.

77.2 Toxicokinetics

PG has been shown to be rapidly absorbed form the gastrointestinal tract and following dermal absorption through damaged skin. It is metabolised to lactic acid and pyruvic acid, which enter the energy production, or for pyruvic acid is further metabolised to CO_2 and water. 20- 45% PG is found unchanged in urine.

77.3 Human toxicity

77.3.1 Single dose toxicity

No reports of deaths caused by PG were found. Acute effects included CNS depression and acidosis was reported from exposure to very high doses.

77.3.2 Irritation and sensitisation

Numerous patch tests have been performed to examine the incidence and nature of PG skin reactions. However, the results were difficult to interpret. PG showed weak irritative and none to slight allergenic response.

77.3.3 Repeated dose toxicity

No effects were seen from repeated exposure to up to 94 mg PG/m^3 over several weeks. Ingestion by a child of 114-228 mg/kg b.w./day for 13 months resulted in CNS-depression with seizures and unconsciousness.

No further data regarding effects in humans were found.

77.4 Animal toxicity

77.4.1 Single dose toxicity

Rabbits exposed to aerosol concentrations of 10% PG showed degeneration of the goblet cells in the trachea, and the ciliated cells were affected. Reported oral LD_{50}-values for rats and mice range from approximately 20 to 30 g/kg b.w., while the LD_{50}-values for rabbits and guinea pigs ranged from 18 to 20 g/kg b.w.; an LD_{50}-valus of 22 g PG/kg b.w. in dog was reported. Signs of toxicity include CNS-depression and respiratory depression and reversible haematological effects. Slight hyperaemia or the gastrointestinal tract and one case of haemorrhagic enteritis in rats have also been reported. A dermal LD_{50}-value in the rabbit was reported to be 20.8 g/kg b.w.

77.4.2 Irritation and Sensitisation

Various reports describe PG as not irritating to mildly irritating to the skin and eyes of rabbits. No sensitisation studies in animals were found.

77.4.3 Repeated dose toxicity

Nose bleeding was reported from inhalation exposure of rats to aerosols at concentrations of 160, 1000 and 2200 mg PG/m^3 over 90 days.

Repeated inhalation exposure of rats to aerosols with 1000 or 2200 mg PG/m^3 over 90 days caused multiplication and enlargement of the goblet cells and their mucous content in the nasal epithelium.

Rats exposed by inhalation to 2200 mg PG/m^3 as an aerosol over 90 days showed an effect (not dose-related) on haematological parameters.
Dogs treated via the diet with 5000 mg PG/kg b.w/day over 2 years and cats treated for shorter periods of time (2, 3, 5, 13 or 17 weeks) with doses of 1100 to 8000 mg PG/kg b.w./day showed haematological changes with decreased erythrocyte count and Heinz body formation.

No effects were seen in rats and in dogs treated with 2000 mg/kg b.w./day for 2 years. An ADI for PG from food of 25 mg/kg b.w/day has been defined on basis of this NOAEL.

77.4.4 Toxicity to reproduction

PG did not cause fertility effects in rats by inhalation or oral administration. No developmental effects in rats, mice, hamsters or rabbits treated by the oral route. No dermal data were available.

77.4.5 Mutagenic and genotoxic effects

PG was tested in *in vitro* bacterial assays and in mammalian cells for chromosome aberrations, DNA-damage and repair and cell transformation. All tests but one, a chromosome aberration assay in Chinese hamster lung cells conducted without metabolic activation, were negative.
An *in vivo* chromosome aberration test in rats and two dominant lethal *in vivo* tests, in rats and mice, were negative.

77.4.6 Carcinogenic effects

No carcinogenic effect was reported from PG in a 2-year oral study in rats or a 120-week dermal study in mice.

78 Evaluation

Data on human toxicity of propylene glycol (PG) are scarce. The toxicological data on experimental animals indicate that PG has a low order of toxicity.

PG has a very low acute oral toxicity in several species with LD_{50}-values of 18-33.5 g/kg b.w.. At high oral doses, central nervous system depression occurs as demonstrated in dogs treated with 8000 mg/ kg b.w.

No effects on the respiratory tract have been reported from human exposure to PG used as a vehicle for medical purposes. No systemic effects in rabbits and dogs were reported from short-time inhalation exposure of 10% PG. However, no details on exposure conditions or particle size of the aerosol were given in the reference. Local effects in the respiratory tract involving goblet cells have been reported in rats and rabbits. Haemorrhage from the nose and hyperaemia of the intestines is probably related to the highly hygroscopic character of PG and the effects are probably due to dehydration of the tissues.

PG is a mild irritant to rabbit skin. No data on sensitisation to PG in animals were found. Contact dermatitis in humans from PG has been reported. The majority of these reactions are of irritative nature. However, a few may be of allergic nature. On basis of the low number of persons sensitised to PG in comparison with widespread exposure due to extensive use of the substance, the sensitising potential of PG is considered to be low.

Effects on the erythrocytes with decreased erythrocyte counts and formation of Heinz bodies was described at high doses in dogs treated orally with 5000 mg PG/kg b.w./day over 2 years, and more consistently and at lower doses in cats exposed to PG by the oral route for 2-17 weeks to doses as low as 1100 mg/kg b.w./day. Cats thus appear to be more sensitive to PG, which could be explained by a different metabolism in cats possibly yielding a higher level of the parent compound in the blood. No haematological signs have been reported in humans from exposure to PG. Therefore, the effect is considered of lesser relevance to humans.

Short-term studies in rats and dogs showed no adverse effects at levels of around 10 % of the diet (equivalent to approximately 13200 mg/kg b.w. in rats). The NOAEL from long-term feeding studies in rats and dogs was 2000 mg/kg b.w./day. PG is considered of low toxicity following repeated exposure.

No effects on reproduction were reported from fertility and developmental studies in mice, rats, hamsters and rabbits.

Several negative *in vitro* assays for different mutagenicity end-points are available. One in vitro chromosome aberration test showed a positive result without metabolic activation. However, 3 *in vivo* tests were negative. On basis of all the evidence available, PG is evaluated not to be a mutagenic substance.

No carcinogenic effect has been reported.

Based on the available data, the critical effects of PG are considered to be the effects on the skin and its dehydrating potential to mucous membranes.

79 References

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Appendix 11: 2-Butoxyethanol (EGBE)

80 General description

80.1 Identity

Molecular formula:	$C_6H_{14}O_2$
Structural formula:	CH_3-CH_2-CH_2-CH_2-O-CH_2-CH_2-OH
Molecular weight:	118.2
CAS-no.:	111-76-2
Synonyms:	2-Butoxyethanol *n*-Butoxyethanol Butyl Cellosolve Butylglycol Glycol butyl ether Monobutyl ethylene glycol ether

80.2 Physical / chemical properties

Description:	Colourless liquid with a faint, mild ethereal odour.
Melting point:	-70, -75 °C
Boiling point:	171 °C
Density:	0.9019 g/ml (at 20°C)
Vapour pressure:	0.76 mmHg (101.3 Pa) (at 20°C) 0.88 mmHg (117.3 Pa) (at 25°C)
Concentration of saturated vapours:	4900 mg/m^3 (at 20°C and 760 mmHg) (calculated).
Conversion factor:	1 ppm = 4.91 mg/m^3 (at 20°C and 760 mmHg). 1 mg/m^3 = 0.204 ppm
Solubility:	Water: Miscible.
$LogP_{octanol/water}$:	0.81
References:	EPA (1999), ATSDR (1998).

81 Toxicokinetics

81.1 Absorption, distribution, excretion

81.1.1 Inhalation

When seven male volunteers were exposed to EGBE (20 ppm (98 mg/m^3)) for 2 hours during light physical exercise (bicycle ergometer), the respiratory uptake of EGBE averaged 1.2 mg/minute (57% of the inspired amount). The concentration of EGBE in blood reached a plateau level of 0.9 mg/l within 1-2 hours and was no longer detectable 2-4 hours after the end of exposure. The apparent values of elimination half-time, mean residence time, total blood clearance, and steady-state volume of distribution were 40 minutes, 42 minutes, 1.2 l/minute, and 54 l, respectively, for EGBE. The half-life for EGBE in urine was 1.36 hours. The amount of EGBE excreted in urine was less than 0.03% of the total uptake, while that of 2-butoxyacetic acid (2-BAA) ranged from 15-55%. Relative to the uptake of EGBE, the excretion averaged 41% on an equimolar basis. (Johanson et al. 1986 – quoted from ATSDR 1998, ECETOC 1995b).

In a similar study in five healthy male volunteers, the minimum and maximum observed concentrations of 2-BAA in blood ranged from 2.4 to 6.7 mg/l; the blood level peaked after 2-4 hours. (Johanson & Johanson 1991 – quoted from ATSDR 1998).

Healthy male volunteers (four individuals) were exposed to 50 ppm (245 mg/m^3) EGBE vapour for 2 hours by mouth (through a respiratory valve). The concentration of EGBE increased during the first hour and appeared to approach steady state at about 0.35 mg/l at the second hour. The respiratory uptake rate was 1.3 mg/minute. (Johanson & Boman 1991 – quoted from ATSDR 1998).

When rats (three to five animals per group) were exposed (nose-only) to 4.3, 49, or 438 ppm (21, 240, or 2150 mg/m^3) [^{14}C]EGBE for 6 hours, the respiratory uptake was 531, 541, and 423 mikrogram/ppm at 4.3, 49, and 439 ppm, respectively. A less-than-proportional amount of EGBE was, according to ATSDR, inhaled at the high dose because of decreased minute volume. 2-BAA was the major metabolite of EGBE in plasma. Ratios of ethylene glycol to 2-BAA in plasma were higher than those in urine. The majority of the inhaled [^{14}C]EGBE was eliminated in the urine, with free 2-BAA being the major urinary metabolite, accompanied by lesser amounts of ethylene glycol and EGBE glucuronide. A small proportion (5-8%) of the retained EGBE was exhales as ^{14}C-carbon dioxide. Faeces accounted for 1-2% of the absorbed dose. At 4.3 ppm, about 60% of the urinary radioactivity was excreted during the 6-hour exposure, whereas at 438 ppm, only 10% was excreted during the exposure period. The production of carbon dioxide most closely followed the production of ethylene glycol and may represent the metabolism of this metabolite. (Sabourin et al. 1992 – quoted from ATSDR 1998).

In another study in rats, eight male Sprague-Dawley rats were exposed to 20 or 100 ppm (98 or 490 mg/m^3) EGBE 24 hours per day for 1, 2, 3, 4, 6, 8, 10, or 12 days. The concentrations of EGBE and 2-BAA in blood, muscle, liver, and testis increased rapidly during the first 1-3 days of exposure and continued to increase, but more slowly, during the remaining days. The average peak concentration of EGBE in the blood was 1.8 mg/l in the 20-ppm group and 8.5 mg/l in the 100-ppm group. Respiratory uptake averaged 37 mg/day at 20 ppm and 186 mg/day at 100 ppm. The urinary excretion rate for 2-BAA in the 20-ppm group averaged 24 mg/day, whereas the 100-ppm group excreted 122 mg/day; the observed urinary excretion rate corresponded to 64% of the calculated respiratory uptake and renal clearance of 0.53 l/hour for both groups. (Johanson 1994 – quoted from ATSDR 1998).

81.1.2 Oral intake

Case reports of intentional poisonings with EGBE indicate that EGBE is absorbed in humans following oral exposure.

When male F-344 rats were given a single oral dose of [^{14}C]EGBE at 125 or 500 mg/kg, detectable exhalation of radioactivity began 1-2 hours after treatment indicating rapid absorption and distribution. Approximately 18 or 10% of the respective doses was exhaled as ^{14}C-carbon dioxide in 48 hours. Cumulative urinary excretion of radioactivity amounted to approximately 32 or 18% of the respective doses at 8 hours, to approximately 64 or 30% at 24 hours, and to approximately 70 or 40% at 48 hours. Faecal excretion of radioactivity amounted to 2-3% of the doses in 48 hours. Thus, by adding the 48-hour excretion of radioactivity in the expired air and urine, at least 88% of the 125 mg/kg dose and at least 50% of the 500 mg/kg dose was absorbed. According to ATSDR, the fact that the percentage of radioactivity excreted was less at the higher dose than at the lower dose may indicate saturation of a metabolic pathway rather than lower absorption of the high dose.
EGBE was distributed to all tissues, with the highest levels (determined 48 hours after dosing) detected in the forestomach. The increase in the tissue concentration was not proportional to the increase in dose. While the ratio of the administered doses was 1:4, the ration of the tissue concentration in most tissues was greater than 1:10. According to ATSDR, this may be related to saturation of EGBE metabolism at the high dose.
The major route of elimination was the urine (40-70%), followed by exhalation (11-18%). A small portion was excreted in the bile (8% at the 500-mg/kg dose) 8 hours after dosing, and a small amount was detected in the faeces (2-3%).
(Ghanayem et al. 1987 – quoted from ATSDR 1998).

In male F-344 rats given single gavage doses of 8.6 or 126 mg/kg [^{14}C]EGBE, 59% of the low dose of radioactivity and 38-70% of the high dose of radioactivity was excreted in the urine during the first 24 hours. Excretion of ^{14}C-carbon dioxide in the expired air amounted to 7.2 or 8%, respectively, in 24 hours. Thus, at least 66% of the low dose and 46-78% of the high dose was absorbed. (Corley et al. 1994 – quoted from ATSDR 1998).

Male F-344 rats were administered [^{14}C]EGBE in the drinking water at concentrations of 290, 860, or 2590 ppm (corresponding to 28, 47, or 141 mg/kg b.w./day according to ATSDR) for 24 hours. The majority of the

radioactivity was excreted in urine (50-60% as 2-BAA, 10% as ethylene glycol) or exhaled as ^{14}C-carbon dioxide (8-10% of the dose). Less than 5% of the dose was exhaled as unmetabolised EGBE. Ethylene glycol was excreted in urine, representing approximately 10% of the dose of EGBE. Elimination in the faeces was a minor pathway (1.6-2.8% of the dose). Unmetabolised EGBE and its glucuronide conjugate were also identified in the urine. The maximum excretion occurred during the first 12-24 hours after the start of exposure. (Medinsky et al. 1990 – quoted from ATSDR 1998).

No quantitative or qualitative alterations of metabolism or disposition were observed in rats by repeated exposure to EGBE as compared to treatment with a single dose. Elimination of ^{14}C-carbon dioxide and EGBE-derived radioactivity in the urine of rats receiving multiple doses of EGBE was essentially the same as for rats receiving a single dose. The ratios of free 2-BAA, the EGBE-glucuronide and the EGBE-sulphate conjugates, and parent EGBE excreted in the urine of rates treated for 4 or 8 days were relatively similar to those from rats treated with a single dose. (Ghanayem et al. 1992 – quoted from ATSDR 1998).

81.1.3 Dermal contact

Dermal absorption of EGBE was evaluated in workers using window-cleaning agents containing EGBE in concentrations ranging from 0.9 to 21.2% in volume (Vincent et al. 1993 – quoted in ATSDR 1998). Positive correlations between exposure levels of EGBE and 2-BAA in the urine indicate dermal (and probably some pulmonary) absorption of EGBE by these workers.

Experimental evidence exists that dermal absorption of EGBE vapours may contribute to the overall absorption of EGBE during vapour exposure (Johanson & Boman 1991 – quoted in ATSDR 1998, EPA 1999, ECETOC 1995b). Healthy male volunteers were exposed to 50 ppm (245 mg/m^3) EGBE vapour for 2-hour, followed by 1 hour of no exposure, followed by 2 hours of body-only exposure (exposed in a chamber while breathing fresh air via respirator) to 50 ppm. Capillary blood samples (finger prick, assuming that the finger prick blood samples represented mixed arterial blood) were collected at regular intervals and analysed for EGBE. The average concentration in blood and the calculated rate of uptake of EGBE was about 3-4 times higher during dermal exposure than during inhalation exposure, suggesting that about 75% of the total uptake during normal vapour exposure could be accounted for by dermal absorption.

The results obtained in the above study have been questioned by Corley et al. (1994 – quoted in EPA 1999). They reanalysed the data assuming that the finger prick blood samples represented venous blood draining the skin prior to mixing systemically. These simulations resulted in a dermal absorption contributing no more than 22% of the total uptake of EGBE in a whole-body exposure.

Corley et al. (1997 – quoted in ATSDR 1998, EPA 1999) observed a much lower dermal absorption (<1500 times) of EGBE vapour when blood samples were taken from an unexposed arm compared to blood samples taken from the exposed arm (finger prick sample).

About two-thirds of the 2-BAA excreted in the urine was in the form of the *N*-butoxyacetyl glutamine conjugate. No free EGBE, free og conjugated ethylene glycol ether, or glycolic acid were detected in the urine. 2-BAA was eliminated in the urine primarily during the first 12-hour collection period.

Dermal uptake has been estimated under worst-case resting conditions (respiration rates at their lowest, no clothing worn) and under more realistic conditions (35% of the body surface area exposed). Under the worst-case conditions, 15-27% of the total uptake of EGBE vapour would be through the skin, while dermal uptake would be 4.5-8.5% of the total if only 25% of the body surface was exposed. Under simulated exercise, uptake was estimated as 4.6-8.7% and 1.2-2.3% of the total assuming 100 or 25% of the body surface area was exposed, respectively. (Corley et al. 1997 – quoted from ATSDR 1998).

Percutaneous absorption of liquid EGBE has been investigated in male volunteers keeping two or four fingers immersed in neat EGBE for 2 hours. The presence of EGBE in blood and 2-BAA in urine confirmed that EGBE enters the systemic circulation. The excretion rate of 2-BAA increased during the first hours after exposure, reached a maximum at about 5 hours, and then declined, with an average half-life of 3.1 hours. Seventeen percent of the absorbed dose was excreted as 2-BAA in 24 hours. (Johanson et al. 1988).

EGBE is also absorbed through animal skin. Percutaneous absorption in rats appears to be 20-30% of the applied dose (Bartnik et al. 1987, Sabourin et al. 1992, 1993 – both quoted in ATSDR 1998).
Only a small amount of the applied dose (0.3-2%) was still present at the application site 72 hours following dosing. The majority of the absorbed dose was excreted in the urine (82-83%); 3-5% was found as carbon dioxide, 3-6% in the faeces, and 3-13% in the carcass. Free 2-BAA was the main metabolite (66-70%), followed by the EGBE-glucuronide (13-15%9, and ethylene glycol (4-6%). (Sabourin et al. 1992, 1993 – quoted in ATSDR 1998).

81.2 Elimination

According to ATSDR, there is no available evidence to suggest that the route of administration has any substantial effect on the subsequent metabolism of EGBE in humans, but data from animals indicate that the route of exposure may make some differences in the relative amounts of metabolites produced.

The proposed metabolic pathway in rats and humans for EGBE is shown in Figure 2.2. EGBE is oxidised in the liver by alcohol dehydrogenase to butoxyacetaldehyde (BAL), which is further oxidised to 2-BAA by acetaldehyde dehydrogenase. In humans, 2-BAA can be conjugated with glycine or glutamine to form *N*-butoxyacetyl glycine or *N*-butoxyacetyl glutamine, respectively, or it can be further metabolised (humans, rats) to carbon dioxide. EGBE can also undergo O-dealkylation (by a cytochrome P450 dealkylase CYP2E1) to form ethylene glycol, and butyraldehyde and butanol. In rats, EGBE can also be conjugated with sulphate to form butoxyethanol sulphate, or with glucuronic acid to form the glucuronide. (EPA 1999, ATSDR 1998).

The two main oxidative pathway of EGBE metabolism in rats are alcohol dehydrogenase and O-dealkylation by CYP2E1. EGBE may also form

conjugates with glucuronide and sulphate to some extent. Primarily because 2-BAA is excreted in the urine of both rats and humans following exposure to EGBE, it has been suggested that the former pathway would be applicable to both rats and humans. However, the other three proposed metabolic pathways of EGBE may be applicable only to rats as the metabolites of these pathways (ethylene glycol, EGBE-glucuronide, and EGBE-sulphate) have been observed only in the urine of rats and not in the urine of humans. In addition, approximately two-thirds of the 2-BAA formed by humans is conjugated with glutamine and, to a lesser extent, glycine; these 2-BAA conjugation pathways have not been detected in the rat. (Medinsky et al. 1990, Corley et al. 1997, Bartnik et al. 1987, Ghanayem et al. 1987 – quoted from EPA 1999).

81.3 Mode of action

The most characteristic toxic effect of EGBE in experimental animals is on the haematological system and includes elevated osmotic fragility of erythrocytes, haemolysis, decreased haematocrit (Hct), haemoglobinuria, haemoglobinaemia, and haemolytic anaemia. Compensatory erythropoiesis occurring in response to haemolysis results in an increase in reticulocytes, increased mean cell volume, increased or normal mean cell haemoglobin, and decreased mean cell haemoglobin concentrations. Most of the other toxic effects of EGBE observed in animal studies may be secondary to the haemolytic effects. (ATSDR 1998, NTP 2000).

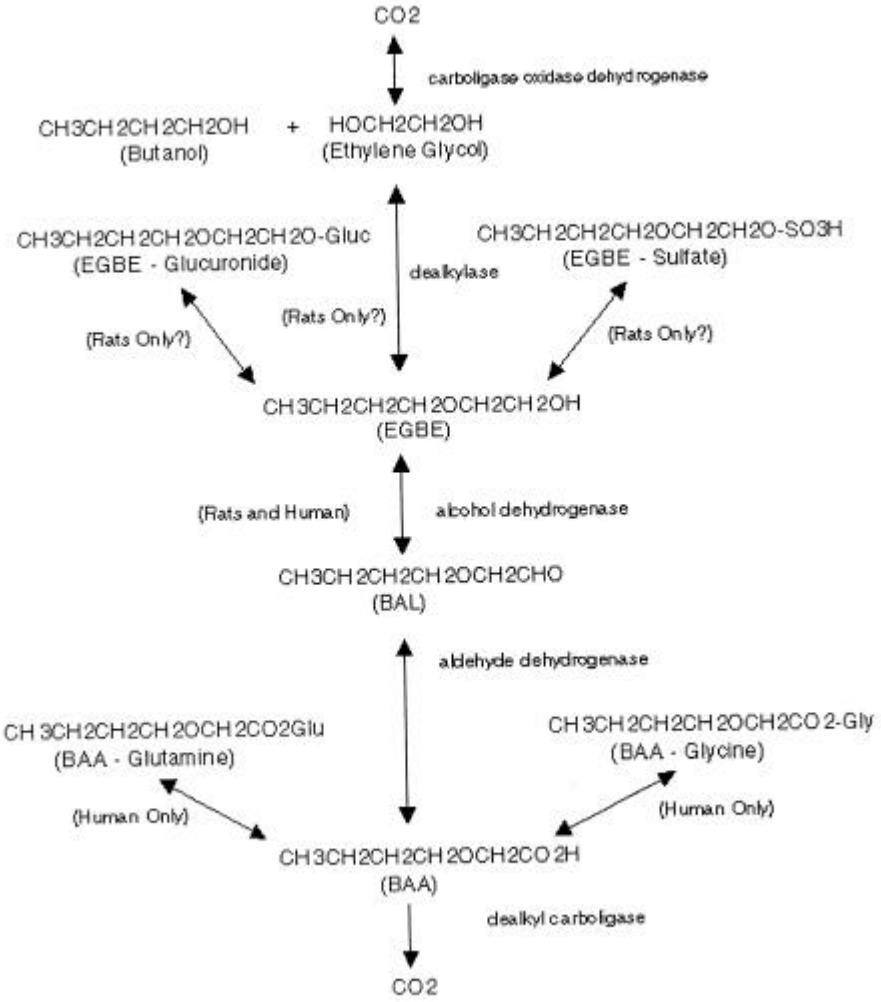

Figure 2.2. Proposed metabolic pathway for EGBE in rats and humans. From EPA (1999 – adapted from Medinsky et al. 1990 and Corley et al. 1997).

The EGBE-induced haemolysis is a direct effect on the erythrocyte membrane causing osmotic fragility and intravascular lysis. Significant species differences have been reported with rats appearing to be particularly sensitive to EGBE induced haemolysis, whereas humans appear to be more resistant to this effect. (ECETOC 1995a, ATSDR 1998, NTP 2000). The mechanism(s) of action is not fully understood, but the metabolite of EGBE, 2-butoxyacetic acid (2-BAA), shows a significantly greater haemolytic effect *in vitro* than EGBE (ECETOC 1995a, EPA 1999, ATSDR 1998). Furthermore, studies in rats showed that pyrazole and cyanamide as metabolic inhibitors of alcohol and aldehyde dehydrogenases, respectively, protected or significantly reduced the EGBE-induced haematotoxicity (EPA 1999).

Spermatogenesis is adversely affected by the lower ethylene glycol ethers (EGME, EGEE) and their acetates with EGME being the most potent glycol

ether inducing testicular toxicity. There is no evidence however, that higher homologues, as EGBE, are testicular toxicants. (ECETOC 1995a).

The developmental effects of ethylene glycol ethers are mediated by alkoxy acetic acid metabolites. However, ethylene glycol ethers with alkyl chains of more than 2 carbon atoms, as EGBE, do not appear to be selectively toxic to the foetus. For EGBE, foetotoxic effects have been observed only in the presence of maternal toxicity. In an *in vitro* study, the alkoxy acetic acids methoxyacetic acid (MAA) (from ethylene glycol monomethyl ether (EGME)) and ethoxyacetic acid (EEA) (from ethylene glycol monomethyl ether (EGEE)) showed similar developmental effects, whereas 2-BAA (from EGBE) was only slightly active. (ECETOC 1995a).

82 Human toxicity

Haematological effects have been identified as the critical end-point in toxicological studies on EGBE. Humans appear to be less sensitive to the haematological effects of EGBE than are rats, mice, rabbits, and guinea pigs. (EPA 1999).

82.1 Single dose toxicity

82.1.1 Inhalation

Three controlled studies have been conducted by Carpenter et al. (1956 – quoted from EPA 1999, ATSDR 1998, NTP 2000).
In the first study, two men were exposed for 4 hours to an EGBE concentration of 113 ppm (555 mg/m^3). Effects observed included nasal and ocular irritation, a metallic taste in the mouth, and belching. Erythrocyte osmotic fragility was not affected.
In a second study, two men and one woman were exposed to 195 pm (957 mg/m^3) for two 4-hour periods, separated by a 30-minute recess. There was no change in the blood pressure, erythrocyte fragility, or pulse rate. Irritation of the nose and throat followed by ocular irritation and disturbed taste was noted.
In the third study, two men and two women were exposed for 8 hours to an EGBE concentration of 100 ppm (490 mg/m^3). No changes in blood pressure, erythrocyte fragility, or pulse rate were observed. Irritation of the nose and throat followed by ocular irritation and a disturbing metallic taste were noted.

When seven male volunteers were exposed to EGBE (20 ppm (98 mg/m^3)) for 2 hours during light physical exercise (bicycle ergometer), none of the subjects complained of or showed any signs of adverse effects (pulmonary ventilation, respiratory frequency, heart rate, electrocardiograms) (Johanson et al. 1986 – quoted from ECETOC 1995b, ATSDR 1998, NTP 2000).

82.1.2 Oral intake

In twenty-four children (aged 7 months to 9 years) observed subsequent to oral ingestion of at least 5 ml of glass window cleaner containing EGBE (0.5-9.9%), no symptoms of EGBE poisoning and no haemolysis were observed in any of the children (Dean & Krenzelok 1991 – quoted from EPA 1999, ATSDR 1998).

Poisoning cases by ingestion of household products containing EGBE have been reported (Rambourg-Schepens et al. 1988, Gijsenbergh et al. 1989, Bauer et al. 1992, Gualtieri et al. 1995 – quoted from EPA 1999, ECETOC 1995b, ATSDR 1998, NTP 2000). The intakes of EGBE were 30-60 ml, 25-30 g (corresponding to 400-500 mg/kg b.w.), ca. 46 ml, and 79-105 ml (corresponding to 1130-1500 mg/kg b.w.) in the four case reports, respectively. The symptoms observed in various studies included cardiovascular effects (increased heart rate, decreased blood pressure),

haematological effects (decreased haemoglobin concentration and haematocrit, and thrombocytopenia), metabolic acidosis, haematuria, and haemoglobinuria.

82.1.3 Dermal contact

No data have been located.

82.1.4 *In vitro* studies

Blood from male and female young volunteers was unaffected by 4-hour incubations with 2-butoxyacetic acid (2-BAA, the toxic metabolite of EGBE) at concentrations up to 4.0 mM (470 mg/l) (60-235 mg/l produced total rat erythrocyte haemolysis). There was a slight increase in haemolysis of human erythrocytes incubated with 8 mM (945 mg/l) of 2-BAA with female erythrocytes showing a slightly greater sensitivity than male erythrocytes. (Ghanayem 1989, Ghanayem 1989 et al. 1989 – quoted from EPA 1999, ECETOC 1995b, ATSDR 1998).

Using a sensitive assay for erythrocyte deformability and haemolysis, human erythrocytes were unaffected following exposure to 2-BAA at concentrations up to 2mM (235 mg/l) for up to 4 hours (rat erythrocytes were haemolysed and showed decreased deformability). The possibility that certain human subpopulations, including the aged and those predisposed to haemolytic disorders, might be at an increased risk from exposure to EGBE has been investigated using blood from the elderly (mean age 71.9 years; range 64-79 years, five men and four women), from patients with sickle cell disease (seven patients), and from patients with spherocytosis (three individuals). Erythrocytes from these potentially sensitive groups were unaffected by incubations with 2-BAA at concentrations up to 2.0 mM (235 mg/l) for up to 4 hours. (Udden 1994, Udden & Patton 1994 – quoted from EPA 1999, NTP 2000; Udden 1992 – quoted from ECETOC 1995b).
The deformability of human erythrocytes incubated at 2-BAA concentrations of 7.5-10 mM (885-1180 mg/l) displayed a slight but significant decrease that was accompanied by slight increases in osmotic fragility and MCV. These effects were judged pre-haemolytic and corresponded to similar changes reported in rat erythrocytes, but at approximately 15-fold lower concentrations (0.5 mM) in rat erythrocytes than in human erythrocytes. (Udden 1995 – quoted from EPA 1999).

82.2 Irritation

Irritation of nose and throat, and eyes have been reported following exposure to airborne EGBE at concentrations from 490-957 mg/m^3 for 4-8 hours; irritation was not reported following exposure to EGBE (98 mg/m^3) for 2 hours. See 3.1.1 for further details.

82.3 Sensitisation

Volunteers (214 men and women) were given 0.2 ml 10% EGBE in patches applied to the intra-scapular area of the back. The dosed area was occluded. Testing included dosing, removal of the patch within 24 hours, evaluating the exposed area at 48 hours, and application of an identical patch to the same location. The subjects received nine such applications over a 6-week period. By the end of the 6 weeks, 25% of the subjects showed slight or definite

erythema. Following application to a naïve area at 6 weeks, slight erythema was observed in seven subjects at 48 hours and 12 at 72 hours; one subject had definite erythema at 72 hours. No other effects were noted. It was concluded that there were no dermal effects of 10% EGBE under the conditions of the study. (Greenspan et al. 1995 – quoted from ATSDR 1998, NTP 2000).

82.4 Repeated dose toxicity

In a cross-section study, 31 male workers (22-45 years old, employed for 1-6 years) exposed to low levels of EGBE were monitored by Haufroid et al. (1997 – quoted from EPA 1999, ATSDR 1998). The average airborne concentration of EGBE was 2.9 mg/m^3 (20 workers were exposed to an average concentration of 3.7 mg/m^3 and 11 workers to 2.3 mg/m^3). Red blood cell (RBC) count, haemoglobin (Hgb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), haptoglobin (Hp), reticulocyte numeration (Ret), and osmotic resistance (OR, a measure of osmotic fragility) as well as hepatic and renal creatinine and urinary retinal binding protein parameters was investigated. Single determinations of 2-BAA (toxic metabolite of EGBE) in post-shift urine samples were used to assess exposure to EGBE. A significant correlation was observed between post-shift free urinary 2-BAA concentrations and EGBE in air. No difference between exposed and control workers was observed for RBC counts, Hgb, MCV, MCH, Hp, Ret, and OR. Statistically significant changes were observed for Hct (3.3% decrease) and MCHC (2.1% increase). No significant differences were observed in hepatic and renal biomarkers. According to ATSDR (1998), the differences in Hct and MCHC may be consistent with haemolysis and may be early indicators of potential adverse effects, but because the changes were in the range of normal clinical values, the concentration in the air of EGBE (2.9 mg/m^3) was considered as a NOAEC.

82.5 Toxicity to reproduction

No data have been located regarding toxicity to reproduction in humans following exposure to EGBE.

82.6 Mutagenic and genotoxic effects

No increases in the frequencies of micronuclei or sister chromatid exchanges were observed in peripheral blood lymphocytes of varnish production workers exposed to both EGBE and to 2-ethoxyethanol (EGEE) (Söhnlein et al. 1993 – quoted from ATSDR 1998, NTP 2000).

82.7 Carcinogenic effects

No data have been located regarding carcinogenic effects in humans following exposure to EGBE.

83 Animal toxicity

Numerous studies have examined the effects of EGBE in experimental animals. Haematological effects have been identified as the critical end-point in the toxicological studies following both acute and repeated exposures. In addition to the haematological effects, effects in the liver, spleen, and kidney have also been observed following exposure to EGBE; the available data indicate that these effects are secondary to the haematological effects. Below, the focus has therefore been put on the most relevant studies for an evaluation of the haematological effects primarily based on the citations of the relevant studies in EPA (1999), ATSDR (1998), and ECETOC (1995b).

83.1 Single dose toxicity

83.1.1 Inhalation

For rats, an LC_{50}-value (4 hours exposure, whole-body) of 2385 mg/m^3 has been reported for males and of 2210 for females; haemoglobinuria and effects in kidneys were observed. In another study, the LC_{50}-value (4 hours exposure) was reported to be about 2455 mg/m^3. (ECETOC 1995b, ATSDR 1998, NTP 2000).

An LC_{50}-value (7 hours exposure) of 3440 mg/m^3 has been reported for mice. One of six guinea pigs died after 4 hours of exposure to saturated vapour (ca. 4900 mg/m^3); no haemoglobinuria was observed. (ECETOC 1995b, ATSDR 1998, NTP 2000).

Haematological effects of EGBE have been observed in animals after acute inhalation exposure. Female rats exposed to 432 ppm (2120 mg/m^3) for 2-8 hours exhibited haemolysis at 2 hours, haemoglobinuria at 3 hours, and hemin crystalluria at 4 hours during exposure. Elevation of erythrocyte fragility was seen in rats exposed to 62 ppm (305 mg/m^3) for 4 hours. Haemoglobinuria has been observed in rats exposed to 203 ppm (995 mg/m^3) for 7 hours and in mice exposed to 200 ppm (980 mg/m^3) for 7 hours. One male monkey and one female monkey exposed to 200 ppm (980 mg/m^3) EGBE for 7 hours did not show any alteration of erythrocyte fragility or evidence of haemoglobinuria. (Carpenter et al. 1956 – quoted from ATSDR 1998).

83.1.2 Oral intake

LD_{50}-values ranging from 530 to 3000 mg/kg have been reported for rats; the LD_{50}-value was age and sex dependent, with females and older animals being more susceptible to toxicity. Signs of toxicity included congested or haemorrhagic lungs, mottled livers, congested kidneys, and haemoglobinuria (ECETOC 1995b, ATSDR 1998, NTP 2000).

LD_{50}-values of 1230 and 1519 mg/kg have been reported for mice, from 320 to 3100 mg/kg in rabbits, and from 950 to 1415 mg/kg in guinea pigs (ECETOC 1995b, ATSDR 1998, NTP 2000).

Administration of a 2000 mg/kg oral dose of EGBE to guinea pigs caused complete mortality of females and 60% mortality of males. A 20% mortality was observed following administration of 1000 mg/kg. Clinical signs and gross necropsy indicated toxicity was due to irritation of the stomach. There was no evidence of haemolytic toxicity. (Shepard 1994 – quoted from EPA 1999).

Haemolysis and haemoglobinuria are common observations in animals treated orally with EGBE for acute duration (ATSDR 1998).
In rats, haemoglobinuria was observed following a single dose (gavage) of 3000 mg/kg in male rats and 1500 mg/kg in female rats (Carpenter et al. 1956 – quoted from ATSDR 1998).
Male rats receiving a single dose of 500 mg/kg by gavage exhibited haemolysis of erythrocytes accompanied by a drastic increase in the concentration of free haemoglobin in the plasma and haemoglobinuria was observed in all rats. These effects were also observed following a single dose of 125 mg/kg, although to a lesser degree. (Ghanayem et al. 1987 – quoted from ATSDR 1998).
In male rats given 250 mg/kg by gavage, haemolysis was noted, characterised by an increase in mean cell volume and haematocrit, followed by a decline in haemoglobin concentration and RBC count. MCH and MCHC initially increased and then declined. Guinea pigs dosed once with 250 mg/kg showed no adverse haematological effects. (Ghanayem & Sullivan 1993 – quoted from ATSDR 1998).

The effect of age on toxicity of EGBE has been assessed by comparing effects in treated young male rats (4-5 weeks old) and adult male rats (9-13 weeks, 5-6 months, or 16 months) given single doses (gavage in water) of 0, 32, 63, 125, 250, or 500 mg/kg). Evaluations included haematology (red blood cell (RBC) counts and white blood cell (WBC) counts), organ weights, and histology (liver, spleen, bladder, kidney, and testes). Haematological effects were found to be dose- and age-dependent, with older rats being more sensitive than younger rats. Significant decreases in RBC counts, Hct and Hgb occurred in both adult and young rats, with the younger rats exhibiting significantly less pronounced responses. Histopathological changes in the liver, consisting of focal coagulative necrosis of hepatocytes were observed in adult rats at the two highest dose levels; no effects were seen in the liver of young rats. Phagocytised haemoglobin was found in the hepatic parenchymal cells and Kupffer cells, which is consistent with the role of these cells in haemoglobin degradation. Histopathological changes in the kidney, consisting of haemoglobin casts in the proximal tubules, were observed in mature rats from 125 mg/kg b.w./day; no effects were seen in young rats. (Ghanayem et al. 1987 – quoted from EPA 1999, ATSDR 1998).
Based upon the findings in this study, a LOAEL of 32 mg/kg for haematological effects in the aged rats has been used for establishing an acute oral 'Minimal Risk Level MRL' of 0.4 mg/kg (ATSDR 1998).

83.1.3 Dermal contact

LD_{50}-values ranging from 406 to 1804 mg/kg have been reported for rabbits, from 1200 to 4800 mg/kg (1 week occluded exposure) for guinea pigs, of 255 or 271 mg/kg for guinea pigs on intact or abraded skin, respectively, and of 2273 mg/kg for rats (ECETOC 1995b, ATSDR 1998).

Dermal exposure of animals has also resulted in haemolysis of red blood cells and haemoglobinuria (ATSDR 1998).
Haemolytic effects were observed in 2/3 rats within 6 hours following a single application of 260 mg/kg EGBE placed on the dorsal shaved skin and covered with a glass capsule, and haemoglobinuria in 1/3 rats. A dose of 500 mg/kg increased MCV, decreased RBC count and haemoglobin level, and haemoglobinuria; no effects were observed at 200 mg/kg (Bartnik et al. 1987 – quoted from EPA 1999, ATSDR 1998).

83.1.4 *In vitro* studies

Ghanayem (1989 – quoted from EPA 1999, ECETOC 1995b) has studied the metabolic and cellular basis of EGBE-induced haemolysis of rat erythrocytes *in vitro*. EGBE is not metabolised when incubated with blood from rats and caused no haemolysis or metabolic alterations at concentrations up to 10 mM (1180 mg/l). A concentration of 20 mM (2360 mg/l) EGBE was required to produce significant haemolysis of rat blood. Incubation of rat blood with the metabolites of EGBE 2-butoxyacetaldehyde (2-BAL) and 2-butoxyacetic acid (2-BAA) caused a time- and concentration-dependent increase in cell swelling (increased Hct) followed by haemolysis. This response was more pronounced for 2-BAA, with nearly complete haemolysis observed following a 4-hour incubations at 2.0 mM (235 mg/l); BAL produced only slight haemolysis under the same conditions. The addition of aldehyde dehydrogenase and its cofactors to rat blood followed by BAL produced a potentiation of the haemolytic effects. Addition of cyanamide, an aldehyde dehydrogenase inhibitor, significantly decreased the effects either with or without added aldehyde dehydrogenase.

83.2 Irritation

83.2.1 Skin irritation

Dermal irritation from EGBE has been studied in rabbits using both the Draize protocol (24-hour occluded exposure) and the EU protocol (4-hour occluded exposure. For both protocols, 0.5 ml of undiluted EGBE was placed on the skin. EGBE was considered a severely irritant by the Draize protocol and an irritant by the EU protocol. (Zissu 1995 – quoted from ATSDR 1998).

EGBE has been reported to be slightly irritating to rabbit skin (4 hour, non occluded exposure) and moderately irritating in percutaneous toxicity studies over 24 hours (Tyler 1984 – quoted from ECETOC 1995b).

Several other studies cited in ATSDR (1998) have also reported EGBE to be a skin irritant in rabbits.

Female rabbits exposed to EGBE (≥72 mg/kg) for 8 hours developed cutaneous lesions accompanied by necrosis of epidermis and dermis on the 4th day after exposure; skin lesions healed within a 2-week period (Duprat & Gradiski 1979 – quoted from ATSDR 1998).

Severe skin irritation has been noted in guinea pigs after application of EGBE (dose not specified) (Eastman Kodak 1988 – quoted in ATSDR 1998).

83.2.2 Eye irritation

In several studies cited in ATSDR (1998), EGBE has been reported to be severely irritating when instilled (undiluted) in the eyes of rabbits; moderate to extensive conjunctivitis, moderate corneal damage, and/or slight iritis were observed.

According to ECETOC (1995b), EGBE is severely irritating to the rabbit eye.

Moderate corneal injury was observed in rabbits in which 0.5 ml of a 15% dilution of EGBE was placed in the conjunctival sac; no effects were observed with a dilution of 5% (Union Carbide 1980 – quoted from ATSDR 1998).

In a Draize test performed in rabbits, scores for different concentrations (given in parenthesis as percent) tested at 24 hours post-instillation (0.1 ml in polyethylene glycol) were 66 (100%) indicating severe irritation, 49 (70%) and 39 (30%) indicating moderate irritation, and 2 (20%) and 1 (10%) indicating mild irritation (the maximal obtainable score in the system is not stated in the reviews). In an assessment that measured corneal thickness, the highest concentrations was classified as severely irritating, the 70% concentration was moderately irritating, and the lower concentrations were mildly irritating. (Kennah et al. 1989 – quoted from EPA 1999, ATSDR 1998).

In another test for ocular irritation in rabbits (no further details given), mean erythema scores and percent corneal thickening indicated that the substance should be classified as an eye irritant (Jacobs & Marten 1989 – quoted from EPA 1999).

83.2.3 Respiratory tract irritation

Male mice exposed to 153-1666 ppm (750-8200 mg/m^3) EGBE for 10-15 minutes exhibited a 20% decreased in respiratory rate at the lowest concentration and a 40% decrease at the highest concentration; the decrease occurred during the first few seconds of exposure and is an indicator of respiratory irritation (Kane et al. 1980 – quoted from EPA 1999).

83.3 Sensitisation

EGBE has been tested for dermal sensitisation in guinea pigs using the maximized Magnusson and Kligman test. The animals were given an injection of EGBE with Freund's adjuvant behind the shoulders at the beginning of the 1st week of the study. On the 8th day, the animals received a 48-hour topical application of EGBE (doses not stated). On the 24th day of the study, 0.5 ml of a 1% EGBE solution was applied for 48 hours to the left sheared flank using an occlusive patch. The intensity of the erythema and oedema was scored, and histopathological examinations were completed 24 hours after the removal of the patch. EGBE did not result in dermal sensitisation under the conditions of this study. (Zissu 1995 – quoted from ATSDR 1998).

EGBE was not sensitising in a guinea pig maximisation test (0.5% injection induction, 25% application induction, 10% application challenge) (Unilever 1989 – quoted from ECETOC 1999b).

83.4 Repeated dose toxicity

83.4.1 Inhalation

83.4.1.1 Rats

Wistar rats (23 animals/group) were exposed by inhalation to 0, 135, or 320 ppm EGBE (0, 663, or 1570 mg/m^3) 7 hours/day, 5 days/week for 5 weeks. The highest dose level resulted in an increased percentage of circulating immature granulocytes, a decrease in Hgb concentration and RBC count, and an increase in RTC count. These haematological changes were, according to the authors, not severe and reversed 3 weeks after discontinuing exposures. No effect on the WBC count was observed. (Werner et al. 1943 – quoted from EPA 1999, NTP 2000).

Rats (15 animals/sex) were exposed to EGBE vapours at concentrations of 54, 107, 203, 314, or 432 ppm (265, 525, 997, 1540, or 2120 mg/m^3) 7 hours/day, 5 days/week for 6 weeks. Erythrocyte osmotic fragility was observed immediately after a single 7-hour exposure to 107 ppm or higher; fragility in females exceeded that for males. In almost all animals, these high fragility values returned to normal after the overnight exposure free period. (Carpenter et al. 1956 – quoted from EPA 1999, NTP 2000).

In a 90-day study, F344 rats (16 rats/sex) were exposed to EGBE vapours for 6 hours/day, 5 days/week at concentrations of 0, 5, 25, or 77 ppm (0, 25, 123, or 378 mg/m^3). High-dose males exhibited slight (5%) but statistically significant decreases in red blood cell (RBC) counts and haemoglobin (Hgb) levels that were accompanied by increases in mean corpuscular haemoglobin (MCH). At the end of the study, these effects had either decreased or returned to the range of the control values. The NOAEC was determined to be 25 ppm (123 mg/m^3). (Dodd et al. 1983 – quoted from EPA 1999; ATSDR 1998, NTP 2000).

In a 14-week NTP study (NTP 2000), F344 rats (10 animals/sex/group) were exposed via inhalation to concentrations of 0, 31, 62.5, 125, 250, or 500 ppm of EGBE (0, 152, 307, 614, 1228, or 2455 mg/m^3) 6 hours/day, 5 days/week.
One female rat in the 250 ppm group was killed moribund during week 8; four females in the 500 ppm group were killed moribund during week 1 and one during week 5. Final mean body weights of females exposed to 500 ppm were significantly less than those of the controls. Clinical findings included abnormal breathing, pallor, red urine stains, nasal and eye discharge, lethargy, and increased salivation and/or lachrymation.
The haematological evaluation showed an exposure concentration-related anaemia, evidenced by decreases in Hct, Hgb, and RBC counts, occurred from 125 ppm in males and in all exposed groups of females. Females appeared to be more slightly sensitive than males as evidenced by approximately 25-35% decreases in Hct and Hgb in females compared to approximately 20-25% decreases in males, and a 44% decreased in RBC counts for 500 ppm females compared to a 34% decreased for 500 ppm

males. The anaemia was characterised as microcytic, normochromic, and responsive.
Liver and kidney weights were significantly increased in females from 125 ppm and in males from 250 ppm and at 500 ppm, respectively; thymus weights of 500 ppm females were significantly decreased. The histopathological lesions noted in rats at terminal sacrifice were similar between males and females and were consistent with haemolytic anaemia and haemoglobinuria and included haematopoietic cell proliferation of the spleen, primarily erythroid (in females from 62.5 ppm; in males from 250 ppm); bone marrow hyperplasia (in females from 62.5 ppm; in males from 250 ppm); pigmentation (haemosiderin) of the hepatic Kupffer cells (in females from 62.5 ppm; in males from 125 ppm); and deposition of pigment (haemosiderin) in the renal cortical tubules (in females from 125 ppm; in males from 250 ppm). In addition, minimal forestomach inflammation and epithelial hyperplasia were noted in male rats from 250 ppm and epithelial hyperplasia in one female at 250 and at 500 ppm. The 31 ppm exposure level (152 mg/m^3) was a LOAEC for haematological changes in female rats and 62.5 ppm (307 mg/m^3) a NOAEC in male rats.

NTP (2000) has also performed a 2-year inhalation study in F344 rats (50 animals/sex/group) exposed to concentrations of 0, 31, 62.5, or 125 ppm of EGBE (0, 152, 307, or 614 mg/m^3) 6 hours/day, 5 days/week. For haematology and bone marrow analysis, additional groups of rats (27/sex/group) were exposed similarly to 0, 62.5 or 125 ppm of EGBE (0, 307, or 614 mg/m^3) for evaluation at 3, 6, and 12 months and nine male and nine female rats were exposed to 31 ppm (152 mg/m^3) for evaluation at 3 (haematology only) and 6 months.
No effect on survival was observed. Mean body weight of 125 ppm female rats were generally less than those of controls. Non-neoplastic effects included hyaline degeneration of the olfactory epithelium (males: 13/48, 21/49, 23/49, 40/50; females: 13/50, 18/48, 28/50, 40/49); Kupffer cell pigmentation in the livers (males: 23/50, 30/50, 34/50, 42/50; females: 15/50, 19/50, 36/50, 47/50), and splenic fibrosis in males (11/50, 14/50, 19/50, 20/50). The severity of the nasal lesion was not affected by exposure and is considered to be the most common age-related change in the nasal passages of rats; overall, the lesion was considered to be incidental and likely not related to EGBE exposure. The Kupffer cell pigmentation resulted from haemosiderin accumulation, a recognised secondary effect of the haemolytic activity of EGBE. Neoplastic findings are described in 4.7.
The most consistent exposure-related effect on the haematopoietic system was an exposure concentration-related mild macrocytic, normochromic, and responsive anaemia, evidenced by decreases in Hct, Hgb, and RBC counts, with females being more affected than males. Significant decreases in Hct values, Hgb concentration and RBC counts occurred at 3 and 6 months in females from 31 ppm and in males at 125 ppm, and at 12 months in males and females from 62.5 ppm. Evidence of macrocytosis was demonstrated by increases in MCV (at 3 months from 31 ppm in both males and females) accompanied by elevation in the MCHV. Increases were also observed in reticulocyte and nucleated erythrocyte counts (both sexes), which is consistetnt with an erythropoietic response to the anaemia. Increases in bone marrow cellularity occurred in 125 ppm females at all time points.
The LOAEC for haematological changes was 31 ppm (152 mg/m^3).

83.4.1.2 Mice

Mice (10 males per group) were exposed to EGBE vapours at concentrations of 100, 200, or 400 ppm (490, 980, or 1965 mg/m^3) 7 hours/day for 30, 60, or 90 days. An increase in erythrocyte osmotic fragility occurred at all concentrations and was consistent throughout the exposures. In all animals, erythrocyte osmotic fragility was normal after a 17-hour rest period. (Carpenter et al. 1956 – quoted from EPA 1999, ATSDR 1998, NTP 2000).

In a 14-week NTP study (NTP 2000), B6C3F1 mice (10 animals/sex/group) were exposed via inhalation to concentrations of 0, 31, 62.5, 125, 250, or 500 ppm of EGBE (0, 152, 307, 614, 1228, or 2455 mg/m^3) 6 hours/day, 5 days/week.
An exposure concentration-related anaemia, evidenced by decreases in Htc, Hgb, and RBC counts, occurred in males from 125 ppm and in all exposed groups of females; the anaemia was slightly more pronounced in females than in males. The anaemia was characterised as normocytic, normochromic, and responsive; nocrmocytic and normochromic erythrocytes were demonstrated by the lack of change in MCV and MCHC, and responsiveness by increased reticulocyte counts. Platelet counts were increased from 250 ppm in females and at 500 ppm in males.
Histopathological effects observed in mice surviving to the end of the study consisted of exposure-related increases in the incidences of haematopoietic cell proliferation and haemosiderin pigmentation of the spleen (in females from 250 ppm; in males from 125 ppm); haemosiderin pigmentation in hepatic Kupffer cells (in females from 250 ppm; in males at 500 ppm); and renal tubular haemosiderin pigmentation (in males and females at 250 ppm); and epithelial hyperplasia and inflammation (in females from 125 ppm; in males at 500 ppm).
The NOAEC for haematological changes was 62.5 ppm (307 mg/m^3) in males and the LOAEC was 31 ppm (152 mg/m^3).

NTP (2000) has also performed a 2-year inhalation study in B6C3F1 mice (50 animals/sex/group) exposed to concentrations of 0, 62.5, 125, or 250 ppm of EGBE (0, 307, 614, or 1228 mg/m^3) 6 hours/day, 5 days/week. For haematology and bone marrow analyses, additional groups of mice (30/sex/group) were exposed similarly and evaluated at 3, 6, and 12 months. Survival was significantly decreased in male mice at 125 and 250 ppm (54 and 53%, respectively). Mean body weights were generally less in exposed animals than for controls, with females experiencing greater and earlier reductions. Non-neoplastic effects included forestomach ulcers and epithelial hyperplasia (in males at 125 ppm; in females in all exposed groups); haematopoietic cell proliferation in the spleen (in males from 125 ppm; in females at 250 ppm); haemosiderin pigmentation in the spleen (in males in all exposed groups; in females from 125 ppm); Kupffer cell pigmentation (haemosiderin) in the livers (in males from 125 ppm; in females in all exposed groups); hyaline degeneration of the olfactory epithelium (in females in all exposed groups); and bone marrow hyperplasia (in males from 125 ppm). Neoplastic findings are described in 4.7.
An exposure-related anaemia, evidenced by decreases in Htc, Hgb, and RBC counts, occurred in males from 125 ppm and in all exposed groups of females; the anaemia was slightly more pronounced in females than in males. The anaemia was characterised as minimal normocytic, normochromic, and

regenerative. The LOAEC for haematological changes was 62.5 ppm (307 mg/m^3).

83.4.1.3 Dogs

Dogs (2 animals/group) were exposed by inhalation to 0, or 415 ppm EGBE (0 or 2040 mg/m^3) 7 hours/day, 5 days/week for 12 weeks. Decreased Hgb concentration and RBC count, and increased hypochromia, polychromatophilia, and microcytosis were observed. These haematological changes were, according to the authors, not severe and reversed 5 weeks after the end of exposure. (Werner et al. 1943 – quoted from EPA 1999, ATSDR 1998, NTP 2000).

83.4.1.4 Monkeys

One rhesus monkey exposed to 210 ppm (1030 mg/m^3) EGBE for up to 30 days exhibited elevated osmotic fragility after the fourth exposure and reduced RBC count and haemoglobin values after 30 exposures. At the end of the study, RBC counts and haemoglobin concentration were reduced to levels half that at the start of the study. A male and a female monkey exposed to 100 ppm (490 mg/m^3) for 90 days had increased erythrocyte fragility and decreased numbers of erythrocytes; the female was more severely affected. (Carpenter et al. 1956 – quoted from NTP 2000, ATSDR 1998).

83.4.2 Oral intake

83.4.2.1 Rats

Rats (24 male F344 rats per group) were gavaged with EGBE in water at doses of 0, 500, or 1000 mg/kg for 4 days. Six rats per dose were examined at 1, 4, 8, and 22 days after the last dose. Haematology evaluations showed marked dose-related effects on circulating RBCs and WBCs. Most of the RBC changes subsequently returned to normal. Body weight gain was reduced throughout the post-treatment period at the highest dose level. Changes in relative organ weights (increased liver and spleen weights at both dose levels; increased kidney and reduced thymes weights at the highest dose level) were evident on post-treatment day 1. These changes returned to normal by post-treatment day 22 except for liver and spleen weights at the highest dose level, which remained increased about 5 and 20%, respectively. (Grant et al. 1985 – quoted from EPA 1999, NTP 2000).

When rats were administered EGBE via gavage for a 12-day period at dose levels of 0 or 125 mg/kg, significant haemolysis occurred, which became more pronounced up to the third day of dosing; gradual recovery was observed up to day 12. Relative spleen weights increased up to the sixth day of dosing and declined thereafter. (Ghanayem et al. 1992 – quoted from EPA 1999).

Ten adult male rats were given 222, 443, or 885 mg/kg b.w./day undiluted EGBE by gavage 5 days/week for 6 weeks. Haematology parameters were determined following the last treatment. Dose-related changes were observed in the RBC counts (decreased RBC counts and Hgb concentration, and increased MCH and haemoglobinuria) of all treatment groups. At the two higher dose-levels, decreased MCHC was observed as well. Increased relative

liver weight was observed at all dose levels; histological examination revealed haemosiderin deposition from 443 mg/kg b.w./day and hepatocytomegaly at 885 mg/kg b.w./day. Focal haemosiderin accumulation was observed in the proximal tubules at the two higher dose levels. Splenic congestion was seen at all doses and enlarged dark spleens were observed in 3/9 mid-dose rats and in 4/8 high-rats. The LOAEL was determined to be 222 mg/kg b.w./day, the lowest dose tested. (Krasavage 1986 – quoted from EPA 1999, ATSDR 1998, NTP 2000).

In a 13-week NTP study (NTP 1993 – quoted from EPA 1999, NTP 2000), F344 rats (10 animals/sex/group) received EGBE in the drinking water at doses of 0, 69/82, 129/151, 281/304, 367/363, or 452/470 mg/kg b.w./day in male/female rats, respectively. Male rats evaluated at 13 weeks showed significantly reduced RBC counts from 281 mg/kg b.w./day, and reduced Hgb concentration and reduced platelets from 367 mg/kg b.w./day. Female rats showed significantly reduced RBC counts and Hgb concentration from 82 mg/kg b.w./day, and increased RTCs and reduced platelets from 304 mg/kg b.w./day. Histopathological examination of the livers revealed hepatocellular alterations at all dose levels in both sexes; centrilobular hepatocellular degeneration from 281 and 304 mg/kg b.w./day in males and females, respectively; and brown to green granular pigment staining strongly positive for iron in Kupffer cell cytoplasm in males at 452 mg/kg b.w./day and in females from 151 mg/kg b.w./day. These lesions, particularly in the females, displayed both increased dose-related incidence and increased dose-related severity. Bone marrow hyperplasia was found from 281 and 363 mg/kg b.w./day in males and females, respectively; haematopoietic cell proliferation and congestion in the spleen from 367 and 363 mg/kg b.w./day in males and females, respectively; and increased haemosiderin pigmentation in the spleen was found from 129 and 151 mg/kg b.w./day in males and females, respectively. No histopathological changes were observed in the kidneys, urinary bladders, thymus, lymph nodes, testes, or epididymides. The lowest dose level tested (69/82 mg/kg b.w./day in males/females, respectively) was considered a LOAEL.

In a study designed to investigate effects in the immune system, rats (6 animals/group) were exposed to EGBE in the drinking water at doses of 0, 180/204, or 506/444 mg/kg b.w./day in males and females, respectively, for 21 days. All rats were injected subcutaneously with heat aggregated aqueous keyhole limpet haemocyanin (KLH) antigen on days 7 and 13 following the start of dosing. No dose-related changes in organ weights (spleen, thymus, liver, kidney, and testis) or histology (thymus, liver, kidney, and testis) were observed. Natural killer cell cytotoxic response was significantly enhanced in the low-dose group, but not in the high-dose group (a decrease in this parameter is an indication of compromised non-specific immune system integrity; an increased is not considered an adverse effect). No significant alterations were noted in other immune parameters (serum anti-KLH IgG antibody levels, delayed-type hypersensitivity reaction, interleukin 2 and interferon production, and spleen cell counts). (Exon et al. 1991 – quoted from EPA 1999, ATSDR 1999).

83.4.2.2 Mice

EGBE was administered by gavage to male mice at doses of 0, 357, 714, or 1430 mg/kg b.w./day 5 days/week for 5 weeks. All of the high-dose animals

died before study termination; no mortality was observed in the lower dose groups. Haematology was evaluated at the end of the study. Mean RBC counts were significantly lower than the control value in the 357 and 714 mg/kg b.w./day groups; WBC counts were not affected. No differences in testes weight or histology was found. The LOAEL was 357 mg/kg b.w./day, the lowest dose tested. (Nagano et al. 1979 – quoted from EPA 1999).

In a 13-week NTP study (NTP 1993 – quoted from EPA 1999, NTP 2000), B6C3F1 mice (10 animals/sex/group) received EGBE in the drinking water at doses of 0, 118/185, 223/370, 553/676, 676/861, or 694/1306 mg/kg b.w./day in male/female mice, respectively. Mean final body weight and body weight gain were slightly reduced at the three highest dose levels. Haematological evaluations were not performed. No histopathological effects were observed in the liver, kidneys, urinary bladders, spleen, thymus, lymph nodes, bone marrow, testes, or epididymides.

83.4.3 Dermal contact

Repeated application of EGBE either neat or as a dilute (aqueous) and occluded to rabbits at dose levels of 18, 90, 180, or 360 mg/kg 6 hours/day, nine consecutive applications, produced haemoglobinuria in high-dose males and in females at the two highest dose levels. Recovery was noted following a 14-day observation period. (Tyler 1984 – quoted from EPA 1999).

In a 13-week study, occluded dermal administration of EGBE to rabbits (6 hours/day, 5 days/week) at exposure levels of 10, 50, or 150 mg/kg produced no observable haematological effects (Tyler 1984 – quoted from EPA 1999, ATSDR 1998).

In guinea pigs, dermal administration of EGBE at 2000 mg/kg produced no clinical signs of toxicity or treatment-related signs of organ toxicity (Shepard 1994 – quoted from EPA 1999).

83.5 Toxicity to reproduction

Due to the known testicular and developmental toxicity of two lower ethylene glycol ethers (EGME, EGEE), the reproductive and developmental toxicity of EGBE has been studied in a variety of well-conducted oral, inhalation and dermal studies using rats, mice and rabbits.

EGME seems to be the most potent glycol ether inducing testicular toxicity, but there is no evidence however, that higher homologues, as EGBE, are testicular toxicants. The developmental effects of ethylene glycol ethers are mediated by alkoxy acetic acid metabolites. However, ethylene glycol ethers with alkyl chains of more than 2 carbon atoms, as EGBE, do not appear to be selectively toxic to the foetus. For EGBE, foetotoxic effects have been observed only in the presence of maternal toxicity. In an *in vitro* study, the alkoxy acetic acids methoxyacetic acid (MAA) (from ethylene glycol monomethyl ether (EGME)) and ethoxyacetic acid (EEA) (from ethylene glycol monomethyl ether (EGEE)) showed similar developmental effects, whereas 2-BAA (from EGBE) was only slightly active. (ECETOC 1995a).

83.5.1 Inhalation

83.5.1.1 Rats

In a 90-day study, F344 rats (16 rats/sex) were exposed to EGBE vapours for 6 hours/day, 5 days/week at concentrations of 0, 5, 25, or 77 ppm (0, 25, 123, or 378 mg/m^3). No changes in testicular weight or in the pathology of the epididymides and testes of male rats were observed at any exposure level; reproductive organs of female rats were not examined histologically. Other findings are described in 4.4.1.1. (Dodd et al. 1983 – quoted from EPA 1999; ATSDR 1998).

In a 14-week NTP study (NTP 2000), no effects were noted in reproductive organs of F344 rats (10 animals/sex/group) exposed via inhalation to concentrations of 0, 31, 62.5, 125, 250, or 500 ppm of EGBE (0, 152, 307, 614, 1228, or 2455 mg/m^3) 6 hours/day, 5 days/week. Other findings are described in 4.4.1.1.

NTP (2000) has also performed a 2-year inhalation study in F344 rats (50 animals/sex/group) exposed to concentrations of 0, 31, 62.5, or 125 ppm of EGBE (0, 152, 307, or 614 mg/m^3) 6 hours/day, 5 days/week. No effects were noted in the reproductive organs. Other findings are described in 4.4.1.1.

Rats (15/group) were exposed to 0, 150, or 200 ppm (0, 737, or 980 mg/m^3) EGBE via inhalation for 6 hours/day during gestation days 7-15. High-dose rats showed some evidence of haematuria on the first day of exposure, but no effects were noted thereafter. No adverse effects were seen in the offspring. (Nelson et al. 1984 – quoted from EPA 1999, ECETOC 1995b, NTP 2000).

Pregnant F344 rats (36/group) were exposed to 0, 25, 50, 100, or 200 ppm (0, 123, 246, 491, or 982 mg/m^3) EGBE via inhalation for 6 hours/day on gestational days 6-15. A 50% decrease in viable implants and in live foetuses per litter, and an eight-fold increase in non-viable implants were observed at 200 ppm, but not at the lower concentrations. Foetotoxicity was observed in the form of retarded skeletal ossification of vertebral arches or centra, sternebrae, or phalanges at 100 and 200 ppm. There were no significant increases in external, visceral, skeletal, or total malformations in the foetuses due to treatment. Maternal toxicity was also evident at 100 and 200 ppm as an increased incidence of haematuria, reduced RBC count, decreased weight gain, and reduced food consumption. The NOAEL for maternal and developmental toxicity was 50 ppm (246 mg/m^3). (Tyl et al. 1984 – quoted from EPA 1999, ATSDR 1998, ECETOC 1995b, NTP 2000).

83.5.1.2 Mice

In a 14-week NTP study (NTP 2000), no effects were noted in reproductive organs of B6C3F1 mice (10 animals/sex/group) exposed via inhalation to concentrations of 0, 31, 62.5, 125, 250, or 500 ppm of EGBE (0, 152, 307, 614, 1228, or 2455 mg/m^3) 6 hours/day, 5 days/week except for testicular degeneration in 2/4 mice from the 500 ppm group that died or were killed moribund. Other findings are described in 4.4.1.2.

NTP (2000) has also performed a 2-year inhalation study in B6C3F1 mice (50 animals/sex/group) exposed to concentrations of 0, 62.5, 125, or 250 ppm of EGBE (0, 307, 614, or 1228 mg/m^3) 6 hours/day, 5 days/week. No effects were noted in the reproductive organs. Other findings are described in 4.4.1.2.

83.5.1.3 Rabbits

Pregnant rabbits (24/group) were exposed to 0, 25, 50, 100, or 200 ppm (0, 123, 246, 491, or 982 mg/m^3) EGBE via inhalation for 6 hours/day on gestational days 6-18. Decreases in total implants and implant viability were observed at 200 ppm. Foetal skeletal ossification of sternebrae and rudimentary rib was delayed at 200 ppm. Maternal toxicity was also evident at 200 ppm as an increased incidence of clinical signs, reduced gravid uterine weight, and decreased weight gain; no effect on haematological parameters was observed. The NOAEL for maternal and developmental effects was 100 ppm (491 mg/m^3). (Tyl et al. 1984 – quoted from EPA 1999, ATSDR 1998, ECETOC 1995b, NTP 2000).

83.5.2 Oral intake

83.5.2.1 Rats

Male rats were given single gavage doses of 0, 174, 434, or 868 mg/kg b.w. of 2-butoxyacetic acid (BAA, a metabolite of EGBE). Occasional significant decreases were observed in the weight of the prostate and seminal vessels, but the decreases were not time or dose related. No treatment-related lesions were noted following histological examination of the testes, epididymides, and prostate. (Foster et al. 1987 – quoted from EPA 1999, NTP 2000).

In male F344 rats (24/group) gavaged with EGBE in water at doses of 0, 500, or 1000 mg/kg for 4 days, no testicular effects were observed. Other findings are described in 4.4.2.1. (Grant et al. 1985 – quoted from EPA 1999, ATSDR 1998).

When male and female F344 rats were exposed to EGBE in the drinking water for 2 weeks at doses of 0, 73/77, 108/102, 174/152, 242/203, or 346/265 mg/kg b.w./day in males and females, respectively, there were no effects on testicular weight and no histopathological lesions in the testes or epididymides (NTP 1993 – quoted from EPA 1999, ATSDR 1998).

In male rats (6 animals/group) exposed to EGBE in the drinking water at doses of 0, 180, or 506 mg/kg b.w./day for 21 days, no effects were observed regarding testis weight, testicular histopathology, and fertile parameters. Other findings are described in 4.4.2.1. (Exon et al. 1991 – quoted from EPA 1999, ATSDR 1998).

Ten adult male rats were given 222, 443, or 885 mg/kg b.w./day undiluted EGBE by gavage 5 days/week for 6 weeks. No effects were found on testicular weight and in histopathological lesions in the testes, seminal vesicles, epididymides, or prostate at any exposure level. Other findings are described in 4.4.2.1. (Krasavage 1986 – quoted from EPA 1999, ATSDR 1998).

In a 13-week NTP study (NTP 1993 – quoted from EPA 1999, ATSDR 1998), no effects on testicular weight and no histopathological lesions in the testes, epididymides, or seminal vesicles were found in F344 rats (10 animals/group) administered EGBE in the drinking water at doses of up to 452 mg/kg b.w./day. Spermatozoa concentrations were significantly decreased compared to controls from 281 mg/kg b.w./day but not in a dose-related manner; there were no significant effects on spermatid heads, spermatid counts, or percent mobile spermatozoa. No histopathological lesions were found in the mammary glands, ovaries, or uterus at doses of up to 470 mg/kg b.w./day. Other findings are described in 4.4.2.1.

Pregnant F344 rats (28-35 per group) were administered EGBE by gavage in distilled water at doses of 0, 30, 100, or 200 mg/kg b.w./day on gestation days 9-11, or doses of 0, 30, 100, or 300 mg/kg b.w./day on gestation days 11-13. Maternal effects in either dosing sequence included marked reductions in body weight and/or weight gain; increases in kidney and spleen weights; severe haematotoxicity as evidenced by a decrease in HCT, Hgb, and RBC counts and an increase in RCTs at doses greater than or equal to 100 mg/kg b.w./day. These effects were dose related. No indications of developmental toxicity were observed at the two lower dose levels. Viability of embryos was reduced at 200 but not 300 mg/kg b.w./day. A decreased platelet count was noted in the foetuses at 300 mg/kg b.w./day. No foetal malformations, including cardiovascular malformations, were noted at any dose. The NOAEL for maternal toxicity was 30 mg/kg b.w./day and for developmental toxicity 100 mg/kg b.w./day. (Sleet et al. 1989 – quoted from EPA 1999, ATSDR 1998).

83.5.2.2 Mice

When male and female B6C3F1 mice were exposed to EGBE in the drinking water for 2 weeks at doses of 0, 93/150, 148/237, 210/406, 370/673, or 627/1364 mg/kg b.w./day in males and females, respectively, there were no treatment-related gross lesions in any of the reproductive organs, histopathological examinations were not performed (NTP 1993 – quoted from EPA 1999).

When EGBE was administered by gavage to male mice at doses of 0, 357, 714, or 1430 mg/kg b.w./day 5 days/week for 5 weeks, no changes in testes weight or histology were found. Other findings are described in 4.4.2.2. (Nagano et al. 1979 – quoted from EPA 1999, ATSDR 1998).

In a 13-week NTP study (NTP 1993 – quoted from EPA 1999, ATSDR 1998), no effect was observed in testis weight, no histopathological changes were observed in the seminal vesicle, testes, and epididymides, and no consistent treatment-related effects on sperm parameters in B6C3F1 mice (10 animals/group) administered EGBE in the drinking water at doses of up to 694 mg/kg b.w./day. No histopathological lesions were found in the mammary glands, ovaries, or uterus at doses of up to 1306 mg/kg b.w./day. Other findings are described in 4.4.2.2.

A continuous breeding study has been performed in Swiss CD-1 mice exposed to EGBE in drinking water at doses of 0, 700, 1300, or 2000 mg/kg b.w./day for 7 days pre-mating and 98 days as breeding pairs (Heindel et al. 1990 – quoted from EPA 1999, ATSDR 1998, ECETOC 1995b, NTP

2000). In the high-dose and mid-dose groups, 13/30 and 6/20 females, respectively, died during the study. Toxic effects in adults at these dose levels included decreased body weight gain, increased kidney and liver weights, and dose-related decreases in water consumption. No significant effects on the weights of testes, seminal vesicles, or prostate, and no effects on sperm parameters were observed. Decreased pup weight and a decrease in the number of litters produced per pair and in the size of each litter were observed in the mid- and high-dose groups. A significant reduction (5%) of live pup weight was also observed in the low-dose group, but no adverse effect on fertility. At the completion of the continuous breeding phase, a crossover mating trial was performed to determine which sex was more affected by treatment. F_0 males and females from the 1300 mg/kg b.w./day group were mated with male and female control animals. The exposed mice had significantly lower body weights and increased relative kidney weights, but reproductive organ weights, sperm motility and morphology, and oestrous cycle length and frequency did not differ from controls. The proportion of successful copulation was the same in all groups, and no developmental effects were observed in the offspring of any group. However, the number of fertile females was significantly reduced in the groups where treated females were mated with control males. No adverse effect on fertility was noted when offspring of the low-dose groups were mated; there were insufficient numbers of offspring to assess the two highest dose groups. The NOAEL for maternal and reproductive effects is 700 mg/kg b.w./day, which is a LOAEL for developmental effects as a slight decrease in pup weight was observed at this dose.

CD-1 mice received 0, 350, 650, 1000, 1500, or 2000 mg/kg b.w./day EGBE via gavage on days 8-14 of gestation. Maternal toxicity included mortality of 3/6 animals in the 1000 mg/kg b.w./group and 6/6 in the 2000 mg/kg b.w./day group. Treatment-related clinical observations were lethargy, abnormal breathing, and green or red vaginal discharge. An increased number of resorptions and a reduced number of viable foetuses were observed from 1000 mg/kg b.w./day. The LOAEL for maternal effects was 350 mg/kg b.w./day and the NOAEL for prenatal effects was 650 mg/kg b.w./day. In a post-natal study, reproductive effects were evaluated in CD-1 mice administered EGBE via gavage at 0, 650, or 1000 mg/kg b.w./day on days 8-14 of gestation. Maternal body weight was lowered at the high dose. No adverse reproductive or developmental effects were observed. (Wier et al. 1987 – quoted from EPA 1999, ATSDR 1998, ECETOC 1995b, NTP 2000).

83.5.3 Dermal contact

Females rats were given EGBE by dermal application to the shaved interscapular skin during days 6-15 of gestation, four time per day at 1800 or 5400 mg/kg b.w./day. In the high-dose group, 10/11 rats died between days 3 and 7 of treatment. Signs associated with treatment included red-stained urine, ataxia, inactivity, rough coats, and necrosis of the tail tip. At the lower dose, body weight was slightly reduced; there was no evidence of embryo- or foetotoxicity, nor were any gross malformations or variations observed. (Hardin et al. 1984 – quoted from EPA 1999, NTP 2000).

83.6 Mutagenic and genotoxic effects

83.6.1 *In vitro* studies

NTP has reported negative responses for mutagenicity when EGBE was tested in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 at concentrations up to 10 mg/plate with or without metabolic activation (Zeiger et al. 1992 – quoted from EPA 1999, ECETOC 1995b, NTP 2000).

EGBE was negative for mutation in *Salmonella typhimurium* strains TA98, TA100, and TA102 both with and without rat S9 activation at concentration up to 9 mg/plate; a weak mutagenic response was seen in strain TA97a at 4.5 mg/plate with (S9 mix) and without metabolic activation (Hoflack et al. 1995 – quoted from EPA 1999, ATSDR 1998, ECETOC 1995b, NTP 2000).
In order to confirm this result, testing was conducted in *Salmonella typhimurium* strains TA97a and TA100, as well as *Escherichia coli* WP2*uvr*A. EGBE was negative in these strains when evaluated at 0.5, 1.0, 2.5, 5.0, 8.5, and 10 mg/plate in the presence and absence of Arochlor-induced rat liver S9 mix. (Gollapudi et al. 1996 – quoted from EPA 1999, ATSDR 1998, NTP 2000).

No mutagenic activity was observed when EGBE was investigated using bacteriophage T4D with *Escherichia coli* B, CR63, and K12 when tested at concentrations from 20 to 111 µl/ml, but EGBE had a severe toxic effect on phage yield (Kvelland 1988 – quoted from EPA 1999, ECETOC 1995b).

When tested for 5 hours at up to toxic doses, EGBE or its metabolite 2-butoxyacetaldehyde (BAL) were not found to be mutagenic in an *in vitro* gene mutation assay using Chinese hamster ovary (CHO) cells. High toxicity was reported at 4.5 mg/ml. (Chiewchanwit & Au 1995 – quoted from EPA 1999, ATSDR 1998, NTP 2000).
Both EGBE and BAL weakly induced gene mutations in Chinese hamster V79 cells at high concentrations (from 7.5 mg/l) (Elias et al. 1996 – quoted from EPA 1999).

EGBE did not induce sister chromatid exchanges (SCEs) or chromosomal aberrations in CHO cells with or without liver S9 mix; EGBE induced cell cycle delay (an indication of cytotoxicity) (NTP 1993 – quoted from EPA 1999, NTP 2000).
EGBE was reported not to induce SCEs in Chinese hamster ovary cells exposed to concentrations up to 0.25% (2.5 mg/ml) for 2 hours in the presence of metabolising enzymes (S9) and for 5 hours in the absence of S9 (Tyler 1982 – quoted from ATSDR 1998).
EGBE did not induce chromosomal aberrations in Chinese hamster V79 fibroblast cells. At high concentrations (from 8.5 mM, ca. 1 mg/ml), EGBE weakly induced aneuploidy (numerical chromosomal anomalies), SCEs and micronuclei, and potentiated the clastogenicity induced by methyl methanesulfonate. (Elias et al. 1996 – quoted from EPA 1999).
EGBE did not induce chromosomal aberrations in human lymphocytes when tested at concentrations up to 3000 ppm, but induced sister chromatid exchanges (SCEs) (Villalobos-Pietrini et al. 1989 – quoted from ECETOC 1995b).

83.6.2 *In vivo* studies

EGBE did not increase the incidence of micronuclei in the bone marrow cells of male rats or mice. Animals were given three intraperitoneal injections of EGBE 24 hours apart and sacrificed 24 hours after the last injection. Rats were dosed at 0, 7, 14, 28, 56, 112.5, 225, or 450 mg/kg, and mice were dosed at 0, 17, 34, 69, 137.5, 275, or 550 mg/kg. (NTP 1998 – quoted from EPA 1999, NTP 2000).

No increase in DNA adducts was detected by ^{32}P post-labelling in the brain, liver, kidney, testis, or spleen of rats following oral administration of 120 mg/kg EGBE (NTP 2000).

83.7 Carcinogenic effects

In a 2-year inhalation study (NTP 2000), F344 rats (50 animals/sex/group) were exposed to concentrations of 0, 31, 62.5, or 125 ppm of EGBE (0, 152, 307, or 614 mg/m^3) 6 hours/day, 5 days/week. No effect on survival was observed. Benign and/or malignant pheochromocytoma of the adrenal medulla was observed in female rats (3/50, 4/50, 1/49, 8/49); the increase in high-dose animals relative to controls was not significant. Based upon these findings, NTP concluded "no evidence of carcinogenic activity in male F344 rats, and equivocal evidence of carcinogenic activity in female F344 rats". Non-neoplastic findings are described in 4.4.1.1.

In a 2-year inhalation study (NTP 2000), B6C3F1 mice (50 animals/sex/group) were exposed to concentrations of 0, 62.5, 125, or 250 ppm of EGBE (0, 307, 614, or 1228 mg/m^3) 6 hours/day, 5 days/week. Survival was significantly decreased in male mice at 125 and 250 ppm (54 and 53%, respectively). Significantly increased incidences of hepatocellular carcinomas (10/50, 11/50, 16/50, 21/50) and haemangiosarcomas (0/50, 1/50, 2/49, 4/49) were observed in high-dose male mice relative to controls. When hepatocellular adenomas and carcinomas were combined, no significant increase was observed in any exposure group. No significant increase in benign or malignant hepatocellular tumours or haemangiosarcomas was noted in female mice. Forestomach squamous cell papillomas and carcinomas (combined) were significantly increased (Trend Test = 0.017) in female mice relative to controls (0/50, 1/50, 2/50, 6/50) but not in male mice. Based upon these findings, NTP concluded "some evidence of carcinogenic activity in male and female B6C3F1 mice". Non-neoplastic findings are described in 4.4.1.2.

84 Regulations

84.1 Ambient air

Denmark (C-value): 0.04 mg/m^3 (MST 2002)

84.2 Drinking water

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84.3 Soil

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84.4 Occupational Exposure Limits

Denmark:	20 ppm (98 mg/m^3) H (At 2002)
ACGIH:	20 ppm (98 mg/m^3) skin (ACGIH 2001)
Germany:	20 ppm (98 mg/m^3 H (MAK 1998)

84.5 EU Classification

EGBE is classified for acute toxic effects (Xn;R20/21/22 – harmful by inhalation, in contact with skin and if swallowed) and for irritative effects (Xi;R36/38 – irritating to eyes and skin) (MM 2002).

84.6 IARC

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84.7 US-EPA

Because of the uncertain relevance to humans of the tumour increases observed in the NTP inhalation studies, that EGBE is generally negative in genotoxic tests, and the lack of human data to support the findings in rodents, the human carcinogenic potential of EGBE, in accordance with the recent 'Proposed Guidelines for Carcinogen Risk Assessment', cannot be determined at this time, but suggestive evidence exists from rodent studies. Under existing EPA guidelines, EGBE is judged to be a possible human carcinogen, Group C. (EPA 1999).

85 Summary

85.1 Description

EGBE is a colourless liquid with a low vapour pressure (0.76 mmHg at 20°C); it is miscible with water.

85.2 Toxicokinetics

EGBE is rapidly absorbed and distributed throughout the body (humans and rats) following inhalation, oral administration and dermal contact. Following inhalation, peak blood levels were observed in humans within 1-2 hours following exposure; one study in human volunteers indicates an uptake of about 60% of the inspired amount during light physical exercise.
In humans, EGBE is eliminated primarily as 2-butoxyacetic acid (2-BAA) in the urine either free or conjugated to glutamine and, to a lesser extent, glycine. In rats, EGBE can also be converted to ethylene glycol or conjugated to glucuronide and sulphate to some extent. Excretion also takes place via expired air in form of carbon dioxide.

85.3 Human toxicity

85.3.1 Single dose toxicity, irritation

In human volunteers exposed by inhalation to EGBE at concentrations from 490-957 mg/m^3 for 4-8 hours, irritation of the nose and throat, and eyes was noted; no haematological effects were observed. When seven male volunteers were exposed to EGBE (98 mg/m^3) for 2 hours, none of the subjects complained of or showed any signs of adverse effects.
Poisoning cases are available reporting haematological changes and metabolic acidosis following acute ingestion of large doses of EGBE in household products and combined with other solvents.

85.3.2 Irritation, sensitisation

In a patch test, human volunteers showed no dermal effects following application of 10% aqueous EGBE.

85.3.3 Repeated dose toxicity

No differences between exposed and control workers (average airborne concentration of EGBE: 2.9 mg/m^3) were observed for haematological parameters except for small changes for haematocrit (3.3% decrease) and mean corpuscular haemoglobin concentration (MCHC) (2.1% increase).

85.3.4 Toxicity to reproduction

No data have been located.

85.3.5 Mutagenic and genotoxic effects

No increases in micronuclei or sister chromatid exchanges were observed in workers exposed to both EGBE and to 2-ethoxyethanol (EGEE).

85.3.6 Carcinogenic effects

No data have been located.

85.4 Animal toxicity

85.4.1 Single dose toxicity

LC_{50}-values (4-hour exposure) of 2200-2400 mg/m^3 have been reported for rats and of 3440 mg/m^3 (7-hour exposure) for mice. One of six guinea pigs died after 4 hours of exposure to saturated vapour (ca. 4900 mg/m^3).
Oral LD_{50}-values of 530-3000 mg/kg have been reported for rats, of 1230 and 1519 mg/kg for mice, of 950-1415 for guinea pigs, and of 320-3100 for rabbits.
Dermal LD_{50}-values of 406 to 1804 mg/kg have been reported for rabbits, of 255-4800 mg/kg for guinea pigs, and of 2273 mg/kg for rats.

Following inhalation, increased erythrocyte fragility was seen in rats exposed to 305 mg/m^3 for 4 hours and haemoglobinuria was observed in rats and mice exposed to about 1000 mg/m^3 for 7 hours; no such effects were observed in monkeys exposed to about 1000 mg/m^3 for 7 hours.
Following oral administration to rats, haemoglobinuria was observed at 3000 and 1500 mg/kg in male and female rats, respectively. Haemolysis of erythrocytes accompanied by haemoglobinuria have been observed following a single dose of 500 mg/kg, and at 125 mg/kg although to a lesser degree. Guinea pigs dosed once with 250 mg/kg showed no adverse haematological effects. The haematological effects in rats were found to be dose- and age-dependent, with older rats being more sensitive than younger rats.
Following dermal exposure, haemolytic effects have been observed in rats by application of 260 mg/kg to the shaved skin.

85.4.2 Irritation

In rabbits, EGBE has been considered a severe irritant by the Draize protocol and an irritant by the EU protocol. Several other studies have also reported EGBE to be a skin irritant in rabbits. Severe skin irritation has been noted in guinea pigs after application of EGBE.
Female rabbits exposed to EGBE (≥72 mg/kg, 8 hours) developed cutaneous lesions accompanied by necrosis of epidermis and dermis on the 4th day after exposure; skin lesions healed within a 2-week period.

EGBE has in several studies been reported to be severely irritating when instilled (undiluted) in the eyes of rabbits. Moderate corneal injury was observed in rabbits in which 0.5 ml of a 15% dilution of EGBE was placed in the conjunctival sac; no effects were observed with a dilution of 5%. In a Draize test performed in rabbits, undiluted EGBE caused severe irritation, dilutions at 70 and 30% caused moderate irritation, and dilutions of 20 and 10% caused mild irritation.

Male mice exposed to 750-8200 mg/m^3 EGBE for 10-15 minutes exhibited a 20% decreased in respiratory rate at the lowest concentration and a 40% decrease at the highest concentration.

85.4.3 Sensitisation

EGBE did not result in dermal sensitisation when tested in the guinea pig maximisation test.

85.4.4 Repeated dose toxicity

Following repeated inhalation exposure to EGBE vapours, haematological effects (decreased RBC counts and Hgb levels, increased MCH) have been observed in rats exposed for 90 days to 378 mg/m^3; these effects had either decreased or returned to the range of the control values. The NOAEC was 123 mg/m^3.
In 14-week NTP studies in rats and mice, haematological evaluations showed an exposure concentration-related anaemia (in female rats and mice from 152 mg/m^3; in male rats and mice from 614 mg/m^3). Histopathological effects were observed in the spleen, bone marrow (rats only), liver, kidneys, and forestomach from 307 mg/m^3 in female rats, and from 614 mg/m^3 in male rats and mice (both sexes). The lowest concentration used in the study, 152 mg/m^3, was a LOAEC for haematological changes in female rats and mice; the NOAEC for male rats and mice was 307 mg/m^3.
In chronic NTP-studies in rats and mice, exposure-related anaemia was observed in both species; female animals were generally more sensitive than male animals. The LOAEC for haematological changes was 152 mg/m^3 for rats and 307 mg/m^3 for mice, the lowest concentrations tested in the studies of rats and mice, respectively.

Following oral administration of EGBE by gavage to male rats for 6 weeks, dose-related changes (decreased RBC counts and Hgb concentration, and increased MCH and haemoglobinuria) were observed from 222 mg/kg b.w./day, the lowest dose level used in the study. Histopathological changes were noted in the spleen from 222 mg/kg b.w./day, and in the liver and kidneys from 443 mg/kg b.w./day.
In a 13-week NTP drinking water study, haematological effects were observed in female rats from 82 mg/kg b.w./day and in male rats from 281 mg/kg b.w./day. Histopathological changes were observed in the spleen from 129 and 151 mg/kg b.w./day in males and females, respectively; and in the liver from 69 and 82 mg/kg b.w./day in males and females, respectively. The lowest dose level tested (69/82 mg/kg b.w./day in males/females, respectively) was a LOAEL for haematological effects.
When EGBE was administered by gavage to male mice for 6 weeks, the LOAEL for haematological effects was 357 mg/kg b.w./day, the lowest dose tested.

In a 13-week study, occluded dermal administration of EGBE to rabbits at dose levels up to 150 mg/kg produced no observable haematological effects.

The immune system did not appear to be a sensitive target of EGBE in rats exposed to EGBE in the drinking water at doses up to 506/444 mg/kg b.w./day in males and females, respectively, for 21 days.

85.4.5 Toxicity to reproduction

The reproductive toxicity of EGBE has been studied in a variety of studies in rats, mice and rabbits following inhalation or oral administration. EGBE did not cause adverse effects in any reproductive organ, including testes, in any of the studies. In a two-generation reproductive toxicity study, fertility was reduced in mice only at maternally toxic doses (above 1000 mg/kg b.w./day) when EGBE was administered in the drinking water.
In addition, several developmental toxicity studies have addressed the potential of EGBE to induce embryo- and/or foetotoxicity following oral, inhalation, and dermal exposures to rats, mice, and rabbits. Toxicity to embryos and/or foetuses was observed only at dose levels, which also resulted in maternal toxicity. No malformations were observed in any of the studies.

85.4.6 Mutagenic and genotoxic effects

EGBE was negative for mutation in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA1535, and TA1537 both with and without metabolic activation. A weak mutagenic response was seen in one test in strain TA97a both with and without metabolic activation. When this test was repeated, a negative response was obtained.
No mutagenic activity was observed when EGBE was investigated using bacteriophage T4D with *Escherichia coli* B, CR63, and K12, and in a test with *Escherichia coli* WP2*uvr*A in the presence and absence of Arochlor-induced rat liver S9 mix.

EGBE or its metabolite 2-butoxyacetaldehyde (BAL) were not found to be mutagenic in an *in vitro* gene mutation assay using Chinese hamster ovary (CHO) cells, whereas both EGBE and BAL weakly induced gene mutations in Chinese hamster V79 cells at high concentrations.

EGBE did not induce sister chromatid exchanges (SCEs) or chromosomal aberrations in CHO cells with or without liver S9 mix or in Chinese hamster V79 fibroblast cells. At high concentrations, however, EGBE weakly induced aneuploidy (numerical chromosomal anomalies), SCEs and micronuclei, and potentiated the clastogenicity induced by methyl methanesulfonate.

EGBE did not increase the incidence of micronuclei in the bone marrow cells of male rats or mice given three intraperitoneal injections of EGBE.

85.4.7 Carcinogenic effects

NTP has performed 2-year inhalation studies in rats and mice. No evidence of carcinogenic activity was found in male rats and equivocal evidence of carcinogenic activity in female rats based on increased combined incidences of benign and malignant pheochromocytoma of the adrenal medulla. In mice, some evidence of carcinogenic activity was found based on increased incidences of forestomach squamous cell papillomas and carcinomas in female mice and increased incidences of hemangiosarcoma of the liver in male mice.

86 Evaluation

EGBE is absorbed and distributed throughout the body (humans and rats) following inhalation, oral administration and dermal contact. EGBE is metabolised to 2-butoxyacetic acid (2-BAA), which probably is responsible for the haemolytic effect identified as the critical end-point in toxicological studies on EGBE.

Only limited data on the toxic effects of EGBE in humans are available. These data comes largely from case reports dealing with accidental poisonings and/or workplace exposure. EGBE seems of low acute toxicity in humans. The available data show that after acute oral ingestion of large doses of EGBE (combined with other solvents), haematological changes and metabolic acidosis are the primary effects. The only indication of haemolysis (small changes for haematocrit and MCHC) following repeated inhalation was observed in workers exposed to an average airborne concentration of EGBE of 2.9 mg/m^3; however, the changes were within the range of normal clinical values and are not considered as being adverse.

EGBE is of moderate acute toxicity whether animals are exposed via the oral, dermal, or respiratory routes with oral LD_{50}-values ranging from 320 to 3100 mg/kg (rats, mice, guinea pigs, rabbits), dermal LD_{50}-values ranging from 406 to 4800 mg/kg (rabbits, guinea pigs, rats), and LC_{50}-values of 2200-2400 mg/m^3 (4-hour exposure) for rats and of 3440 mg/m^3 (7-hour exposure) for mice.

Irritation of the nose and throat, and eyes has been noted in human volunteers exposed by inhalation to EGBE at concentrations from 490-957 mg/m^3 for 4-8 hours, but not in volunteers exposed to EGBE (98 mg/m^3) for 2 hours. The NOAEC for irritative effects of EGBE in humans is above 100 mg/m^3.
In rabbits, EGBE is severely irritating when instilled (undiluted) in the eyes; dilutions at 70 and 30% caused moderate irritation, and dilutions of 20 and 10% caused mild irritation. In rabbits, EGBE has been considered a severe irritant by the Draize protocol and an irritant by the EU protocol. Severe skin irritation has also been noted in guinea pigs. Male mice exposed to 750-8200 mg/m^3 EGBE for 10-15 minutes exhibited a 20% decreased in respiratory rate at the lowest concentration and a 40% decrease at the highest concentration.

Human volunteers showed no dermal effects of 10% EGBE in a patch test and EGBE did not result in dermal sensitisation when tested in the guinea pig maximisation test.

Intravascular haemolysis is the primary response elicited in sensitive animal species following inhalation, oral, and dermal exposure to EGBE.
Changes in haematological parameters have been observed in rats and mice following acute inhalation exposure (increased erythrocyte fragility in rats at 305 mg/m^3 for 4 hours; haemoglobinuria in rats and mice at about 1000 mg/m^3 for 7 hours). Following oral administration, haemolysis of erythrocytes

accompanied by haemoglobinuria was observed in rats following a single dose at dose levels from about 125 mg/kg. Following dermal exposure, haemolytic effects have been observed in rats by application of 260 mg/kg to the shaved skin.
Following repeated inhalation exposure to EGBE vapours, the NOAEC for haematological changes was 123 mg/m^3 in a 90-day study in rats; in the 2-year NTP-study, the LOAEC for haematological changes was 152 mg/m^3 for rats and 307 mg/m^3 for mice, the lowest concentrations tested in the studies of rats and mice, respectively. In the 14-week NTP-study, the lowest concentration used in the study, 152 mg/m^3, was a LOAEC for haematological changes in female rats and mice; the NOAEC for haematological changes in male rats and mice was 307 mg/m^3. Following oral administration of EGBE by gavage to male rats for 6 weeks, the LOAEL for haematological changes was 222 mg/kg b.w./day, the lowest dose level used in the study. In a 13-week NTP drinking water study, the lowest dose level tested (69/82 mg/kg b.w./day in males/females, respectively) was a LOAEL for haematological effects. When EGBE was administered by gavage to male mice for 6 weeks, the LOAEL for haematological effects was 357 mg/kg b.w./day, the lowest dose tested. In a 13-week study, occluded dermal administration of EGBE to rabbits at dose levels up to 150 mg/kg produced no observable haematological effects.

The available data indicate that certain species are more susceptible to the haemolytic effects of EGBE. The sensitivities range from that of guinea pigs, which displayed no haemolytic effects following a single oral administration of 1000 mg/kg b.w EGBE, to that of rats, which displayed increased osmotic fragility of erythrocytes at a single-inhalation exposure to 305 mg/m^3 and haemolysis of erythrocytes following a single oral dose at dose levels from about 125 mg/kg. Humans appear to be less sensitive than rats are to the haemolytic effect of EGBE as haemolysis has not been observed in acute inhalation exposures of human volunteers to up to about 950 mg/m^3; reversible haemolytic effects have been observed in cases where humans consumed single oral doses of 400 to 1500 mg/kg b.w. This difference in sensitivity between rats and humans is supported by *in vitro* studies, which have shown that erythrocytes from humans were unaffected by incubations with 2-butoxyacetic acid (2-BAA, the toxic metabolite of EGBE) at concentrations, which produced total rat erythrocyte haemolysis.

With respect to gender sensitivity, the available data consistently showed that female rats are more sensitive to EGBE-induced haemolysis than male rats are. Female mice also appeared to be slightly more sensitive than male mice. *In vitro*, female erythrocytes have shown a slightly greater sensitivity than male erythrocytes following incubation with 2-BAA.

Several studies have assessed the effect of age on the haemolytic effects in young and adult rats. Older rats appeared to be more sensitive than the younger rats following acute doses of EGBE; however, chronic exposures appear to impart a certain level of tolerance to rats and mice over time as apparent tolerance to EGBE-induced haemolysis in rats and mice was observed in the 2-year NTP inhalation studies.
The possibility that certain human subpopulations, including the aged and those predisposed to haemolytic disorders, might be at an increased risk from exposure to EGBE has been investigated using blood from the elderly, from patients with sickle cell disease, and from patients with spherocytosis.

Erythrocytes from these potentially sensitive groups were unaffected by incubations with 2-BAA at concentrations, which produced total rat erythrocyte haemolysis.

Effects have also been observed in the liver (hepatocellular degeneration and haemosiderin pigmentation of Kupffer cells), kidneys (haemosiderin accumulation, renal tubular degeneration, and intracytoplasmic haemoglobin), bone marrow (hyperplasia), and spleen (haematopoietic cell proliferation, congestion, and increased haemosiderin pigmentation) following exposure to EGBE. These effects, which generally occur at higher exposure levels than those causing haemolysis, are secondary to the haematotoxicity of EGBE and a result of a compensatory response to the haemolysis.

The reproductive and developmental toxicity of EGBE has been studied in several studies in rats, mice and rabbits following inhalation, oral administration, or dermal application (developmental toxicity only). It can be concluded from these studies that EGBE does not affect the reproductive organs of parents (both males or females), and only results in adverse reproductive and developmental effects at dose levels, which also result in parental toxicity. No malformations were observed in any of the studies. No data have been located regarding toxicity to reproduction in humans.

No increases in micronuclei or sister chromatid exchanges were observed in workers exposed to both EGBE and to 2-ethoxyethanol (EGEE). EGBE has been tested for its potential to induce gene mutations in *in vitro* systems and cytogenetic damage in both *in vitro* and *in vivo* systems. In most of the tests, EGBE has given negative results. One laboratory, however, has reported weak genotoxicity responses, but only at toxic doses. Overall, the available data do not support a mutagenic or clastogenic potential for EGBE.

In 2-year NTP inhalation studies, no evidence of carcinogenic activity was found in male F344 rats, equivocal evidence in female F344 rats based on increased combined incidences of benign and malignant pheochromocytoma of the adrenal medulla, and some evidence in B6C3F1 mice based on increased incidences of forestomach squamous cell papillomas and carcinomas in female mice and increased incidences of hemangiosarcoma of the liver in male mice. These tumour increases are, according to EPA (1999), of uncertain relevance to humans. As EGBE is generally negative in the genotoxicity tests and as glycol ethers generally appear unlikely to be carcinogenic, the concern for a carcinogenic potential of EGBE is low. No data have been located regarding carcinogenic effects in humans.

86.1.1 Conclusion

The critical effects following exposure to EGBE are the irritative effects on the respiratory tract and eyes observed in humans and in experimental animals and the haemolytic effect observed in experimental animals and probably also indicated by the sparse human data available.

Irritation of the nose and throat, and eyes was noted in human volunteers exposed by inhalation to EGBE at concentrations from 490-957 mg/m^3 for 4-8 hours, but not in volunteers exposed to EGBE (98 mg/m^3) for 2 hours. The NOAEC for irritative effects of EGBE in humans is thus above 100 mg/m^3.

Male mice exposed to 750-8200 mg/m^3 EGBE for 10-15 minutes exhibited a 20% decreased in respiratory rate at the lowest concentration and a 40% decrease at the highest concentration. EGBE is irritating to the eyes and skin of experimental animals.

Haemolysis has been identified as the critical end-point in toxicological studies of EGBE. Certain species differences in sensitivity have been observed regarding the haemolytic effects of EGBE, with rats being particularly sensitive, mice sensitive, and guinea pigs appearing relative insensitive. The only indication of haemolysis (small changes for Hct and MCHC) following repeated inhalation was observed in workers exposed to an average airborne concentration of EGBE of 2.9 mg/m^3; however, the changes were within the range of normal clinical values and are not considered as being an adverse effect.
Data indicate that humans are less sensitive to the haemolytic toxicity of EGBE than are rats as no or very slight haemolytic effects were observed in the poisoning cases after acute oral ingestion of large doses. This difference in sensitivity between rats and humans is supported by *in vitro* studies, which have shown that erythrocytes from humans were unaffected by incubations with 2-butoxyacetic acid (2-BAA, the toxic metabolite of EGBE) at concentrations, which produced total rat erythrocyte haemolysis.

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Appendix 12: 1-Methoxy-2-propanol (2PG1ME)

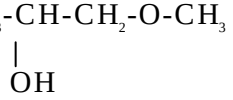
88 General description

Most of the available toxicological data on 1-methoxy-2-propanol (2PG1ME) relate to the commercial compound methoxy-propanol (PGME), which consists of 95-99% 2PG1ME and less than 5% 2-methoxy-1-propanol (1PG2ME).

88.1 Identity

Molecular formula: $C_4H_{10}O_2$

Structural formula:



Molecular weight: 90.1

CAS-no.: 107-98-2

Synonyms: 1-Methoxy-2-propanol;
1-Methoxypropan-2-ol;
Methoxypropanol, alpha isomer;
Propylene Glycol Monomethyl Ether;
PGME;
2-Propylene glycol 1-methyl ether;
2PG1ME;
methoxy ether of propylene glycol;
alpha-propylene glycol monomethyl ether;
propylene glycol 1-methyl ether;
(+/-)-1-methoxy-2-propanol;
1-Methoxy-2-hydroxypropane

88.2 Physical / chemical properties

Description: 1-Methoxy-2-propanol (2PG1ME) is the α-isomer of propylene glycol monomethyl ether (PGME). It is a colourless liquid with a sweet ether-like odour.

Melting point: - 96 °C

Boiling point: 120 °C

Density: 0.924 g/ml (at 20°C)

Vapour pressure: 9 mmHg (12 hPa) (at 20°C)

Concentration of saturated vapours: 44348 mg/m^3 (calculated at 20°C and 1 atm)

Conversion factor:	1 ppm = 3.75 mg/m^3 (calculated at 20°C and 1 atm) 1 mg/m^3 = 0.267 ppm
Solubility:	Water: completely soluble
Partition coefficient:	log $P_{o/w}$ = -0.49 to -0.43 (BUA 1997)
References:	A&H (1990), ACGIH (1991), BUA (1997), DECOS (1993), ECETOC (1995).

89 Toxicokinetics

89.1 Absorption

The toxicity data of 2PG1ME indicate uptake by all routes of exposure. In anaesthetised rats exposed to 1000 ppm 2PG1ME (3750 mg/m^3), the respiratory uptake was 87% (Stott & McKenna 1984 – quoted from A&H 1990).

89.2 Distribution

Exposure by inhalation of rats over 6 hours to 300 -3000 ppm 2PG1ME (equivalent to 1125-11250 mg/m^3) resulted in a continuous increase in blood concentration, which ranged from 109-2133 µg/g. The blood/air partition coefficient of 2PG1ME was 12400 in one study and 403 in another study (Johanson & Dynesius 1988 and Stott & McKenna 1984, respectively – quoted from A&H 1990).

Oral administration to rats of 0.09 or 0.78 g/kg ^{14}C-labelled 2PG1ME resulted in recovery of 92-98 % of the radioactivity. Forty-eight hours after dosing, the liver had the highest percentage (1.4 %) of the radioactive dose. The fat/blood partition coefficient was 0.09, indicating that 2PG1ME does not accumulate in adipose tissue. There were no indications of accumulation of radioactivity in other main body tissues, or in testes. (Miller et al. 1983).

89.3 Metabolism and elimination

Rats were given an oral dose of 0.09 g/kg b.w. ^{14}C-labelled 2PG1ME. Sixty-six percent of the dose was recovered in the expired air, 63% of which as carbon dioxide. Urinary excretion accounted for 11%, while 0.9% of the radioactivity was found in faeces. The predominant urinary metabolite was the glucuronic acid conjugate of 2PG1ME, followed by the sulphate conjugate, propylene glycol and unchanged 2PG1ME. The urinary recovery increased to 19-25% after exposure to a dose of 0.78 g/kg b.w. (Miller et al. 1983). A metabolism scheme for 2PG1ME is given in Figure 1.

In rats exposed by inhalation to 300 - 3000 ppm 2PG1ME (1125-11250 mg/m^3) for 6 hours, the kinetic was non-linear. The half-time in the blood was 2.4 hr after the lowest exposure level and 15.7 hr after the highest, indicating saturation of the metabolic pathways. (Morgott & Nolan 1987– quoted from A&H 1990).

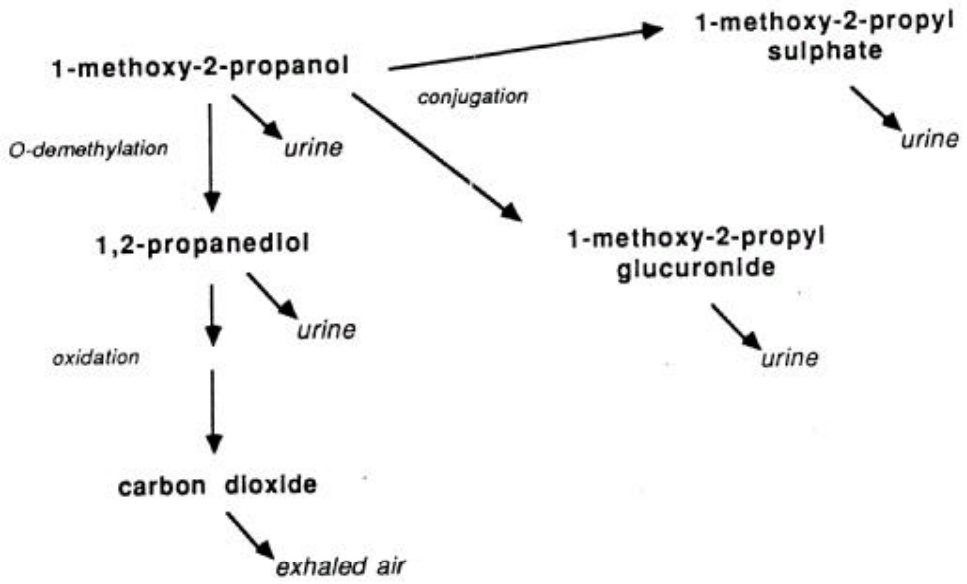


Figure 1. Proposed metabolism for 2PG1ME (modified from A&H 1990).

89.4 Mode of action

Glycol ethers capable of giving rise to alkoxyacetic acids have been shown to exhibit bone marrow depression and developmental, testicular and immunological toxicity. 2PG1ME is a secondary alcohol which does not form methoxy propionic acid but is primarily metabolised to carbon dioxide. No propylene glycol ethers being secondary alcohols have been shown to cause developmental toxicity. (ECETOC 1995).

90 Human toxicity

90.1 Single dose toxicity

90.1.1 Inhalation

Two subjects exposed to rising concentration of PGME over two hours reported subjective signs of mild narcotic effects (headache, dizziness) at 300-400 ppm (corresponding to 1125-1500 mg/m^3). When the concentration reached 1000 ppm, signs of CNS impairment occurred in one of the two subjects in a balancing test. (Stewart et al. 1970).

90.1.2 Oral intake

No data were found.

90.1.3 Dermal contact

No data were found.

90.1.4 Irritation

Two male volunteers were exposed to steadily increasing PGME concentration from 1 to 2050 ppm (equivalent to 3.75 - 7700 mg/m^3) over 2 hours. The odour was noted at less than 25 ppm (94 mg/m^3) and reported as objectionable at 50-75 ppm (188 - 281 mg/m^3). Mild eye irritation occurred at 300-400 ppm (1125 – 1500 mg/m^3), eye, nose and throat irritation from 500 ppm (1875 mg/m^3), and lachrymation and rhinorrhea from 750 ppm (2813 mg/m^3). Severe eye irritation occurred in one of the two subjects at 1000 ppm (3750 mg/m^3) and the man had to leave the chamber. The other man experienced severe lachrymation, blepharospasm, throat irritation and pain hindering him to breath through the nose at 2050 ppm (7700 mg/m^3). The pain was reversible within 15 min. after ended exposure, while the eye irritation lasted for 1 hour and the nasal congestion was present for 24 hours. (Stewart et al. 1970).

Twenty-three men were exposed to 250 ppm (equivalent to 938 mg/m^3) PGME for 1-7 hr. After 15-30 minutes, eight men experienced eye irritation, three had nose irritation, one had a headache and one had nausea. After 45-60 min, 20 men had developed eye irritation and increased blinking, 15 complained of nasal irritation, 2 of throat irritation and one had a headache. Ten men remained in the chamber after 2 hours, 8 of whom complained of eye and 5 of nasal irritation. All four subjects remaining in the chamber for 7 hr had eye irritation and lachrymation, while none of them could still detect the odour of PGME. (Stewart et al. 1970).

90.2 Repeated dose toxicity

No data were found.

90.3 Toxicity to reproduction

No data were found.

90.4 Mutagenic and genotoxic effects

No data were found.

90.5 Carcinogenic effects

No data were found.

91 Animal toxicity

91.1 Single dose toxicity

91.1.1 Inhalation

Groups of rats varying from 10 to 20 animals were exposed to single exposures of 7000, 10000 or 15000 ppm PGME (corresponding to 25500, 36400 and 54600 mg/m^3, respectively) for 1 to 8 hours.
Five out of 10 rats exposed to 54600 mg/m^3 for 4 hours died, while 3/20 rats exposed to 36400 mg/m^3 and 2/20 at 25500 mg/m^3 died. Dose-related CNS depression was seen from 25500 mg/m^3. At 54600 and 36400 mg/m^3, there were signs of respiratory tract irritation with interstitial oedema, congestion and areas of emphysema in the lung.
Death occurred in 2/20, 15/25 and 15/20 rats exposed once for 6 hours to 25500, 36400 or 54600mg/m^3 PGME, respectively. CNS-depression was seen at all exposure levels, and all rats at the highest exposure level, were unconscious. (Rowe et al. 1954).

Groups of 10 -15 rats exposed once to 10000 ppm (36400 mg/m^3) for 6 hours, 6000 ppm (21800 mg/m^3) or 3000 ppm (10900 mg/m^3) for 7 hours were examined with respect to injury. At the highest exposure level, body weights were moderately depressed and liver, kidney and lung weights were increased. Microscopy of the animals at the highest concentration level revealed slight irritation of the lungs, but there were no effects on other organs. The mid-concentration level caused slight histopathological effects including granulation of the cytoplasm of centrolubolar cells and non-fatty deposits in 5 out of 10 rats one day after exposure, while no effect was seen on the liver after 3 days. At the low exposure level, no effects were seen. (Rowe et al. 1954).

Five out of 10 guinea pigs exposed to PGME for 10 hours died. Lethargy was reported at sublethal concentrations (Rowe et al. 1954).

Groups of 2 or 4 rabbits were exposed to one single exposure of 10000 or 15000 ppm (36400 or 54600 mg/m^3) PGME for 4, 6 or 7 hours. One out of 4 animals died following 7 hours exposure to the high dose (Rowe et al. 1954).

One male monkey was exposed in a single exposure to 10000 ppm PGME (36400 mg/m^3) for 6 hours. It showed eye and nasal irritation, ataxia and depressed respiration, followed by excitation and lethargy after the animal was removed from the exposure chamber. The effects were reversible. (Rowe et al. 1954).

91.1.2 Oral intake

An LD_{50} – value of 6100 mg PGME/kg b.w. was reported on basis of administration of 9 dosage levels of undiluted PGME to rats by gavage.

Dyspnoe and symptoms of CNS depression, including somnolence, uncoordinated gait and ataxia, were seen. (Rowe et al. 1954).

For rats, LD_{50}– values of >5000, 5200, 5710 and 5900 mg/kg b.w. were reported without any details (IUCLID 2000).

An LD_{50} – value for mice of 10800 mg/kg b.w. was reported without any details (IUCLID 2000).

For rabbits, LD_{50} – values of >1840 and 5300 mg/kg b.w. were reported without any details (IUCLID 2000).

One cat survived a single exposure to 2 ml/kg b.w. (corresponding to 1840 mg/kg b.w.), but showed behavioural changes for 2 days after exposure (IUCLID 2000).

For dogs, and LD_{50} – value of 9200 mg/kg b.w. was reported. Signs of toxicity were nausea, vomiting and respiratory arrest. (Shideman and Procita 1951- quoted from ECETOC 1995).

91.1.3 Dermal contact

Groups of 5 or 10 rabbits were exposed to 5.0, 7.0, 10.0, 12.0 or 15.0 ml PGME/kg b.w. (equivalent to 4.6, 6.4, 9.2, 11.1 or 13.9 g/kg b.w.) under occlusive bandage for 24 hours. The exposed areas were then washed with soap and water, and the animals were observed for two weeks. Four of 5 animals of the high dose group, and 2 out of 10 animals of the second highest dose group died within 5 days. All rabbits showed narcosis. (Rowe et al. 1954). An LD_{50} – value of ca. 13000 mg/kg b.w. was reported from this experiment (IUCLID 2000).

Another study reported an LD_{50} – value for rabbits of 14200 mg/kg b.w. (Smyth et al. 1962 – quoted from IUCLID 2000).

91.1.4 Irritation

Respiratory tract irritation was seen in rats and guinea pigs exposed by inhalation to high concentrations (from 10000 ppm – equivalent to 37500 mg/m^3) of PGME for 4-10 hours (Rowe et al. 1954).

PGME was reported to be not irritating to the skin of rabbits. No details were given. (IUCLID 2000).

Repeated contact to rabbit skin over 13 weeks with2, 4 , 7 or 10 ml PGME/kg b.w. (equivalent to 1.85, 3.70, 6.47 or 9.24 g/kg b.w.) caused only occasional scaling and erythema, and no difference to controls could be demonstrated. (Rowe et al. 1954).

One drop of undiluted PGME was placed in the eyes of rabbits on five consecutive days. Mild transient irritative response of the conjunctiva was noted following each dose. No corneal injury was revealed by fluorescein staining. (Rowe et al. 1954).

PGME was reported to be not irritating or slightly irritating to rabbits eyes. No further details were given. (IUCLID 2000).

91.1.5 Sensitisation

No sensitisation was seen in Guinea pigs in an adjuvant test with topical applications described by Maguire (1973) (Carreon et al. 1984 – quoted from IUCLID 2000).

91.2 Repeated dose toxicity

91.2.1 Inhalation

Male and female Fischer 344 rats and B6C3F1 mice were exposed to 0, 300, 1000 or 3000 ppm PGME (equivalent to 0, 1125, 3750 or 11250 mg/m^3) 6 hr/day for 9 days. At the high concentration, central nervous system depression and decrease in urine specific gravity was seen in rats, and liver weights were increased in male rats and mice. No effects were reported at any other concentration in rats or in mice and no gross pathological or histopathological effects were reported at any concentration in either species. (Miller et al. 1981).

Rats were exposed to 2500, 5000 or 10000 ppm PGME (9375, 18750 or 37500 mg/m^3) 4 hours/day, 5 days/week for 2 weeks. No effects were seen in the low exposure group. At the two highest exposure levels, transient non-specific CNS-depression was reported, with tolerance development at the end of the exposure period. Body weights were decreased in the high dose group. (Goldberg 1964 – quoted from IUCLID 2000).

Groups of 5 male and 5 female rats were exposed to 10000 ppm (corresponding to 36400 mg/m^3) PGME 30 minutes daily for 107 days (79 exposures), 1 hour daily for 106 days (78 exposures) or 2 hours daily for 116 days (84 exposures). Drowsiness and unsteadyness were seen after each 2 hour-exposure, and slight CNS depression was seen in the 1 hour-exposure group. The 2-hour group showed moderately increased liver and kidney weights. No other effects on organ weights haematology or histology were seen in this group or in the two other treatment groups. (Rowe et al. 1954).

In a 90-day-study, groups of 10 male and 10 female Fischer 344 rats were exposed to 0, 300, 1000 or 3000 ppm PGME (corresponding to 0, 1090, 3620 or 10900 mg/m^3), 6 hours/day, 5 days/week. No effects were seen in the two lowest exposure groups. In the high exposure group, transient sedation in relation to the exposure was recorded during the first weeks of the study. Increased relative liver weights and, in females, slight hypertrophy of the liver in the centrilobular area were also reported at this concentration. No effects were seen in other organs or in haematology. (Landry et al. 1983).

A 90-day-study using the same exposure regime as used above for the rats was performed using 7 New Zealand White rabbits/sex/exposure concentration. No effects were seen at the two lowest exposure groups, while animals exposed to 10900 mg/m^3 exhibited transient central nervous depression. No other effects were reported (Landry et al. 1983).

Exposure for 4 hours daily to 10000 ppm (corresponding to 36400 mg/m^3) of two male monkeys 66 times over 91 days and of one female monkey 26 times in 38 days caused CNS depression after each exposure. Body weights were depressed and liver weights increased significantly. Histology revealed cytoplasma granulation in the central area and non-fatty degeneration. Slight congestion of the lungs was seen. No further adverse effects were reported. (Rowe et al. 1954).

Groups of rats were exposed 7 hours/day, to 6000 ppm (corresponding to 21800 mg/m^3) for 114 days (81 exposures) or to 3000 or 1500 ppm (corresponding to 10900 or 5460 mg/m^3) for 198 days (141 exposures). A group of controls was also used. At the high concentration, 4/10 males and 7/10 females died. The survivors exhibited narcotic effects after each exposure, although tolerance to the material developed during the last 2 months. Slight body weight depression and increased liver and kidney weights were reported, but no histopathological findings were seen. At the mid concentration, mortality was not higher than in the control group. Reversible, mild CNS depression was reported from exposure during the first week at this concentration. No effects were seen on body weights in this group, but the liver weights were significantly increased in both males and females. No microscopic changes were found. At the low dose, no adverse effects were seen. (Rowe et al. 1954).

Groups of at least 5 male and 5 female Guinea pigs were exposed 7 hours/day, 5 days/week to either 6000 ppm PGME (corresponding to 21800 mg/m^3) for 113 days, to 3000 or to 1500 ppm (corresponding to 10900 and 5450 mg/m^3, respectively) for 184 days. In the high dose group, all animals survived, but marked narcotic effects were reported. Body weights were significantly depressed. Only very slight vacuolation and granulation in liver cells were seen. No adverse effects were seen at the two lower exposure levels. (Rowe et al. 1954).

One female rabbit was exposed 7 hours daily, 5 days/week to 6000 ppm PGME over a period of 113 days, two rabbits to 3000 ppm and 4 rabbits to 1500 or 800 ppm PGME over 184 days. The concentration levels correspond to 21800, 10900, 5450 or 2906.7 mg/m^3, respectively. The one female treated at the high concentration showed narcotic effect and body weight depression. Liver and kidney weights were slightly increased, but no significant pathological effects were seen in the liver. Very slight local irritation and congestion and oedema were seen in the lungs. At 10900 and 5450 mg/m^3, liver weights were slightly increased, microscopy revealing slight changes as described above for the guinea pigs. Slight congestion and oedema and areas of emphysema were seen in the lungs. No adverse effects were seen at the low concentration. (Rowe et al. 1954).

91.2.2 Oral intake

Groups of five male rats were given gavage doses of 0, 0.1, 0.3, 1.0 or 3.0 ml PGME/kg b.w. in olive oil (equivalent to 0, 0.09, 0.28, 0.92 or 2.77 g/kg b.w.) 26 times over 35 days. No animals died. In the high dose group, the liver and kidney weights were increased, while the lung, heart, spleen and testes weights were normal. No effects were seen in the 3 lower dose groups. (Rowe et al. 1954).

Dogs were exposed by gavage to 0, 0.5, 1 or 2-3 ml/kg b.w. (equivalent to 0, 0.46, 0.92 or 1.85-2.77 g/kg b.w.) PGME for 14 weeks. Mild central nervous system depression was observed at the two highest doses. Male dogs of the high dose group had spermiophages (possibly macrophages) in the epididymis and testis. No further information are given. (Stenger et al. 1972).

91.2.3 Dermal contact

Five male and 5 female New Zealand white rabbits were exposed dermally to 1000 mg/kg b.w., 5 days/week for 3 weeks. Slight scaling of the skin was reported. (Calhoun et al. 1984 - quoted from ECETOC 1995).

Groups of 5 rabbits were exposed to 0, 2.0, 4.0, 7.0 or 10.0 ml PGME/kg b.w. (equivalent to 0, 1.85, 3.70, 6.47 or 9.24 g/kg b.w.) under occlusion, 5 times /week for 13 weeks. Mortality is reported in Table 4.2.3.

Table 4.2.3 Mortality in dermal 90-day study with PGME

Dose (g/kg b.w.)	0	1.85	3.70	6.47	9.24
No. deaths/No. treated	0/5	1/6	2/7	8/9	11/11

Decreased food consumption and body weight loss as well as narcosis were recorded prior to death in the two highest dose groups. In the lower dose groups, the deaths were associated with respiratory infections. Organ weights and gross pathology were normal, except for gastric retention and occasional haemorrhagic foci in the gastric mucosa in narcotic animals. Histological examination revealed renal tubular necrosis in 3 animals from the two highest dose groups who had died of the treatment, while other rabbits showed only a slight granular degeneration in the tubules. (Rowe et al. 1954).

91.3 Toxicity to reproduction

91.3.1 Inhalation

Ten male Wistar rats exposed for 10 days to 0, 200 or 600 ppm (equivalent to 0, 750 or 2250 mg/m^3) PGME 6 hours/day showed no effects on testicular weights or histology. (Doe et al. 1983).

Pregnant Wistar rats were exposed to 0, 200 or 600 ppm PGME (equivalent to 0, 750, 2250 mg/m^3) 6 hours/day on days 6-17 of gestation. No effects on dam weights, on number of pups, proportion of live pups or mean group pup weights were reported. (Doe et al. 1983).

Groups of 30-32 pregnant Fischer 344 rats were exposed to 0, 500, 1500 or 3000 ppm PGME (equivalent to 1875, 5625 or 11250 mg/m^3) 6 hours/day from day 6-15 of gestation. The study was conducted in accordance with OECD guideline 414. Mild central nervous system depression was reported at the high exposure level at the beginning of the exposure period, but accommodation developed. In this group, food consumption was decreased the first 3 days, and body weight gain was depressed at the end of the exposure period. No differences on pregnancy rates, litter size, resorption rates or feral body weights were seen when compared to controls.

Significantly increased delayed sternebral ossification was reported in pups of the high concentration group dams. There was no significant increase in incidence of malformations in any groups.
(Hanley et al. 1984).

Groups of 31-33 pregnant New Zealand white rabbits were exposed to 0, 500, 1500 or 3000 ppm PGME (equivalent to 1875, 5625 or 11250 mg/m^3) 6 hours/day from day 6-18 of gestation, in a study in accordance with OECD guideline 414. Mild lethargy was seen in the high concentration group during the first two days of exposure. The overall weight gain was statistically reduced. Six rabbits died, four of which at the high concentration. No significant differences in relative number of pregnancies, litters, resorptions or live foetuses or in foetal body weights were reported in any treatment group when compared to controls. No significant difference in incidence of malformations or variations in the pups of any treatment group was seen when compared to controls. (Hanley et al. 1984).

91.3.2 Oral intake

Male mice administred 2% PGME in the drinking water (approximately equivalent to 2.5 g/kg b.w.- assuming a water consumption of 3.5 ml/mouse/day and a mouse weight of 25 g) for 25 days did not show any significant changes in testes or in seminal vesicle weights (Nagano et al. 1984 – quoted from ACGIH, 1991).

Male dogs exposed by gavage to 462 - 2772 mg/kg b.w. PGME for 14 weeks developed numerous spermiophages (macrophages) in the epididymis. The study is also described under point 4.2.2 (Stenger et al. 1972).

Female rats were dosed by gavage to 0, 0.05, 0.1, 0.2, 0.4 or 0.8 ml/kg b.w. PGME (equivalent to 0, 46, 92.4, 185, 370 or 739 mg/kg b.w.) on days 1-21 of gestation. There was no effect on the number of pups. The offspring showed significant delayed ossification of the skull at the highest dose. No information on maternal toxicity was given. (Stenger et al. 1972).

A continuous breeding study was performed in CD-1 mice. Twenty males and 20 females/group were dosed in the drinking water from 7 days before mating with 0, 0.5, 1.0 or 2.0 % PGME (according to DECOS 1993, the dose levels correspond to 0, 950, 1890 and 3330 mg/kg b.w. for males); the offspring dosed continuously with 2.0 % PGME in the drinking water (approximately corresponding to 3330 mg/kg b.w.). In the F1- generation, birth weights of the pups of the high dose group were reduced. In the F2 – generation, reduction in birth weights and reduced epididymis and prostate gland weights, but no effect on sperm morphology, motility or density was reported. (Unpublished data by Dow, 1986 – quoted from IUCLID 2000 and DECOS 1993).

Female mice were exposed by gavage to 0, 0.5, 1 or 2 ml/kg b.w. (equivalent to 462, 924 and 1848 mg/kg b.w, respectively) PGME on days 1-18 of gestation. No evidence of foetotoxicity was seen. (Stenger et al. 1972).

Pregnant rabbits were exposed by gavage to 0, 0.25, 0.5 or 1 ml/kg b.w. PGME (equivalent to 0, 231, 462 or 924 mg/kg b.w.) on day 6-18 of gestation. No evidence of toxicity were seen in pups. (Stenger et al. 1972)

91.3.3 Dermal contact

No data were found.

91.4 Mutagenic and genotoxic effects

91.4.1 *In vitro* studies

An Ames test conducted in *Salmonella typhimurium* strains TA98, TA 100, TA 1535, TA 1537 and TA 1538 with and without metabolic activation using 2 - 6250 µg/plate was negative in all strains (unpublished report by Dow 1983a- quoted from ECETOC 1995).

A mammalian unscheduled DNA synthesis test in vitro using primary rat hepatocytes with PGME concentrations of 3.16×10^{-5} to 0.1 M (2.85×10^{-3} - 9.01 g/l) showed no effect on grain counts. (Mendrala 1983 - quoted from ECETOC 1995 and DECOS 1993).

A chromosomal aberration test conducted in Chinese Hamster ovary cells at PGME concentrations of 1.25, 2.5, 5.0 and 10.0 mg/ml was negative (unpublished report by Dow 1983b - quoted from ECETOC 1995).

91.4.2 *In vivo* studies

No data were found.

91.5 Carcinogenic effects

91.5.1 Inhalation

Groups of Fischer-344 rats were exposed to 0, 300, 1000 or 3000 ppm PGME (equivalent to 1125, 3750 or 11250 mg/m^3) 6 hrs/day, 5 days/week for 3, 6 or 12 months (10 rats/sex/conc. + 5 males/conc. for investigation of α_{2U}-globulin nephropathy), 18 months (20 rats/sex/conc.) or 24 months (50 rats/sex/conc.).
At the high exposure level, CNS-depression including decreased activity, incoordination and transient sedation was observed during the first week until 1-2 hours after each treatment. These symptoms disappeared, but reappeared at 12-18 months of exposure. At this exposure level,mortality increased in male rats from approximately 18 months of treatment, but the finding was not statistically significant. Body weights were significantly decreased in the animals treated for 12, 18 or 24 months at the high concentration level.
There was no effect on haematology in any group. In males of the high concentration group, serum creatinine was increased 78% and urea nitrogen 100% at 24 months. Serum alkaline phosphatase was significantly increased from 6 months at 11250 mg/m^3 and at 24 months at 3750 mg/m^3. Liver enzymes (alanine and aspartate amino-transferases) were mildly elevated in males of the high concentration group during the first, but not during the second year of the two year- treatment period.
Gross and microscopic pathology was performed on the animals treated for 2 years. Liver weights were increased in the high exposure group from 2 weeks to 2 years of dosing. The incidence of eosinophilic hepatocellular foci was increased in males of the two highest concentration groups exposed for 24

months. Male rats treated with the high concentration showed significantly elevated hepatic S-phase DNA-synthesis.
Kidney weights of high-exposure males were significantly increased in a time-dependent way from 12 months of exposure. Also female kidney weights were increased, although less consistently than in males. The kidneys of male rats treated with the highest concentration showed epithelium damage in the tubules with multifocal tubular basophilia, slight decrease of cell height and cell debris in the tubular lumen at one week. The effect was less clear at 13 weeks. Levels of α_{2u}-globulin in the epithelial cells of the cortical tubules were increased in all treated male groups (investigated until 12 months exposure period). The effect was most prominent during the first 2 weeks. At 13 weeks, α_{2u}- globulin staining involved about half the cortical tubules in the high concentration group, while only 2/5 males of the low concentration group showed slight staining. α_{2u}-globulin staining remained in males of the two highest dose groups at 6 and 12 months. No effect was seen in females at 1 and 2 weeks and the parameter was not investigated in females at later intervals. No difference in incidence of chronic progressive glomerulonephritis was seen compared to controls, but the effect was more pronounced in the affected animals in the high concentration group, especially in males. No significant increase in tumour incidence was seen in the 24 months-study. (Spencer et al. 2002).

Groups of B6C3F1-mice were exposed to 0, 300, 1000 or 3000 ppm PGME (equivalent to 1125, 3750 or 11250 mg/m^3), 6 hrs/day, 5 days/week for 1 or 2 weeks, 3, 6, 12, 18 months (5-10 mice/sex/conc.) or 24 months (50 mice/sex/conc.).
Decreased activity, incoordination and transient sedation was reported at the high concentration for the first week of exposure. In the 24 months-study, mortality was non-significantly increased in male mice of the high exposure group from around 18 months when compared to controls, while no effect on mortality was seen in studies of shorter duration or in females. Body weights were significantly depressed 2-7% in mice exposed for 24 months to the high concentration. Also mice treated with the mid-concentration had significantly depressed body weight, although the effect was less marked.
Liver weights were increased in high exposure group animals following 2 weeks to 2 years. Hepatic S-phase DNA-synthesis was slightly, but significantly increased in males of the high concentration group.
No effects on kidney weight, gross appearance or histopathology were reported.
In the adrenal glands, pathological examination revealed atrophy of a female specific zone in all female mice exposed for 3 months to the highest concentration and in 2/10 females exposed to the mid-concentration level, but not in females treated for 24 months. The authors report that the toxicological significance of the effect is unknown. (Spencer et al. 2002).

92 Regulations

92.1 Ambient air

Denmark (C-value): 0.03 mg/m^3 (MST 2002)

92.2 Drinking water

-

92.3 Soil

-

92.4 Occupational Exposure Limits

Denmark: 185 mg/m^3 (50 ppm) (At 2002)

ACGIH: 100 ppm (ACGIH 2001)

Germany: 100 ppm (MAK 1998)

92.5 Classification

Flammable with R10 (MM 2002)

92.6 IARC

-

92.7 US-EPA

-

93 Summary

93.1 Description

1-methoxy-2-propanol (2PG1ME) is a water soluble colourless volatile liquid with an ether like odour. 2PG1ME is the α-isomer of propylene glycol monomethyl ether (PGME) and makes out at least 95% of commercial PGME where the β-isomer is present in less than 5%.

93.2 Toxicokinetics

2PG1ME appears to be absorbed by all routes of exposure. Toxicological studies did not indicate that accumulation should occurr. The primary metabolic pathway was via demethylation and oxidation to CO_2, which is then exhaled. Conjugation and urinary excretion played a minor role. The developmental toxicant methoxypropionic acid is not formed by 2PG1ME as it is a secondary alcohol, but only from the β-isomer 2-methoxy-1-propanol.

93.3 Human toxicity

93.3.1 Single dose toxicity

Groups of male volunteers were exposed by inhalation to different concentrations of PGME vapours for 1-7 hours. Symptoms of slight CNS-depression (dizziness, headache) were reported from 1125-1500 mg/m^3 in subjects exposed to rising concentration PGME. Overt CNS-impairment was seen in one of two subjects exposed to 3750 mg/m^3
Volunteers exposed to 938 mg/m^3 for 7 hours, complained of eye and nose irritation after 15-30 minutes, the irritation symptoms increasing and including complaints of throat irritation after 45 minutes. The symptoms were so strong that only four subjects remained in the chamber for 7 hours.

No reports of repeated dose toxicity, reproductive and developmental effects, mutagenic and genotoxic effects or carcinogenic effects in humans were found.

93.4 Animal toxicity

93.4.1 Single dose toxicity

Five out of 10 rats died following a 4 hour-exposure of 54600 mg/m^3 PGME.. Dose-related CNS depression was reported in rats treated with single exposure from 25500 to 56600 for 1 to 8 hours, including unconsciousness in rats exposed to 54600 mg/m^3 for 6 hours.
Histopathological examination of rats dosed 10900-36400 mg/m^3 for 6-7 hours showed slight granulation of liver cell cytoplasm and non-fatty liver deposits in half the rats exposed to 21800 mg/m^3, while liver, kidney and lung weights increase.
Five out of 10 Guinea pigs died following 10 hours exposure to 54600 mg/m^{3}PGME. One out of four rabbits died following 7 hours exposure to

54600 mg/m^3.A monkey exposed to 36400 mg/m^3 PGME for 6 hours showed CNS- symptoms including depressed respiration, excitation and lethargy.

Oral LD_{50}-values of 5000 to 6100 mg PGME/kg b.w. for rats, 10800 mg/kg b.w. for mice, 1840-5300 for rabbits and 9200 mg/kg for dogs were reported. In rats and dogs, general intoxication symptoms and symptoms of CNS depression were reported preceeding death.

Dermal application of 4600-13900 mg PGME /kg b.w. under occlusion for 24 hours caused narcosis in all animals, and 4 out of 5 rabbits at the high dose died. Dermal LD_{50}-values for rabbits of 13000 and 14200 mg/kg b.w were reported.

Respiratory tract irritation was reported from single 4 hour exposures of rats to 36400 or 54600 mg PGME /m^3 . Slight irritation of the lungs was seen from a 7 hour exposure to 109000 mg PGME/m^3, while no effects were reported at lower concentrations.
A monkey exposed to 36400 mg/m^3 PGME for 6 hours showed eye and nose irritation.
PGME was reported to be not irritating or slightly irritating to rabbits skin following single or repeated exposure. PGME was reported to be non-irritating or mildly irritating to the eyes of rabbits.

No sensitisation was seen in an adjuvant test in Guinea pigs.

93.4.2 Repeated dose toxicity

In an inhalation study including, rats, mice, rabbits and monkeys, the animals were exposed to PGME at different regimes, with concentrations from 300 to 10000 ppm (equivalent to 1125 - 37500 mg/m^3), over 9 days to 6 months, 30 minutes to 7 hours daily. All species showed CNS-depression decreasing in studies of longer duration. Slight lung congestion and slight non-fatty degeneration and cytoplasma granulation of the liver in some of the studies. Overall, the effect level in rats and mice was approximately 11000 mg/m^3, while rabbits showed effects in the liver and the lung at 5450 mg/m^3..Guinea pigs appeared slightly less sensitive, with showing CNS depression and slight liver effects at 22000 mg/m^3, and monkeys showed CNS-depression at 36400 mg/m^3.

In a 90-day inhalation study in rats and rabbits exposed to 1090-10900 mg/m^3, rats of the high concentration level exhibited CNS depression, increased liver weights and slight liver hypertrophy, while rabbits of the high exposure level showed CNS depression .

In dogs, oral ingestion of PGME over 14 weeks resulted in CNS-depression at 920 and 1850/2770 mg/kg b.w. Rats dosed 2.77 g/kg b.w. over 35 days showed increased liver and kidney weights.

Daily dermal doses of 6.47 and 9.24 g/kg b.w. over 13 weeks were toxic to rabbits. Occasional gastric retention and renal tubular necrosis were reported.

93.4.3 Toxicity to reproduction

Inhalation of up to 2250 mg/m^3 PGME for 10 days did not result in testicular effects in rats.
No effects on reproduction of female rats were reported from exposure up to 2250 mg/m^3 PGME from days 6-17 of gestation.

No changes in testes or seminal vesicle weights were seen in male mice exposed to 2.5 g/kg b.w. PGME in drinking water for 25 days.Dogs exposed orally to 462-2772 mg/ kg b.w. for 14 weeks developed numerous spermiophages (macrophages) in the epididymis.

Delayed sternebral ossification was reported in pups of rats dosed 11250 mg/m^3 from days 6-15 of gestation. Mild depression of the central nervous system and decreased body weight gain were seen in the dams of this treatment group, while no effect was seen on dams, reproduction parameters or pups at lower concentrations.
Pregnant rabbits exposed up to 11250 mg PGME/m^3 had increased mortality, CNS depression and reduced weight gain at the high concentration, while there were no effects on reproduction or on development at this or lower concentration levels.

Delayed ossification of the skull was reported in pups of rats exposed orally to 739 mg/kg b.w. PGME on days 1-21 of gestation. No effect on the number of pups was seen. No details are given on maternal effects.
The F_1 and F_2-generation of mice exposed through the drinking water to approximately 3.3 g/kg b.w PGME in a continuous breeding study showed reduction in birth weights and reduced epididymis and prostate gland weights.
No evidence of foetotoxicity was seen from exposure of female mice by gavage to up to 1848 mg/kg b.w from days 1-18 of gestation.
No foetoxicity resulted from oral exposure of rabbits on days 6-18 of gestation to up to 924 mg/kg b.w.

93.4.4 Mutagenic and genotoxic effects

PGME was negative in an Ames test, in a unscheduled DNA synthesis test in rat hepatocytes and in a chromosome aberration test in Chinese hamster ovary cells. No *in vivo* studies were found.

93.4.5 Carcinogenic effects

Rats exposed by inhalation to 1125-11250 mg/m^3 up to 24 months showed increased liver weights and incidence of eosinophilic heptocellular foci were increased in male of the high exposure group. Chronic progressive glomerulonephritis was more pronounced, and tubular epithelium damage in males treated at the high concentration compared to controls, but no there was no increase in tumour incidence. Levels of α_{2u}-globulin were elevated at 3 months in all the treated males compared to controls

Mice exposed to the same regime as the rats also had liver weights increase. No adverse effects on the kidney were seen, but females exposed for 3 months at the high concentration showed changes in the adrenal gland.

94 Evaluation

Most of the available toxicological data on 1-methoxy-2-propanol (2PG1ME) relate to the commercial compound methoxy-propanol (PGME), which consists of 95-99% 2PG1ME and less than 5% 2-methoxy-1-propanol (1PG2ME). On the basis of these data, it appears that 2PG1ME is of low systemic toxicity; irritation of the eyes, the mucous membranes and the respiratory tract as the critical effects. The substance is also a CNS-depressant.

2PG1ME appears to be absorbed by all routes of exposure. It is primarily metabolised via demethylation and oxidised to CO_2, a minor part being excreted through the urine in conjugated form. The toxic metabolite methoxyacetic acid is not formed by 2PG1ME, but only by its β-isomer 2PG1ME.

PGME is of low acute toxicity. No poisoning cases in humans were found. LC_{50}-values in animals were approximately 54600 mg/m^3 following 4 hours exposure in rats and10 hour exposure in Guinea pigs, and higher in rabbits and monkeys. Oral LD_{50}-values of 5000-10800 mg/kg b.w. were reported for rats, mice, rabbits and dogs. The dermal LD_{50}–value for rabbits was around 13000 mg/kg b.w.

PGME caused dizziness and headache in human volunteers from 1125 mg/m^3, and one case of loss of ability to balance is reported at 3750 mg/m^3. CNS-depression was also the major symptom reported from acute studies in rats, mice, Guinea pigs, rabbits and monkeys from 25500 mg/m^3. Oral and dermal exposure at sublethal doses also resulted in CNS depression in rats, rabbits and dogs.

In humans. The substance was irritating to the eyes, nose and throat at 938 mg/m^3.
In animals, the substance was reported to be irritating to the respiratory tract of rats from 37500 mg/m^3. PGME was not irritating or mildly irritating to the eyes of rabbits. No information on skin irritation in humans were available, and no or slight irritation occurred in rabbits following exposure to PGME. The substance was not sensitising in Guinea pigs.

No human data were available on effects of repeated exposure to PGME. Animal data showed increased liver weights, hypertrophy of the liver and occasional slight non-fatty degeneration and granulation of the liver of rats, mice, guinea pigs, rabbits following repeated exposure to 5450 to 21800 mg/m^{3}up to 6 months. In a two-year inhalation study in rats and exposed to 1125-11250 mg/m^3, the liver weights and the incidence of eosinophilic hepatocellular foci were increased in the high dose animals.

Development of glomerulonephritis was significantly increased in male rats exposed to 11250 mg/m^3 over 24 months compared to controls. There was no increase in tumour incidence. No increase was seen in females. The mechanism of nephrotoxicity in males was probably related to the measured

increased levels of α_{2u}-globulin, which is specific to male F344-rats and considered not to be a relevant mechanism for humans.

No effect on testis resulted from inhalation exposure of rats up to 2250 mg/m^3 PGME or oral exposure of mice to 2.5 g/kg b.w.. Dogs treated orally with 462 mg/kg b.w. had macrophages in the testes and epididymis. The finding was so scarcely described in the reference that it cannot be evaluated. However, as no similar pathological change were found in any other study, it is not considered to be toxicologically significant.

No foetotoxicity was seen from exposure by gavage of mice or rabbits during gestation at doses of 1848, respective 924 mg/kg b.w. Delayed ossification of the sternebrae and the skull occurred in pups of rats dosed 11250 mg/m^3 by inhalation and orally to 739 mg/kg b.w., respectively. Maternal toxicity was seen in rabbits and rats at these levels. In a continuous breeding study in mice where PGME was given in the drinking water, reduced birth weights, epididymis and prostate weights were seen at 3.3 g/kg b.w. PGME while no effects were seen in the dams. On this basis, PGME is considered not to have any adverse effect on fertility or to cause developmental toxicity.

PGME was negative in three different *in vitro* mutagenicity tests. No *in vivo* tests were available.

No increased incidence of tumours was found in a 2-year inhalation study in rats and mice exposed up to 11250 mg/m^3.

In conclusion, 2PG1ME is considered to be of low systemic toxicity and its critical effects being irritative effects to the eyes, the mucous membranes and the respiratory tract and depression of the central nervous system.

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Appendix 13: Diethylene glycol mono-*n*-butyl ether (DEGBE)

96 General description

Diethylene glycol mono-*n*-butyl ether (DEGBE) has been selected for evaluation within this project. Because ethylene glycol ether acetates are rapidly metabolised by plasma esterases to the parent ethylene glycol ethers following absorption, the biological fate and thus, the toxicological effects of the acetate (DEGBEA) of DEGBE are expected to be very similar to those of DEGBE. Therefore, data on DEGBEA are included in this document when considered relevant.

96.1 Identity

Molecular formula: $C_8H_{18}O_3$

Structural formula:

Molecular weight: 162.23

CAS-no.: 112-34-5

Synonyms: Butoxy diethylene glycol
2-(2-n-Butoxyethoxy)ethanol
Butyl carbitol
Butyl diglycol
Diethylene glycol butyl ether
Diglycol butyl ether
Ethanol, 2,2´-oxybis-, monobutyl ether
1-Hydroxy-3,6-dioxadecan
2-Hydroxy-2-butoxy-diethylether
2,2-Oxybisethanol monobutyl ether
Dowanol DB

96.2 Physical / chemical properties

Description: Colourless liquid with a weak odour and bitter taste.

Melting point: -68.1°C

Boiling point: 226-234°C

Density: 0.95 g/ml (at 20°C)

Vapour pressure: 0.02 mmHg (2.7 Pa) (at 20°C)

Concentration of saturated vapours: 27 ppm (182 mg/m^3) (calculated) at 20°C and 760 mmHg

Conversion factor:	1 ppm = 6.75 mg/m^3 (at 20°C and 760 mmHg) 1 mg/m^3 = 0.148 ppm
log$P_{octanol/water}$:	0.3; 0.56; 0.82; 4.69
Solubility:	Water: miscible. Also soluble in acetone, alcohol, benzene, and ether.
References:	A&H (1995), ChemFinder (2001), DECOS (1996), HSDB (2000), IUCLID (2000).

97 Toxicokinetics

97.1 Absorption, distribution and excretion

No data regarding the absorption and excretion of DEGBE following inhalation or oral intake, or the distribution of DEGBE have been found.

The *in vitro* percutaneous absorption of DEGBE has been measured in rat and human skin. The absorption rate through rat skin (incubation time 8 hours) was 0.506 ± 0.193 mg/cm^2/hour (n = 12); a permeability constant was calculated to be 5.30 ± 2.02 x 10^{-4} cm/hour. Under the same experimental conditions, the absorption rate through human skin was 0.292 ± 0.133 mg/cm^2/hour (n = 10); a permeability constant was calculated to be 3.06 ± 1.42 x 10^{-4} cm/hour. (Barber et al. 1992 – quoted from DECOS 1996).

When ^{14}C-DEGBEA was given as a single oral dose (200 or 2000 mg/kg) to male Sprague-Dawley rats (5 animals per group), about 85% of the total ^{14}C-dose was excreted in the urine, about 2 to 3% in the faeces, and about 5% as carbon dioxide in expired air by 72 hours after the administration. A proposed metabolic pathway for DEGBEA in the rat is shown in Figure 2.1 below. The asterisks indicate metabolites identified by enzymatic, chromatographic and/or mass spectrometric techniques. The major urinary metabolite was 2-(2-butoxyethoxy)acetic acid, comprising 58.9% and 53.4% of the total urinary radioactivity after the low and the high dose, respectively. No unchanged DEGBEA or DEGBE was detected in rat urine at either dose level and no evidence was found for excretion of 2-butoxyacetic acid (BAA). (Deisinger & Guest 1989 – quoted from DECOS 1996).

Groups of 4 Sprague-Dawley rats of each sex had 0.2 or 2.0 g/kg of undiluted or 0.2 g/kg of a 10% aqueous solution of ^{14}C-labeled DEGBE or its acetate ^{14}C-DEGBEA (only undiluted) applied under occlusion to shaved skin for 24 hours. At the low dose, females excreted about 42/51% (diluted/undiluted) DEGBE and about 40% DEGBEA, and males about 31/27% (diluted/undiluted) DEGBE and about 48% DEGBEA. At the high dose, females excreted about 18% DEGBE and about 12% DEGBEA, and males about 3% DEGBE and about 13% DEGBEA. The excretion in faeces was only a few percent (about 0.2-3%); excretion via expiration was not measured. The main urinary metabolite was 2-(2-butoxyethoxy)acetic acid, which accounted for 61 to 80% of the total urinary radioactivity. The glucuronide of DEGBE was present at levels of about 5 to 8% of the urinary radioactivity. Trace levels of 2-butoxyacetic acid were present in all 8-hour and 24-hour urine samples following application of DEGBE, but not DEGBEA. The dermal absorption rates for DEGBEA were estimated to be 1.58 and 1.28 mg/cm^2/hour for males and females, respectively, and for DEGBE to 0.73 and 1.46 mg/cm^2/hour for males and females, respectively. (Boatman et al. 1993).

97.2 Mode of action

No specific data regarding the toxicological mechanism(s) of DEGBE have been found.

A number of glycol ethers and their acetates have been shown to cause haematological, immunological, testicular, and/or developmental toxicity; these toxic effects appear to be dependent on the formation of alkoxy acetic or alkoxy propionic acids (ECETOC 1995).

Ethylene glycol mono-*n*-butyl ether (EGBE) causes haemolysis; significant species differences have been reported with the rat being the most sensitive species whereas human erythrocytes appear to be the least sensitive of those investigated. The mechanism(s) behind the haemolytic effect is not fully understood, but the metabolite 2-butoxyacetic acid (BAA) has shown a significantly greater haemolytic effect *in vitro* than EGBE whereas DEGBE has shown a considerably less haemolytic activity than EGBE. (ECETOC 1995).

The toxic metabolite 2-butoxyacetic acid (BAA) can be formed via metabolism of DEGBE.

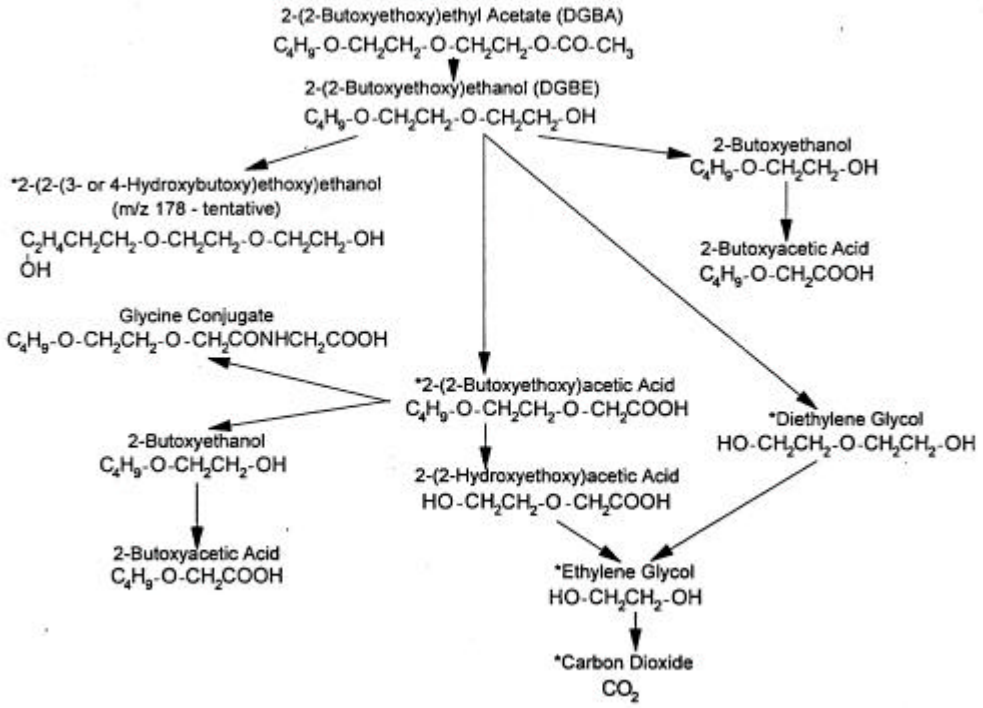

Figure 2.1. A proposed metabolic pathway for DEGBEA in the rat according to Deisinger & Guest (1989 – from DECOS 1996).

98 Human toxicity

98.1 Single and repeated dose toxicity

In one report, two cases of kidney and liver damage have been attributed to single exposures to DEGBE; two persons who were painting in a closed room and at the same time had ingested alcohol (Schwarzbeck et al. 1974 – quoted from A&H 1995 and DECOS 1996). According to A&H (1995), the association with DEGBE is doubtful, and has been questioned.

98.2 Irritation and sensitisation

A case report has described a woman who experienced irritation of the eyes and upper airways, facial erythema, and swollen eyelids when she was in newly painted buildings (water-based painting). She had a positive patch test reaction to an aqueous (20%) solution of DEGBE. (Berlin et al. 1995).

Another case report has described a worker exposed to DEGBE and DEGBEA (over a 20-year period), who had acute, diffuse, erythematous dermatitis on hands, arms, face and neck. The man had a positive response to DEGBEA in a patch test taking during a dermatitis outbreak. One year later, the dermatitis had healed (upon retirement) and he was patch tested with both undiluted and diluted DEGBE and GEGBEA, with negative results. When test was repeated without occlusion both substances yielded a positive reaction, indicating the possibility of contact urticaria (type I allergy). (Dawson et al. 1989 – quoted from A&H 1995 and from ECETOC 1995b).

When 202 house painters were patch tested with a 20% solution of DEGBE in water, 4 of the painters developed an irritative reaction but no sensitisation was observed (Fischer 1993 - quoted from A&H 1995).

98.3 Toxicity to reproduction

No data have been found.

98.4 Mutagenic and genotoxic effects

No data have been found.

98.5 Carcinogenic effects

No data have been found.

99 Animal toxicity

99.1 Single dose toxicity

99.1.1 Inhalation

No rats died when exposed for 7 hours to the maximum attainable vapour concentration (estimated by DECOS to be 122 mg/m^3) of DEGBE (Gingell et al. 1994 – quoted from DECOS 1996 and from ECETOC 1995a). Also rabbits, cats, guinea pigs, rats, and mice survived an exposure period of 2 hours in a saturated atmosphere (DECOS 1996).

An LC_{50}-value of about 73000 mg/m^3 has been reported for rats following exposure for 4 hours to the acetate (DEGBEA) of DEGBE. Signs of toxicity were red foci and congestion in the lungs. (DuPont 1984 – quoted from ECETOC 1995b).

99.1.2 Oral intake

The oral LD_{50}-values reported for DEGBE are between 5080 and 9600 mg/kg b.w. in rats (6 values reported), between 2400 and 6560 mg/kg b.w. in mice (3 values reported), 2200 mg/kg b.w. in rabbits, and 2000 mg/kg b.w. in guinea pigs (Quoted in DECOS 1996, A&H 1995, ECETOC 1995a, and in IUCLID 2000).

99.1.3 Dermal contact

The dermal LD_{50}-value reported for DEGBE is greater than 2000 mg/kg b.w. for rats, and between 2700 and 4120 mg/kg b.w. for rabbits (2 values reported) (Quoted in DECOS 1996, A&H 1995, ECETOC 1995a, and in IUCLID 2000).

99.1.4 Skin irritation

In a skin irritation study in rabbits performed according to directive 84/449/EEC, B.4 “Acute toxicity (skin irritation)”, very slight to moderate erythema and oedema was observed 60 minutes following the application of DEGBE. After 3 days, only very slight irritation was observed and after 9 days, all symptoms had disappeared. (Southwood 1987 - quoted from IUCLID 2000).

DEGBE has been reported to be only very slightly irritating to the skin of rabbits (Rowe et al. 1982 – quoted from DECOS 1996; Gingell et al. 1994 – quoted from ECETOC 1995a) and slightly irritating to the skin of rabbits and guinea pigs (Krasavage & Terhaar 1981 – quoted from Gingell et al. 1996).

99.1.5 Eye irritation

99.1.5.1 Eye irritation

In a primary eye irritation test, 0.1 ml of undiluted or diluted (1, 5, 10, 20, 25, or 50% in water) DEGBE was instilled into the eyes of rabbits (6 animals per group). The eyes were examined after 10 minutes, 1 hour, 24 hours, and daily up to 14 days. Instillation of 1% DEGBE caused a 3% increase in intraocular pressure after 10 minutes, and 20% DEGBE caused a 63% increase; the values returned to normal within an hour. Instillation of 10% DEGBE caused a mild, transient conjunctivitis, and 25% caused increased tear flow, congestion, inflamed eyelids (blepharitis), conjunctival oedema (chemosis), and corneal inflammation (keratitis); these effects disappeared within 2 days. Instillation of 50 and 100% DEGBE caused iritis as well, which lasted 3 and 10 days, respectively. (Ballantyne 1984 – quoted from A&H 1995, DECOS 1996, ECETOC 1995a, and IUCLID 2000).

Following instillation of 0.005 ml of undiluted DEGBE into the eyes of rabbits and observation for 18 to 24 hours, moderate irritation and corneal damage were recorded, graded 5 on a scale of increasing damage 1 to 10 (Smyth & Carpenter 1946 – quoted from IUCLID 2000 and from A&H 1995).

DEGBE has been reported to be capable of causing moderate irritating and moderate transient corneal injury to the eyes of rabbits (Rowe et al. 1982 – quoted from DECOS 1996).

99.1.6 Sensitisation

In a guinea pig maximization test performed according to directive 84/449/EEC, B.6 “Acute toxicity (skin sensitization)”, 10 guinea pigs were induced with 2.5% of the test substance (intradermally) or with the undiluted test substance (covered patch). For the challenge, undiluted test substance was used. The control group consisted of 4 animals. No skin sensitisation was observed. (Basketter 1985 – quoted from IUCLID 2000).

DEGBE was not sensitising in the guinea pig maximisation test (25% injection induction, 100% application induction and application challenge) (Unilever 1984 – quoted from ECETOC 1995).

99.2 Repeated dose toxicity

99.2.1 Inhalation

When rats (20 animals per group) were exposed to DEGBE at concentrations of 0 or 143 mg/m^3, 6 hours a day for 4 days, no changes were induced in clinical chemistry, haematology parameters, or in histology; no further details were given (DFG 1992 – quoted from DECOS 1996).

Wistar rats (5 animals of each sex per group) were exposed (unclear whether whole-body or nose-only) to DEGBE at concentrations of 0, 100, 350, or 1000 mg/m^3, 6 hours a day, 5 days per week for 2 weeks. According to IUCLID, 100 mg/m^3 is the highest attainable vapour concentration. The body weights were decreased at the highest concentration, but not significantly. A dose-related decrease in spleen weight was noted in all male

exposure groups and increased (but not dose-related) lung weights at the two highest concentrations. Histopathology revealed perivascular and peribronchial accumulations of granulocytes and activation of alveolar epithelium at all exposure levels. The NOAEL was considered to be below 100 mg/m^3. (BASF 1987 – quoted from IUCLID 2000).

In a study performed according to OECD Guideline 412, Wistar rats (10 females per group) were exposed (head-nose) to DEGBE at concentrations of 0 or 350 mg/m^3, 6 hours a day, 5 days per week for 2 weeks. Satellite groups were observed for 4 weeks post-exposure. No effects on mortality, organ weights, clinical chemistry, and gross pathology were observed. The body weight gain was significantly decreased in both main and satellite groups. Very slight nasal discharge was observed in some animals during exposure. Histopathology revealed slight to moderate multifocal perivascular and peribronchial accumulation of granulocytes and minimal focal bronchiolisation (no further details are given) in the lungs. After 4 weeks, recovery was observed regarding all effects except minimal to slight granulocytes accumulation in the lungs (regression in severity was evident). The NOAEL was considered to be below 350 mg/m^3. (BASF 1991 – quoted from IUCLID 2000).

Fischer 344 rats (15 animals of each sex per group) were exposed (whole-body) to DEGBE (vapour) at concentrations of 0, 2, 6, or 18 ppm (0, 13, 40, or 122 mg/m^3, 6 hours a day, 5 days per week for 5 weeks. No treatment-related findings were observed regarding mortality, body weights, clinical signs, haematology, and urinalysis. A significantly decreased relative liver weight was observed in males at the two highest exposure levels (dose-related) whereas a dose-related increase was observed in females at these exposure levels (significant only at the highest exposure level). In female rats, slight paleness of the liver was noted in 3/10 animals at the highest exposure level and slight hepatocyte vacuolisation at all dose levels (3/10, 4/10, 9/10, 10/10 of the control, low-, mid-, and high-dose groups, respectively). According to the authors, the slight hepatocytes vacuolisation in females was consistent with fat accumulation and was considered to be of questionable toxicological significance. The NOAEL was considered to be 13 mg/m^3 (DECOS) or 40 mg/m^3 (IUCLID, the decreased liver weight in mid-dose males were not accompanied by histological changes and thus considered not to be treatment-related). (Gushow et al. 1981 – quoted from IUCLID 2000, ECETOC 1995a, and from DECOS 1996).

In a study performed according to OECD Guideline 413, Wistar rats (10 animals of each sex per group in either the main groups and the satellite groups) were exposed (whole-body) to DEGBE at concentrations of 0, 2, 6, or 14 ppm (0, 13, 40, or 95 mg/m^3), 6 hours a day, 5 days per week for 13 weeks. Satellite groups were observed for 4 weeks post-exposure. No mortality and no treatment-related findings were observed for body weight (change), clinical signs, ophthalmology, haematology, urinalysis, organ weights, and pathology; according to IUCLID, no data on spleen weights were reported. The only observed effect was a dose-related decreased level of serum aspartate aminotransferase in males of all exposure groups; this change was, according to IUCLID, not considered as being treatment-related because no effects on liver were observed and the change was within the range of biological variation. Therefore, the NOAEL was considered to be 95

mg/m^3; DECOS has endorsed this conclusion. (BASF 1992 – quoted from IUCLID 2000, Gingell et al. 1996, and from DECOS 1996).

99.2.2 Oral intake

DEGBE was fed to rats (5 animals of each sex per group) at dose levels ranging from 51 to 1830 mg/kg b.w./day for 30 days (according to Gingell et al. (1996), the study is a drinking water study). The lowest dose level that caused loss of appetite was 94 mg/kg b.w./day, and at 650 mg/kg b.w./day histopathological changes were observed in either the liver, kidney, spleen, or testes (no details are given of which organs were affected). (Smyth & Carpenter 1948 – quoted from A&H 1995, ECETOC 1995a, DECOS 1996, and from Gingell et al. 1996).
The NOAEL was considered, by ECETOC, to be 51 mg/kg b.w./day. According to Gingell et al. (1996), DEGBE, in the 1940s, was made by a process using boron trifluoride as a catalyst and may have contained impurities, which could have caused the observed effects.

Sprague-Dawley rats (10 males per group) received 0, 891, 1782, or 3564 mg/kg b.w./day of DEGBE by oral gavage, 5 days a week for 6 weeks. The control group received the vehicle (not specified). Feed consumption and body weight were decreased at the highest dose level and congestion of the spleen was observed. A dose related decrease in haemoglobin, total red cells, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC); increase in absolute and relative spleen weights; decrease in liver weight; and proteinaceous casts were observed at the mid- and high-dose levels. Hyperkeratosis in the stomach was observed in all dosed rats. The NOAEL was lower than 891 mg/kg b.w./day. (Eastman Kodak Company 1984 – quoted from IUCLID 2000). This study is also cited in Gingell et al. (1996); however, not every of the abovementioned effects are included in this citation.

Fischer 344 rats (16 animals of each sex per group for six weeks and 10 for the rest of the study) received oral doses of DEGBE by gavage of 0, 65, 327, or 1630 mg/kg b.w./day (males) or 0, 51, 254, or 1270 mg/kg b.w./day (females) 5 days a week for 13 weeks. At the highest dose level, 83/92% of male/female rats died within the study with 60/30% at the mid-dose level and 0/10% at the low dose level; pulmonary congestion and oedema was observed in the rats that died during the study and, according to ECETOC and Gingell et al., the very high mortality was due to dosing accidents. Due to the high mortality in the high-dose group, statistics were not performed at this dose levels. Food consumption (males and females) and body weight gain (males) were decreased at the highest dose level. Absolute and relative liver weights were increased in the mid-dose males and, according to IUCLID, in the remaining males and females of the high-dose group; the relative kidney weight was, according to IUCLID, increased in the remaining high-dose males; and the relative spleen weight was, according to DECOS, decreased in mid-dose male animals. Haematological changes were found in both males (according to DECOS only: increased erythrocyte count and haemoglobin content at the mid-dose level) and females (dose-related decreased leukocyte and lymphocyte count, and mean corpuscular haemoglobin concentration at the two lowest dose levels). Gross and microscopic lesions were, according to IUCLID, only observed in the thoracic cavity and respiratory tract. (Hobson

et al. 1987 – quoted from IUCLID 2000 and from DECOS 1996). This study is also cited in Gingell et al. (1996) and in ECETOC (1995a); however, the results of the study are very briefly reported in these two sources.

99.2.3 Dermal contact

Sprague-Dawley rats (10 animals of each sex per group) received 2 ml/kg b.w./day of 0, 10, 30 or 100% of DEGBE in water (equal to 0, 200, 600 or 2000 mg/kg b.w./day) by application to the skin (3 cm by 3 cm area on the clipped skin of the back) under occlusion for 6 hours a day, 5 days per week for 13 weeks. No mortality and no treatment-related findings were observed for feed consumption, body weights, clinical chemistry, haematology, organ weights, oestrous cycling in females, and pathology. The only suggestion of a systemic effects was a slightly increased incidence of occult blood in the urine of females in the two highest dose groups; microscopic examination of the urine revealed no urinary casts and no significant numbers of erythrocytes. DEGBE produced skin irritation, which was dose-concentration dependent in incidence, severity, and time of onset and was more severe in females than in males; irritation was minimal at the lower two concentrations whereas application of undiluted DEGBE produced irritation in all animals, some were affected after as few as two doses. Microscopic examination of skin sections from the application site revealed no DEGBE-related histological changes, slight thickening of the epidermis being seen for both control and treated animals. A NOAEL of 2000 mg/kg b.w./day can be considered for systemic effects; for local effects the NOAEL is below 200 mg/kg b.w./day. (Auletta et al. 1993).

In a study performed in accordance with the US-EPA test guidelines for neurotoxicity, Sprague-Dawley rats (12 animals of each sex per group) received 2 ml/kg b.w./day of 0, 10, 30 or 100% of DEGBE in water (equal to 0, 200, 600 or 2000 mg/kg b.w./day) by application to the skin (the test volume was applied to the dorsum and spread over the previously shaven area, approximately 10% of the body surface) under occlusion for 6 hours a day, 5 days per week for 13 weeks. Male and female rats were examined using a functional observational battery (FOB) pre-study, at 1, 6, and 24 hours after the initiation of the first exposure, and prior to treatment on days 7, 14, 35, 63, and 91. Motoractivity was determined pre-study and on non-treated days 34, 62, and 90. At the completion of the treatment, animals from the highest dose level and 6 animals from the control group were perfused for neuropathology. There was no mortality and feed consumption and body weights were unaffected by treatment. Five females at the highest dose level had scab formation at the treatment site during the study; no other treatment-related clinical findings were noted. The FOB and motor activity tests revealed no findings indicative of a neurotoxic effect, and there were no gross or neuropathological treatment-related changes. A NOAEL of 2000 mg/kg b.w./day can be considered for systemic effects, including neurotoxicity; the NOAEL for local effects was 600 mg/kg b.w./day. (Beyrouty et al. 1993).

New Zealand white rabbits (3 animals of each sex per group) had 2 ml/kg b.w./day of 0 or 1.5% of DEGBE in water (equal to 0 or 30 mg/kg b.w./day) applied on abraded skin without occlusion for 7 hours a day, 5 days per week for 4 weeks. Oral exposure was prevented by collars. There were no treatment-related effects regarding clinical signs, urinanalysis, organ weights,

and gross pathology. The water consumption was decreased in males, and haematological (decreased eosinophil and monocyte count in males; decreased haemoglobin, erythrocyte, leukocyte and neutrophil count in females) and clinical chemical (increased alkaline phosphatase in females, but decreased alkaline phosphatase in males; decreased globulin and sodium level in females) changes were observed; these effects were, according to IUCLID, not considered treatment-related because of a wide variation within one group and/or between the groups before and after exposure. No treatment-related effects on the skin were noted. A NOAEL of 30 mg/kg b.w./day can be considered for systemic and for local effects. (Elliot et al. 1982 – quoted from IUCLID 2000).

99.3 Toxicity to reproduction

99.3.1 Inhalation

No data have been found.

99.3.2 Oral intake

In a one-generation study, male Charles River CD rats Groups (25 animals per group) were dosed orally by gavage with 0, 250, 500 or 1000 mg/kg b.w./day of DEGBE for 60 days prior to mating and until sacrifice. Female rats (25 animals per group) were treated similarly for 14 days prior to mating and until sacrifice (one-half of each dose group at gestation day 13 and the reaming animals at lactation day 21). Untreated males were mated with treated females and vice versa. Ten animals died during the study; deaths were attributed to intubation accidents or to the aspiration of the test substance. The numbers of fertile males in the treated groups were not significantly different from controls, nor were there any significant increases in resorptions or decreases in live embryos and live births in untreated females mated with treated males. No significant differences were seen in the body weights of the treated females during the study, and there were no significant differences in the number of fecund females or in resorptions, live embryos, and live pups at birth. The only significant effect observed was a decrease in the weight of the pups at day 14 of lactation in offspring from the high-dose female group. A NOAEL of 1000 mg/kg b.w./day can be considered for reproductive and developmental effects and of 500 mg/kg b.w./day for effects in the offspring (reduced pup weight). (Nolen et al. 1985).

Wistar rats (20 female per group) received 0, 0.04, 0.2, or 1% of DEGBE in their diet (equal to 0, 25, 115, or 633 mg/kg b.w./day) from day 0 to 20 of gestation. On day 20 of gestation, 14-15 rats in each group were sacrificed; the remainder of the rats were allowed to deliver spontaneously and to rear their offspring until weaning where they were sacrificed, the offspring were sacrificed 10 weeks after birth. No deaths or clinical signs of toxicity were observed and the feed consumption in treated animals was similar to that of the control group. The maternal body weight gain was significantly reduced in all exposure groups during the gestation period. No significant differences were found in the pre- and postimplantation losses, the numbers of corpora lutea per litter, implantations per litter, the number of live foetuses per litter, the sex ratio of live foetuses, and the foetal body weight. External, skeletal, and internal examinations of the foetuses revealed no evidence of

teratogenesis. In the postnatal period, a high survival rate and good growth of the offspring were noted. A NOAEL of 633 mg/kg b.w./day can be considered for developmental effects, including teratogenicity. (Ema et al. 1988).

When CD-1 mice (46-48 animals per group) were exposed orally by gavage to 0, 500 or 2050 mg/kg b.w./day of DEGBE from day 7 to 14 of gestation, no indications for developmental effects were observed; however, routine examinations of pups for malformations or skeletal anomalies were, according to IUCLID, not performed. At the high dose, 25% of the dams died. At the low dose, there were no maternal deaths and body weight was similar to controls. (Hardin et al. 1987 - quoted from IUCLID 2000 and from DECOS 1996).

99.3.3 Dermal contact

In a one-generation study, Sprague-Dawley rats (25 animals of each sex per group) received 2 ml/kg b.w./day of 0 or 100% of DEGBE in water (equal to 0 or 2000 mg/kg b.w./day) by application to the skin (3 cm by 3 cm area on the clipped skin of the back) under occlusion for 6 hours a day, 5 days per week for 13 weeks. The animals were then mated within dose groups. The males continued to be treated for 6 hours a day, 5 days per week, until the completion of the mating period. The females were treated for 6 hours a day, 7 days per week during the mating period and on gestational days 0 to 20, and were sacrificed after day 21 of lactation. No mortality and no treatment-related findings were observed for feed consumption and body weights. The male and female mating indices, pregnancy rates, male fertility indices, along with parturition data, pup body weights, and pup survival and viability were not adversely affected by treatment with 100% DEGBE. Examinations of the pups delivered by treated females, pups found dead during lactation, and of the pups at weaning revealed no adverse effects due to the treatment with DEGBE. A NOAEL of 2000 mg/kg b.w./day can be considered for reproductive and developmental toxicity. (Auletta et al. 1993).

In a teratogenicity study performed according to OECD-guideline 414, New Zealand white rabbits (17-19 pregnant females per group) had 0, 100, 300 or 1000 mg/kg b.w. of DEGBE applied to the backs (10 x 20 cm on the dorsal surface, without occlusion) and left for 4 hours a day, on days 7 to 18 of gestation. Oral ingestion was prevented by plastic collars. None of the dams died during the study. There was one abortion and one early delivery, which, according to the authors, not appeared to be related to treatment with DEGBE. All of the dosed dams gained less weight than the controls during gestation, although the reduction was only significant for the mid-dose group. At the two higher dose levels, skin irritation was observed after about 1 week of treatment and persisted until the dams were sacrificed. No significant differences were seen in any of the reproductive parameters (number of corpora lutea, implants, resorptions, or viable embryos), or in the mean foetal body weight. Furthermore, there were no significant differences in the incidence of skeletal anomalies or of gross or visceral malformations. A NOAEL of 1000 mg/kg b.w./day can be considered for developmental toxicity, including teratogenicity; the NOAEL for maternal effects was 100 mg/kg b.w./day (reduced weight gain, skin irritation). (Nolen et al. 1985).

99.4 Mutagenic and genotoxic effects

99.4.1 *In vitro* studies

DEGBE showed negative results when tested in the Ames test in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 with and without metabolic activation at concentrations up to toxicity (20 µl/plate) (Thompson et al. 1984 – quoted from IUCLID 2000, A&H 1995, ECETOC 1995a, Gingell et al. 1996, and from Gollapudi et al. 1993).

DEGBE was negative in a forward gene mutation assay at the HGPRT locus of Chinese Hamster Ovary (CHO) cells (OECD guideline 476) when tested up to a maximum concentration of 5000 µg/ml with and without metabolic activation system (Gollapudi et al. 1993).

When DEGBE was tested for chromosome aberrations in CHO cells, the number of aberrations per cell, including chromatid gaps, were similar to that of negative and solvent controls. A cytotoxicity test was performed with concentrations from 1.06 to 10.56 µl/ml with and without metabolic activation; the toxicity curve was much steeper in the presence of S9 mix and doses of 4.46, 5.94, and 7.92 µg/ml were selected for the cytogenicity assay. (Thompson et al. 1984 – quoted from IUCLID 2000, A&H 1995, ECETOC 1995a, Gingell et al. 1996, and from Gollapudi et al. 1993).

DEGBE also showed a negative result when tested for unscheduled DNA synthesis (UDS) in rat hepatocytes at concentrations from 0.26 to 4.44 µg/ml without metabolic activation. Viability was determined over dose ranges from 0.26 to 10.0 µg/ml; survival figures at 4.44, 6.67, and 10.0 µg/l were 59, 32, and 2%, respectively, relative to solvent control. (Thompson et al. 1984 – quoted from IUCLID 2000, A&H 1995, ECETOC 1995a, Gingell et al. 1996, and from Gollapudi et al. 1993).

A weak, dose-dependent increase in mutations was seen in the mouse lymphoma test (L5178Y) without, but not with, metabolic activation; there was no increase at non-toxic dose-levels. The test was performed with concentrations from 0.42 to 5.6 µg/l without metabolic activation and from 0.56 to 7.5 µg/ml with metabolic activation (S9). The highest concentration used caused 88% toxicity in the absence of S9 and 42% toxicity in the presence of S9. (Thompson et al. 1984 – quoted from A&H 1995, IUCLID 2000, ECETOC 1995a, Gingell et al. 1996, and from Gollapudi et al. 1993).

99.4.2 *In vivo* studies

DEGBE has been evaluated in the *in vivo* mouse bone marrow micronucleus test for cytogenetic damage. DEGBE was administered by oral gavage at dose levels of 0, 330, 1100, or 3300 mg/kg b.w. to CD-1 mice (5 animals of each sex per group). According to the authors, the highest dose level was approximately 80% of the estimated LD_{50}-value. Animals were sacrificed 24, 48, or 72 hours after treatment. DEGBE was ineffective in increasing the incidence of micronucleated polychromatic erythrocytes. (Gollapudi et al. 1993). According to IUCLID (2000), the study was performed according to OECD-guideline 474.

DEGBE was negative when tested for lethal mutations in the Drosophila sex-linked recessive lethal assay following administration of DEGBE in the feed (11000 ppm) for 3 days or by a single injection (14000 ppm) (Thompson et al. 1984 – quoted from IUCLID 2000, A&H 1995, ECETOC 1995a, Gingell et al. 1996, and from Gollapudi et al. 1993).

99.5 Carcinogenic effects

No data have been found.

100 Regulations

100.1 Ambient air

Denmark C-value: 0.02 mg/m^3 L (L means that the C-value is based upon the odour) (MST 2002).

100.2 Drinking water

-

100.3 Soil

-

100.4 Occupational Exposure Limits

Denmark: 100 mg/m^3 (At 2002).

ACGIH: -

Germany: 100 mg/m^3 (as a vapour) (DECOS 1996).

The Dutch Expert Committee on Occupational Standards recommends an occupational exposure limit of 50 mg/m^3 (DECOS 1996).

100.5 Classification

DEGBE is classified for irritant properties (Xi;R36 – irritating to eyes) (MM 2002).

100.6 IARC

-

100.7 US-EPA

-

101 Summary

101.1 Description

DEGBE is a colourless liquid with a low vapour pressure (0.02 mmHg (2.7 Pa, at 20 °C); it is miscible with water.

101.2 Toxicokinetics

When the acetate (^{14}C-DEGBEA) of DEGBE was given as a single oral dose (200 or 2000 mg/kg b.w.) to male Sprague-Dawley rats, about 85% of the total ^{14}C-dose was excreted in the urine, about 2 to 3% in the faeces, and about 5% as carbon dioxide in expired air by 72 hours. The major urinary metabolite was 2-(2-butoxyethoxy)acetic acid, accounting for 53-59% of the total urinary radioactivity. No unchanged DEGBEA or DEGBE was detected and no evidence was found for excretion of 2-butoxyacetic acid.

Following dermal contact to rats (0.2 or 2.0 g/kg of undiluted ^{14}C-DEGBE or ^{14}C-DEGBEA, or 0.2 g/kg of a 10% aqueous solution of ^{14}C-DEGBE) for 24 hours, the excretion in urine at the low dose level was about 40-51% in females and about 27-48% in males. At the high dose, females excreted about 12-18% DEGBE and males about 3-13% DEGBEA. The excretion in faeces was only a few percent (about 0.2-3%). The main urinary metabolite was 2-(2-butoxyethoxy)acetic acid. Trace levels of 2-butoxyacetic acid were present in all 8-hour and 24-hour urine samples following application of DEGBE, but not DEGBEA.

No data regarding the absorption and elimination of DEGBE following inhalation or oral intake, or the distribution of DEGBE have been found.

101.3 Human toxicity

A few case reports of irritation and sensitisation to DEGBE have been reported.

No data on acute and repeated dose toxicity, toxicity to reproduction, mutagenic and genotoxic effects, or carcinogenic effects in humans have been found.

101.4 Animal toxicity

101.4.1 Single dose toxicity

Oral LD_{50}-values for DEGBE ranging from 5080 to 9600 mg/kg b.w. in rats, from 2400 to 6560 mg/kg b.w. in mice, of 2200 mg/kg b.w. in rabbits, and of 2000 mg/kg b.w. in guinea pigs have been reported.
Dermal LD_{50}-values greater than 2000 mg/kg b.w. for rats, and from 2700 to 4120 mg/kg b.w. for rabbits have been reported.
No rats died when exposed for 7 hours to the maximum attainable vapour concentration (estimated to be 122 mg/m^3). Also rabbits, cats, guinea pigs,

rats, and mice survived an exposure period of 2 hours in a saturated atmosphere. An LC_{50}-value of about 73000 mg/m^3 has been reported for rats following exposure for 4 hours to the acetate (DEGBEA) of DEGBE.

In a skin irritation study in rabbits (EU Annex B4), DEGBE was considered to have a non-irritating potential. Other reports have considered DEGBE to have only a very slightly irritating (rabbits) or slightly irritating (rabbits, guinea pigs) potential.

In a primary eye irritation test in rabbits, a concentration of 5% DEGBE did not produce any primary irritant effects. The lowest concentration producing keratitis was 25%, and that causing minor transient conjunctivitis was 10%. Reversible increases in the intraocular tension were observed with concentrations from 1%. Effects were most severe within the first 24 hours and the eye returned to normal within 14 days. Other reports have considered DEGBE to be capable of causing moderate irritating and moderate transient corneal injury to the eyes of rabbits.

DEGBE was not sensitising in the guinea pig maximisation test (EU Annex B6).

101.4.2 Repeated dose toxicity

Wistar rats exposed to DEGBE (0, 100, 350, or 1000 mg/m^3, 6 hours/day, 5 days/week for 2 weeks) showed a dose-related decrease in spleen weight (all male exposure groups), increased (but not dose-related) lung weights at the two highest concentrations, and decreased body weight (not significant) at the highest concentration; histopathology revealed perivascular and peribronchial accumulations of granulocytes and activation of alveolar epithelium at all exposure levels. Similarly, another 2-week inhalation study (OECD Guideline 412) in female Wistar rats exposed (head-nose only) to DEGBE (0 or 350 mg/m^3, 6 hours/day, 5 days/week) showed decreased body weight gain and slight to moderate multifocal perivascular and peribronchial accumulation of granulocytes and minimal focal bronchiolisation in the lungs in exposed animals. In a 5-week inhalation study in Fisher 344 rats exposed (whole-body) to DEGBE (vapour, 0, 13, 40, 122 mg/m^3, 6 hours/day, 5 days/week), only effects on the liver were observed (dose-related decreased relative liver weight in males from 40 mg/m^3, dose-related increase in relative liver weight in females from 40 mg/m^3 (significant only at 122 mg/m^3), and slight paleness of the liver (at 122 mg/m^3) and slight hepatocyte vacuolisation at all dose levels). When Wistar rats were exposed (whole-body) to DEGBE (vapour, 0, 13, 40, or 95 mg/m^3, 6 hours/day, 5 days/week for 13 weeks, OECD Guideline 413), the only observed effect was a dose-related decreased level of serum aspartate aminotransferase in males of all exposure groups.

In a 6-week oral gavage study in male rats, haematological effects (dose related decrease in haemoglobin, total red cells, MCV, MCH, and MCHC) were observed at dose levels from 1782 mg/kg b.w./day; increased spleen weights (absolute and relative), decreased liver weight, and proteinaceous casts were observed as well. Hyperkeratosis in the stomach was observed in all dosed rats (891 mg/kg b.w./day). A 13-week oral gavage study (0, 65/51, 327/254, or 1630/1270 mg/kg b.w./day in males and females, respectively) in rats revealed haematological effects (females: dose-related decrease in MCHC, and white blood cell and lymphocyte counts at the two lowest dose

levels; males: increase in total red cells and haemoglobin content at the mid-dose level); increased liver weights (absolute and relative) in mid- (only males) and high-dose groups) and kidney weight (relative) in high-dose males; decreased spleen weight (relative) in mid-dose males; and decreased food consumption and body weight gain (males) at the highest dose level. Furthermore, a high mortality (88%) was observed at the highest dose level.

The only suggestion of a systemic effect of DEGBE in a 13-week dermal study in rat was a slightly increased incidence of occult blood in the urine of females at dose levels from 600 mg/kg b.w./day; microscopic examination of the urine revealed no urinary casts and no significant numbers of erythrocytes. DEGBE produced skin irritation, which was concentration (2 ml/kg b.w./day of 0, 10, 30 or 100% of DEGBE in water, 6 hours a day, 5 days per week) dependent in incidence, severity, and time of onset and was more severe in females than in males. In another 13-week dermal study in rats, 5/12 females at the highest concentration level (2 ml/kg b.w./day of 100% of DEGBE in water, 6 hours a day, 5 days per week) had scab formation at the treatment site during the study; no other treatment-related clinical findings were noted. FOB and motor activity tests revealed no findings indicative of a neurotoxic effect, and there were no gross or neuropathological treatment-related changes. In a 4-week dermal study in rabbits, haematological changes (decreased eosinophil and monocyte count in males; decreased haemoglobin, erythrocyte, leukocyte and neutrophil count in females) were observed following application of 2 ml/kg b.w./day of 1.5% of DEGBE in water (7 hours a day, 5 days per week); no treatment-related effects on the skin were noted.

101.4.3 Toxicity to reproduction

In a one-generation oral gavage study in rats, the only significant effect observed was a decrease in the weight of the pups at day 14 of lactation in offspring from females administered 1000 mg/kg b.w./day and mated with untreated males; a NOAEL of 1000 mg/kg b.w./day can be considered for reproductive and developmental effects and of 500 mg/kg b.w./day for post-natal effects. Similarly, no reproductive or developmental effects were observed in a one-generation dermal study in rats at a dose level of 2000 mg/kg b.w./day (the only dose level in the study); in contrast to the oral study, no effects were noted in the offspring during the lactation period. A NOAEL of 2000 mg/kg b.w./day can be considered for reproductive, developmental and post-natal effects.

In an oral developmental toxicity study in rats, no developmental effects, including teratogenicity, were observed at dietary dose levels of up to 1% (equal to 633 mg/kg b.w./day, day 0 to 20 of gestation) and in the postnatal period (10 weeks after birth), a high survival rate and good growth of the offspring were noted. Maternal effects (reduced body weight gain) were observed in all exposure groups (equal to 25, 115, or 633 mg/kg b.w./day) during the gestation period. A NOAEL of 633 mg/kg b.w./day can be considered for developmental effects, including teratogenicity, and a LOAEL for maternal effects of 25 mg/kg b.w./day. In mice, no indications for developmental effects were observed at oral (gavage) dose levels of 500 or 2050 mg/kg b.w./day (day 7 to 14 of gestation); 25% of the dams died at the high dose level with no mortality and no effects of body weights being observed at the low dose level. In a dermal teratogenicity study (OECD-

guideline 414) in rabbits, a NOAEL of 1000 mg/kg b.w./day (highest dose level in the study) was observed for developmental toxicity, including teratogenicity. All of the dosed (100, 300 or 1000 mg/kg b.w., days 7 to 18 of gestation) dams gained less weight than the controls during gestation (significant only for the mid-dose group) and at the two higher dose levels, skin irritation was observed after about 1 week of treatment and persisted until the dams were sacrificed; a NOAEL of 100 mg/kg b.w./day can be considered for maternal effects.

101.4.4 Mutagenic and genotoxic effects

DEGBE has shown negative results in the following *in vitro* test systems: in the Ames test (one test in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538), for gene mutation in the HGPRT assay in Chinese Hamster Ovary (CHO) cells, for chromosome aberrations in CHO cells, and for unscheduled DNA synthesis (UDS) in rat hepatocytes. The tests were performed with and without metabolic activation (UDS: only without) and with concentrations up to cytotoxicity. A weak, dose-dependent increase in mutations was seen in the mouse lymphoma test (L5178Y) without, but not with, metabolic activation; there was no increase at non-toxic dose-levels.

Negative results have also been reported in *in vivo* studies: in the mouse bone marrow micronucleus test (OECD-guideline 474) following oral gavage (0, 330, 1100, or 3300 mg/kg b.w.) and for lethal mutations in the Drosophila sex-linked recessive lethal assay following administration in the feed (11000 ppm, for 3 days) or by a single injection (14000 ppm).

101.4.5 Carcinogenic effects

No data have been found.

102 Evaluation

Based upon evaluation of radioactivity excreted in urine and faeces in rats following oral administration of the acetate (^{14}C-DEGBEA) of DEGBE or dermal contact to ^{14}C-DEGBE or ^{14}C-DEGBEA, the absorption is about 85% following oral administration, and about 30-50% at low dose levels (200 mg/kg b.w.) and about 3-18% at high dose levels (2000 mg/kg b.w.) following dermal application. Only minor amounts (a few percent) are excreted in faeces and about 5% as carbon dioxide. Following dermal application, female rats tended to excrete a larger proportion of the applied dose of DEGBE than did male rat. The major urinary metabolite was 2-(2-butoxyethoxy)acetic acid at both exposure routes. No evidence was found for excretion of 2-butoxyacetic acid (BAA) following oral administration of DEGBEA (a single dose of 200 or 2000 mg/kg b.w.), whereas trace levels of BAA were present in urine following dermal application of DEGBE (200 or 2000 mg/kg b.w. under occlusion for 24 hours), but not DEGBEA (2000 mg/kg b.w. under occlusion for 24 hours).

DEGBE is of low acute toxicity following oral administration and dermal application in experimental animals with oral LD_{50}-values ranging from 2000 to 9600 mg/kg b.w. (rats, mice, rabbits, and guinea pigs) and dermal LD_{50}-values greater than 2000 mg/kg b.w. (rats and rabbits). The limited data on acute toxicity following inhalation of DEGBE in experimental animals do not allow an evaluation of this end-point; however, an LC_{50}-value of about 73000 mg/m^3 for rats following exposure to the acetate of DEGBE indicate a low order of acute inhalation toxicity for DEGBE as well. No human data have been found.
Overall, DEGBE is considered to be of low acute toxicity in humans.

A few human case reports of irritation (skin, eyes, and upper respiratory tract) and sensitisation to DEGBE have been reported. In rabbits and guinea pigs, DEGBE has shown a very low skin irritating potential in conventional tests for skin irritation whereas it is a moderate eye irritant in rabbits. DEGBE was not sensitising in the guinea pig maximisation test.
A 13-week dermal study in rats has revealed skin irritation, which was concentration dependent in incidence, severity, and time of onset. In contrast to this study, skin effects (scab formation) were only noted in 5/12 females in another 13-week study at the highest concentration used. No treatment-related effects on the skin were noted in rabbits in a 4-week dermal study; however, the concentration used was low compared to the concentrations used in the two dermal studies in rats. In a dermal teratogenicity study (OECD-guideline 414) in rabbits, skin irritation was observed after about 1 week of treatment and persisted until the dams were sacrificed. The NOAEL for skin irritation is considered to be below 200 mg/kg b.w./day based on the skin irritative effects observed in the first 13-week dermal study in rats although skin irritation was not observed in the other 13-week dermal study in rats at dose levels up to 600 mg/kg b.w./day; according to IUCLID, the second 13-week study cannot by used to conclude a NOAEL for local effects. Furthermore, the NOAEL is considered to be above 100 mg/kg b.w./day based on the dermal teratogenicity study in rabbits in which a NOAEL and a

LOAEL of 100 and 300 mg/kg b.w./day, respectively, was observed for skin irritation.
Overall, DEGBE is considered to be a skin and eye irritant and to have the potential of causing respiratory irritation in humans. As the guinea pig maximisation test was negative, DEGBE is not considered to have a skin sensitising potential although a few human cases have been reported.

No data on repeated dose toxicity in humans following inhalation of DEGBE have been found.
A two-week inhalation study in rats has revealed a dose-related decrease in spleen weight in males and effects indicative of local lung effects (perivascular and peribronchial accumulations of granulocytes and activation of alveolar epithelium) at exposure levels from 100 mg/m^3; increased (but not dose-related) lung weights were observed from 350 mg/m^3. Similarly, another 2-week inhalation study performed according to OECD Guideline 412 (but only with female rats and only one exposure concentration: 350 mg/m^3) also revealed effects indicative of local lung effects whereas no effects were noted on organ weights. These types of effects were, however, not reported in a 5-week or in a 13-week inhalation study in rats. In the 5-week study, only effects on the liver were reported (dose-related decreased relative liver weight in males from 40 mg/m^3, dose-related increase in relative liver weight in females from 40 mg/m^3 (significant only at 122 mg/m^3), and slight paleness of the liver (at 122 mg/m^3) and slight hepatocyte vacuolisation at all dose levels); based on this study, a NOAEL of 13 mg/m^3 for liver effects can be considered. However, in the 13-week study (OECD Guideline 413), the only observed effect was a dose-related decreased level of serum aspartate aminotransferase in males of all exposure groups (13, 40, or 95 mg/m^3), an effect, which is not considered as being toxicologically relevant because, according to IUCLID (2000), no effects on the liver were observed and because the change was within the range of biological variation.
Overall, a NOAEL of 95 mg/m^3 is established for the various effects, both systemic as well as local effects in the lungs, observed in rats following exposure by inhalation to DEGBE.

No data on repeated dose toxicity in humans following oral intake of DEGBE have been found.
Regarding repeated dose toxicity following oral administration in experimental animals, two gavage studies in rats are available. However, the results of these studies have not been cited similarly in the different sources used in this evaluation and, as the results of the studies have not been published, it is impossible to evaluate the results. But, based on the results as they have been cited in the sources used in this evaluation (predominantly IUCLID 2000 but also DECOS 1996), DEGBE appears to induce changes in haematological parameters indicative of a haemolytic effect following oral gavage to rats at dose levels from 1782 mg/kg b.w./day for 6 weeks (only male rats were included in this study) or at dose levels (in females) from about 50 mg/kg b.w./day for 13 weeks. Haematological changes (increase in total red cells and haemoglobin content at the mid-dose level) were, according to DECOS (1996), also observed in male rats in the 13-week study; however, these changes are opposite to those seen in female rats and cannot be interpreted based on the data available. Furthermore, increased spleen weights (absolute and relative) were observed at dose levels from 1782 mg/kg b.w./day in the 6 week-study whereas the relative spleen weight was, according to DECOS, decreased in male animals at 327 mg/kg b.w./day in

the 13-week study. Effects on the liver have also been reported consisting of decreased liver weight at dose levels from 1782 mg/kg b.w./day in the 6 week-study, but increased liver weights (absolute and relative) in male animals at 327 mg/kg b.w./day in the 13-week study. The contradictory results reported in the 6-week and 13-week oral gavage studies cannot be explained based on the data available. Hyperkeratosis in the stomach was observed in all dosed rats (from 891 mg/kg b.w./day) in the 6-week study; this effect was not reported in the 13-week study.
Overall, a NOAEL for effects of DEGBE following oral administration cannot be considered based on the citations of the two oral gavage studies in the sources used in this evaluation. According to Gingell et al. (1996), NOAEL of about 300 mg/kg b.w./day can be justified based upon these two studies.

No data on systemic toxicity in humans following repeated dermal application of DEGBE have been found.
The systemic toxicity of DEGBE following dermal application has been studied in rats and rabbits. A 13-week study in rats revealed no adverse systemic effects at dose levels up to 2000 mg/kg b.w./day. Similarly, no systemic effects, including neurotoxicity, were noted in another 13-week study in rats at dose levels up to 2000 mg/kg b.w./day. In rabbits, haematological changes (decreased eosinophil and monocyte count in males; decreased haemoglobin, erythrocyte, leukocyte and neutrophil count in females) were observed in a 4-week dermal study at a dose level of 30 mg/kg b.w./day (the only dose level in the study); however, these effects were, according to IUCLID (2000), not considered treatment-related because of a wide variation within one group and/or between the groups before and after exposure.
Overall, a NOAEL for systemic effects, including neurotoxicity, of 2000 mg/kg b.w./day following dermal application of DEGBE is considered based on the two 13-week dermal studies in rats because of the limitations in the dermal rabbit study.

No data on toxicity to reproduction in humans following exposure to DEGBE have been found.
No indications of reproductive and developmental effects were observed in rats in two one-generation studies at dose levels of up to 1000 mg/kg b.w./day (oral study) and 2000 mg/kg b.w./day (dermal study); in the oral study, post-natal effects (decreased weight of pups at day 14 of lactation) were observed in offspring from females administered 1000 mg/kg b.w./day and mated with untreated males whereas no post-natal effects were noted in the dermal study.
Overall, a NOAEL of 1000 mg/kg b.w./day can be considered for reproductive and developmental effects, and of 500 mg/kg b.w./day for post-natal effects following oral administration of DEGBE. Following dermal application, a NOAEL of 2000 mg/kg b.w./day can be considered for reproductive, developmental and post-natal effects.

No data on developmental toxicity, including teratogenicity, in humans following exposure to DEGBE have been found.
Developmental toxicity studies have been performed in rats (oral), mice (oral), and rabbits (dermal, OECD-guideline 414). No developmental effects, including teratogenicity, were observed in rats (dietary dose levels of up to 633 mg/kg b.w./day, day 0 to 20 of gestation) or in rabbits (dermal application of dose levels of up to 1000 mg/kg b.w./day, day 7 to 18 of

gestation). In mice, no developmental effects were observed at a dose level of 2050 mg/kg b.w./day (gavage, day 7 to 14 of gestation); according to IUCLID, examinations of pups for malformations were not performed. Maternal effects were observed in rats (reduced body weight gain from 25 mg/kg b.w./day), in mice (mortality (25%) at 2050 mg/kg b.w./day), and in rabbits (reduced body weight gain and skin irritation from 300 mg/kg b.w./day).
Overall, NOAELs for developmental toxicity, including teratogenicity of 633, 2050, and 1000 mg/kg b.w./day can be considered for the rat (oral), mouse (oral, developmental only), and rabbit (dermal), respectively. For maternal effects, NOAELs of 500 and 100 mg/kg b.w./day can be considered for mice and rabbits, respectively; in rats, the NOAEL for maternal effects is below 25 mg/kg b.w./day.

The data on mutagenicity and genotoxicity indicate that DEGBE is not a mutagenic or genotoxic substance neither *in vitro* nor *in vivo*; no data on mutagenic and genotoxic effects in humans have been found.
No data on carcinogenic effects in humans or experimental animals have been found.

102.1.1 Conclusion

The critical effects following exposure to DEGBE are the irritative effects on the skin and eyes and the haemolytic effect observed in studies in experimental animals.

Only a slight skin irritation has been observed in conventional tests for skin irritation; however, skin irritation, which was concentration dependent in incidence, severity, and time of onset was observed in a repeated dermal toxicity study in rats as well as in a teratogenicity study in rabbits. Based upon these two studies, the NOAEL for skin irritation following repeated dermal application of DEGBE is considered to be between 100 and 200 mg/kg b.w./day. DEGBE is a moderate eye irritant in rabbits and is classified for eye irritative properties according to the EU classification criteria.
A few case reports in humans support the skin and eye irritative potential of DEGBE and also suggest that DEGBE has the potential of causing respiratory irritation in humans.

DEGBE appears to induce changes in haematological parameters indicative of a haemolytic effect following oral gavage to rats at dose levels from 1782 mg/kg b.w./day for 6 weeks (only male rats were included in this study) or at dose levels (in females, but not males) from about 50 mg/kg b.w./day for 13 weeks. No changes in haematological parameters have been reported in repeated dose toxicity studies on inhalation and dermal exposure or in the reproductive and developmental toxicity studies using oral or dermal administration routes. As mentioned above, the validity of the results of the two oral gavage studies cannot be evaluated as the study reports are not public available and the citations in the sources used in this evaluation are not consistent.
The mechanism(s) behind the haemolytic effect is not fully understood, but the metabolite 2-butoxyacetic acid (BAA) has shown a significantly greater haemolytic effect *in vitro* than EGBE whereas DEGBE has shown a considerably less haemolytic activity than EGBE. (ECETOC 1995).

BAA is a possible metabolite of DEGBE and trace levels were present in the urine of rats following dermal application of DEGBE (200 or 2000 mg/kg b.w. under occlusion for 24 hours), but not following oral administration of the acetate (DEGBEA) of DEGBE (a single dose of 200 or 2000 mg/kg b.w.) Overall, DEGBE is considered to have the potential of inducing haemolysis in humans, but probably only at high dose levels and following repeated exposure.

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Appendix 14: Dipropylene glycol monomethyl ether (DPGME)

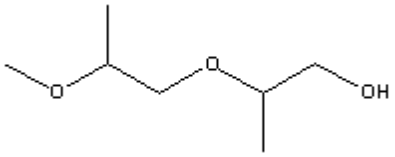
104 General description

Dipropylene glycol monomethyl ether (DPGME) is a solvent produced either by reaction of propylene oxide with methanol or by alkylation of dipropylene glycol with dialkylsulphate in the presence of a catalyst. The result is a mixture of four isomers with the general CAS no: 34590-94-8, in which secondary alcohol isomers are predominant (BUA 1995).

104.1 Identity

Molecular formula: $C_7H_{16}O_3$

Structural formula:



Molecular weight: 148.20

CAS-no.: 34590-94-8

Synonyms: DPGME
Dipropylene glycol monomethyl ether
Methoxymethylethoxypropanol
Dowanol 50B
Methyl ether oxybispropanol
Dowanol DPM
Bis-(2-methoxypropyl) ether
1(or 2)-(2-Methoxymethylethoxy)-propanol
Arcosolve DPM
PPG-2 methyl ether
Solvenon DPM

104.2 Physical / chemical properties

Description: Colourless liquid with ether odour.

Melting point: -80 °C, -83 °C

Boiling point: 189.6 °C
187 – 192 °C

Density: 0.94 g/ml at 20 °C

Vapour pressure: 0.278 mm Hg (0.37 hPa) at 20 °C

Concentration of

saturated vapours:	366 ppm at 20 °C and 1 atm (calculated) 300 ppm at 20°C and 1 atm (max. obtainable conc.)
Conversion factor:	1 ppm = 6.16 mg/m^3 (at 20°C and 760 mmHg) 1 mg/m^3 = 0.162 ppm
Solubility:	Completely miscible with water as well as with many organic solvents, e.g. acetone, benzene, ethanol, ether.
Odour threshold:	35 ppm (215.6 mg/m^3)
References:	A&H (1990), ACGIH (1991), Breslin et al. (1996), BUA (1995), Chemfinder (2002), Merck (1996), HSDB (2001), IUCLID (2000), Rowe et al. (1954).

105 Toxicokinetics

Generally, glycol ethers and their acetates are readily absorbed and distributed throughout the body following inhalation or oral administration and no substantial accumulation of the parent compound has been observed (ECETOC 1995).

No *in vivo* dermal absorption data were found in the literature for DPGME, but toxic effects observed in rabbits and rats after dermal application of the substance indirectly indicate skin uptake (Rowe et al. 1954).

Propylene glycol ethers follow the following major metabolic pathways: Beta isomers, which are primary alcohols, are oxidised to carboxylic acids. Alpha isomers, being secondary alcohols, are oxidised to carbon dioxide after cleavage of the ether bond and O-demethylation. Both alpha and beta isomers are in addition conjugated with sulphate and glucuronic acid. (A&H 1990).

Commercial information from manufacturers states compositions of DPGME to be:
50-55% 1-(2-methoxy-1-propoxy)-2-propanol (CAS no. 13429-07-7), 40-45%1-(2-methoxy-1-methylethoxy)-2-propanol (CAS no. 20324-32-7), 3-5% 2-(2-methoxy-1-methylethoxy)-1-propanol (CAS no. 55956-21-3) and 2-5% 2-(2-methoxy-propoxy)-1-propanol (CAS no. 13588-28-8) (BUA 1995). Miller states that commercial grade DPGME in the US contains at least 95% of the two first-mentioned isomers (Miller 1987).

Analysis of a DOW DPGME-product showed predominance of CAS no. 13429-07-7 (approximately 85%), and only up to 1.6% of each of the primary alcohols (A&H 1990).

Primary alcohol isomers of DPGME, are theoretical substrates for alcohol dehydrogenase with the formation of methoxypropionic acid (ECETOC 1995). However, excretion of methoxypropionic acid in rat urine was not observed in metabolic studies with DPGME (Breslin et al. 1996).

The distribution, metabolism and excretion of DPGME were investigated in three rats dosed orally with 8.7 mmol (1289 mg/kg) radio-labelled material. 60 % of the radioactivity was eliminated in urine, the vast majority (54.3 %) being recovered during the first 24 h after dosing. 27% was recovered in the expired air as $^{14}CO_2$. 2.7% was excreted in faeces, and less than 3% remained in the carcass after 48 h. Levels of ^{14}C in the liver (0.5%) and the skin (1.3%) were higher than in other organs and tissues (kidney, blood, brain and fat). There were no indications of accumulation of radioactivity in adipose tissue, testes or main body tissues. The urine metabolites were identified to be propylene glycol methyl ether and dipropylene glycol (jointly making up 28.8% of the dose) and propylene glycol (9.6%). Sulphate and glucuronide conjugates accounted for respectively 9 and

9.6% of the given dose. 3% of the dose was found in urine as the unchanged parent compound. The authors concluded that microsomal O-demethylation is a significant route of bio-transformation of DPGME. (Miller et al. 1985).

105.1 Mode of action

Propylene glycol ethers and their acetates only show developmental toxicity if the ether bond is present on the secondary carbon atom (e.g. 1PG2ME, 1PG2EE); this allows the primary alcohol to be oxidised to an alkoxypropionic acid. Propylene glycol ethers with the ether bond on the primary carbon atom (e.g., 2PG1ME, 2PG1EE) are secondary alcohols and are not metabolised to alkoxypropionic acids; these ethers and their acetates have not shown developmental toxicity. (ECETOC 1995).

DPGME contains four isomers, a small percentage of which are primary alcohols which could be metabolised to the toxic metabolite methoxypropionic acid (ECETOC 1995). However, metabolic studies carried out with DPGME did not identify the formation of methoxypropionic acid in rat urine (Breslin et al. 1996).

106 Human toxicity

106.1 Single dose toxicity

3.1.1 Inhalation

Exposure by inhalation to 74 ppm (456 mg/m^3) has been reported to be irritating to man (Ruth 1986 – quoted from A&H 1990).

3.1.2 Eye irritation

Application of 0.04 ml of a 20% aqueous solution of DPGME (approx. 7.5 mg) to one eye of ten male volunteers caused a minor stinging sensation accompanied by lachrymation and blepharospasm for about 1 min. Mild injection of the conjunctival vessels persisted for 30-40 min. A temporary increase of up to 33 % in intraocular tension (IOT) was observed. Normal IOT was recorded 1 hour after treatment. (Ballantyne 1983-84).

106.2 Repeated dose toxicity

106.2.1 Inhalation and dermal contact

DPGME has been suggested as one possible cause of bone marrow injury observed in seven lithographers working with multicolour offset and ultraviolet curing printing processes. Personal and area samples revealed air levels of 0.6 - 6.4 ppm (3.7 - 39.4 mg/m^3) DPGME. Gloves were used only intermittently and frequent and prolonged skin contact with wash solutions occurred; no respiratory protective gear was used. Exposure to other solvents, e.g. ethylene glycol monoethyl ether, could not be excluded as the cause of bone marrow injury. (Cullen et al. 1983).

106.2.2 Oral intake

No data were found.

106.2.3 Skin irritation and sensitisation

Undiluted material was applied under patches to the backs of 100 men and 100 women for five days. Three weeks later, the material was applied to the subjects again, for a period of 48 h. In an additional test with repeated insult technique, 25 men and 25 women were treated 10 times, 4-8 h/day, every other day. Challenge was performed three weeks later for 24-48 h. No irritation or sensitisation occurred. (Rowe et al. 1954).

106.3 Toxicity to reproduction

No data were found.

106.4 Mutagenic and genotoxic effects

No data were found.

106.5 Carcinogenic effects

No data were found.

107 Animal toxicity

107.1 Single dose toxicity

107.1.1 Inhalation

Rats exposed to an atmosphere of aerosols and vapour of 500 ppm (3080 mg/m^3) for 7 hours showed mild narcosis (Rowe et al. 1954).

107.1.2 Oral intake

An LD_{50} of 5221 mg/kg (5.54 ml/kg) in rats was established in an experiment including 9 dosage levels and a total of 169 animals (Rowe et al. 1954). Another study reported an LD_{50}-value of 5.13 g/kg in rats (Smyth et al. 1962).

In dogs, the LD_{50}-value was calculated to 7500 mg/kg. Respiratory paralysis was seen. Mortality occurred within 48 h. (Shideman & Procita 1951 - quoted from ECETOC 1995).

107.1.3 Dermal contact

Rabbits were exposed to DPGME under occluded patch for 24 hours to 10, 15 or 20 ml/kg (9.4, 14.1 or 18.8 g/kg) of the substance. No deaths occurred, but slight body weight loss at all dose levels and transient narcosis was observed in the high dose group. (Rowe et al. 1954).

An LD_{50}-value of 10 ml/kg b.w. (9.4 g/kg) was calculated from the result of exposure to DPGME of 4 male albino New Zealand white rabbits under occluded patch for 24 hours (Smyth et al. 1962).

4.1.5 Skin irritation

Data from different studies reported in IUCLID indicate that the substance is not irritating to the skin of rabbits following short time open application of 95 or 500 mg/kg. No further details are provided. (IUCLID 2000).

4.1.6 Eye irritation

One drop (approx 47 mg) undiluted DPGME applied to the eyes of rabbits 5 times with an observation period of 2 weeks produced a mild transitory irritation of the conjunctiva with no cumulative effect nor corneal injury (Rowe et al. 1954).

The substance was reported to score a grade 2 for corneal injury on a 10-grade-scale following an application of 0.5 ml (470 mg) undiluted DPGME to the eyes of rabbits (Smyth et al. 1962).

Application of 0.1 ml (94 mg) DPGME to the eye of 6 female rabbits caused moderately severe conjunctivitis and conjunctoblepharitis. The effect peaked

after about 6 hr and disappeared within a week. Minor keratitis occurred between the first and third day. A 40% solution (37.6 mg) instilled in the eye of 6 additional rabbits produced a mild conjunctival irritation, and a 20% solution (18.8 mg) was without effects in the third group of 6 rabbits. Intraocular tension measured in 60 rabbits and corneal thickness measured in 54 animals increased temporarily in a dose-related way, returning to normal within 3 days. (Ballantyne 1983-84).

107.1.4 Skin sensitisation

No data were found.

107.2 Repeated dose toxicity

107.2.1 Inhalation

In an unpublished range-finding study, 5 male and 5 female Fischer 344 rats and an unreported number of B6C3F1 mice of both sexes were exposed to 0, 50, 140 or 330 ppm (0, 308, 862 or 2033 mg/m^3) DPGME 6 hours/day, 5 days/week for 2 weeks. No treatment-related effects were reported with respect to post-exposure clinical observations, body weights and urinalysis in either species. Absolute liver weights in male rats in the high dose-group were significantly higher than in the concurrent controls. Relative liver weights were significantly increased in male rats at all exposure levels compared to control means (7, 7 and 16% in the low-, mid- and high-dose groups, respectively). Relative liver weights were also significantly increased in female mice. However, the authors evaluated the findings not to be significant adverse effects since no gross or histopathological changes were found in the liver. (Landry et al. 1981 - quoted from Landry et al. 1984).

Groups of 10 male and 10 female Fischer 344 rats were exposed 6 hours/day, 5 days/week for 13 weeks to concentrations of 0, 15, 50 or 200 ppm (92.4, 308 or 1232 mg/m^3) DPGME. There were no clinical effects related to treatment with DPGME. Rat livers exposed to DPGME at gross observation appeared to be enlarged. However, the group liver weight means did not support this observation. Histopathological examination showed only changes considered by the authors to be minimal and spontaneous and not treatment related. Details on histopathology was not reported in the article. Clinical chemistry, haematology and urinalysis parameters were not affected by treatment with DPGME. (Landry et al. 1984).

The above-mentioned study included investigation in groups of 7 male and 7 female New Zealand White rabbits dosed following the same protocol as the rats. Body weights of female rabbits in the mid-dose group (308 mg/m^3) were higher than for the controls, but the authors did not consider this finding to be exposure-related as the high-dose group was not affected. There were several statistical differences in mean organ weights of rabbits in the low- and mid- doses, but not at the high-dose. The absolute mean kidney weights of female rabbits in the mid- and high-dose group were increased. Female rabbits in the high-dose group (1232 mg/m^3) showed 14% increased relative kidney weight compared to controls. However, the findings in the kidney weights were within the range of the historic controls, and no other kidney related parameters were affected. The authors considered the histopathological changes to be minimal spontaneous changes. No statistical

differences to controls were reported from clinical chemistry or haematology. Urinalysis was not performed in the rabbits (Landry et al. 1984).

Rats, guinea pigs, rabbits and monkeys were exposed to nominal concentrations of 0 or 400 ppm (2464 mg/m^3) DPGME 7 hours/day, 5 days/week for six to eight months. However, the measured vapour concentration was around 300 ppm (1848 mg/m^3), as some of the substance was present as aerosols.
20 males and 20 female white rats exposed to around 1848 mg/m^3 showed transient narcosis during the first weeks of exposure. No effects were seen on growth, mortality and final body weights when compared to controls. The relative liver weight was significantly increased. No report of haematological or microscopic examination of the tissues is given.
Eight male and 8 female guinea pigs exposed to around 1848 mg/m^3 DPGME did not show evidence of adverse effects on gross appearance and behaviour, growth, mortality, body weights. The relative liver weights were non-significantly higher in both sexes compared to controls. Microscopic examination of the tissues revealed very slight granulation in the liver cell cytoplasm, mainly in the central area, and significantly numerous non-fatty vacuoles throughout the liver lobules.
Two male and two female rabbits and one male and one female monkey treated with DPGME at around 1848 mg/ m^3 only displayed effects on the liver, namely granulation and vacuolation similar as described for the guinea pigs. (Rowe et al. 1954).

107.2.2 Oral intake

No data were found.

107.2.3 Dermal contact

The subacute percutaneous toxicity of DPGME was evaluated in groups of 8 male Porton-Wistar rats dosed 5 days/week for 4 weeks at 0, 100 and 1000 mg/kg b.w. under occluded as well as unoccluded conditions. DPGME had no effect on body weight gain nor on food intake. With respect to blood parameters, only a slightly, non-significantly elevated red blood cell count (RBC) and packed cell volume (PCV) was seen in the treated groups; however, these changes were not so apparent in the animals treated under occluded conditions. No significant changes were recorded in clinical chemistry or with respect to pathology and histopathology of several organs including the liver, bone marrow and testes. (Fairhurst et al. 1989).

Groups of 5-6 male rabbits were treated with 0, 1, 3, 5 or 10 ml/kg b.w. (corresponding to 0, 0.94, 2.8, 4.7, 9.4 g/kg b.w.) DPGME under occluded patch 5 times/week over a period of 90 days. The experiment was repeated with 0, 3 and 5 ml/kg (≈ 0, 2.8 and 4.7 g/kg). The mortalities recorded are listed in Table 1.

Table 1: Mortality of rabbits in 3 months dermal application

Dose g/kg	Experiment 1 no. deaths / no. treated	Experiment 2 no. deaths / no. treated
0.00	0/5	1/5
0.94	0/5	-
2.82	1/6	1/5
4.70	2/6	2/5
9.40	6/7	-

In the high dose group, 6 animals died following weight loss and narcosis. Gross examination revealed gastric retention and occasional small hemorrhagic areas in the gastric mucosa of the dead animals of this group. Occasional deaths seen at 0.94, 2.82 and 4.70 g/kg b.w. were associated with respiratory infections. There were no abnormal findings in the histological examination except the lungs, which showed pneumonia and/or empyema. Blood urea nitrogen concentrations, determined in experiment 2, were within normal limits. (Rowe et al. 1954).

107.3 Toxicity to reproduction

No specific studies on fertility were found. However, results from repeated dose studies do not indicate effects on the testes. Thus, rat testes have been reported not to be affected pathologically nor histologically following dermal exposure to 100 or 1000 mg/kg b.w., 5 days/week for 28 days (Fairhurst et al. 1962).

No changes in rat or rabbit testes weight or testicular histology were reported following exposure by inhalation to 0, 15, 50 or 200 ppm (0, 92.4, 308 or 1232 mg/m^3) DPGME 6 hours/day, 5 days/week for 15 weeks (Landry et al. 1984).

107.3.1 Inhalation

Groups of 32-37 mated female Fischer 344-rats were exposed by inhalation to nominal concentrations of 0, 50, 150 or 300 ppm DPGME (0, 51, 154 or 285 ppm measured concentrations corresponding to 0, 314.2, 948.6 or 1756 mg/m^3) from gestation-day 6 through 15, 6 hours/day. Clinical signs and changes in general appearance were recorded. Body weights were recorded at day 0, 6, 16 and 21. Following caesarian section, the dams were sacrificed at day 21. Liver weights were recorded. Statistically significant lower body weights at days 0, 6 and 16 in the mid-exposure group were considered insignificant as the finding was not seen at the high exposure level and was present prior to treatment. The authors evaluated that no treatment-related effects were observed in clinical signs, on feed or water consumption, on body weight or body weight gain. The following data were recorded at study termination: gross pathologic alterations in the dams, gravid uterus weight, number of foetuses *in utero*, number of live and dead foetuses, number of resorption sites and corporea lutea, sex and body weight of each foetus. All foetuses were also examined for soft tissue and skeletal alterations. 13 pups (4 in the control group, 2 in the low-, 4 in the mid- and 3 in the high-dose group) had malformations. The malformation types were not specified by the author. However, the numbers being low and the numbers in each of the

treated groups not exceeding the controls, the authors concluded that the malformations were not due to the treatment with DPGME. (Breslin et al. 1996).

Following a similar protocol as described above for rats, groups of 16 inseminated female NZW-rabbits were exposed from day 7 through 19 of gestation, i.e. to concentrations of 0, 314.2, 949.6 or 1756 mg/m^3. No treatment-related clinical signs, changes in general appearance or behaviour were observed at any exposure level. Body weights were recorded at day 0, 7, 20 and 28. One rabbit from the low-dose group was found dead on gestation day 27. A second rabbit form the low-dose group and one rabbit from the high-dose group delivered their litters on gestation day 27. Death and early deliveries were investigated and attributed to pregnancy toxaemia and not to the treatment with DPGME. The surviving dams were sacrificed on day 28 after caesarian section. No treatment-related effects on feed or water consumption or on body weights were observed. There was a large variation in body weight gains within and across groups. However, this was considered insignificant by the authors. Liver weights were not affected by treatment. No treatment related effects on the reproduction in the dams were reported. With respect to foetal parameters, 9 foetuses (3 controls, 2 in the low-, 2 in the mid- and 2 in the high dose group) had malformations. No details on the malformation types are given. As there was no dose-response relationship and the number in each treated groups was lower than in the controls, the authors evaluated that the occurrence of malformations was not treatment related. The same evaluation was made for a statistically increased number of lumbar spurs (regarded by the authors as variations) in the low dose group (314.2 mg/m^3). The authors concluded that there were no treatment-related effects on the reproductive or foetal parameters or on the incidence of malformations or variations. (Breslin et al. 1996).

107.3.2 Other routes

No studies were found.

107.4 Mutagenic and genotoxic effects

107.4.1 *In vitro* studies

A bacterial gene mutation assay (Ames test) conducted according to GLP in Salmonella Typhimurium strains TA1538, TA 1537, TA 1535, TA100 and TA 98 with and without metabolic activation at concentrations of 2, 10, 50, 250, 1250, and 6250 µg/plate was negative (Kirkland et al. 1983 – quoted from IUCLID 2000 and ECETOC 1995).

A cytogenetic assay conducted according to GLP using metaphase analysis of Chinese hamster ovary (CHO) cells treated with DPGME was performed with and without metabolic activation at 1.25, 2.5, 5.0 and 10 mg/ml. The test showed no differences between treated and untreated cells with or without metabolic activation. DPGME is thus not a clastogen for CHO cells. (Kirkland 1983 – quoted from IUCLID 2000 and ECETOC 1995).

DPGME did not produce unscheduled DNA synthesis (OECD guideline 482) in a test conducted in rat hepatocytes at concentrations of 0.01, 0.00316, 0.001, 0.000316, 0.0001 and 0.0000316 M with and without

metabolic activation (Mendrala et al. 1983 – quoted from IUCLID 2000 and ECETOC 1995).

107.4.2 *In vivo* studies

No data were found.

107.5 Carcinogenic effects

No data were found

108 Regulations

108.1 Ambient air

Denmark (C-value): 1 mg/m^3 (MST 2002)

108.2 Drinking water

Denmark: -

108.3 Soil

Denmark: -

108.4 Occupational Exposure Limits

Denmark:	TWA 50 ppm (300 mg/m^3) - skin notation (At 2002)
ACGIH:	TWA 200 ppm (1232 mg/m^3) - skin notation (ACGIH 1991)
Germany:	50 ppm (310 mg/m^3) (MAK 1998)

108.5 EU-Classification

The substance is not listed on the list of dangerous substances (MM 2002).

108.6 IARC -

108.7 US-EPA -

109 Summary

109.1 Description

Dipropylene glycol monomethyl ether (DPGME) is a colourless liquid with ether odour. It is an organic solvent with a relatively low vapour pressure of 0.37 hPa. It is completely miscible with water and a large number of organic solvents.

109.2 Toxicokinetics

DPGME is readily absorbed through all routes of exposure. The substance is a mixture of 4 isomers, two primary and two secondary alcohols. The secondary alcohols are metabolised via the P-cytochrome oxidase of the liver by O-demethylation and excreted as propylene glycol or dipropylene glycol in the urine or further oxidised to carbon dioxide. Sulphate and glucuronide conjugation also occurs. For the primary alcohols metabolism includes dehydrogenation and formation of the teratogenic metabolite methoxypropionic acid (MPA). However, only very small amounts of these isomers are present in commercial DPGME. MPA was not detected in rat urine.

109.3 Human toxicity

109.3.1 Single dose toxicity

Reversible eye irritation has been reported following application of 0.04 ml of a 20% solution of DPGME (7.5 mg). Irritation by inhalation has been reported following exposure to 456 mg/m^3.

109.3.2 Repeated dose toxicity

Neither irritation nor sensitisation occurred following repeated occluded dermal exposure to DPGME.
Bone marrow depression was reported in one study following occupational exposure to DPGME in mixed exposure with a number of other solvents.

109.3.3 Toxicity to reproduction

No data were found.

109.3.4 Mutagenic and genotoxic effects

No data were found.

109.3.5 Carcinogenic effects

No data were found.

109.4 Animal toxicity

109.4.1 Single dose toxicity

Inhalation exposure of rats to a vapour and aerosol concentration of 3080 mg/m^3 DPGME for 7 hours caused narcosis. Reported oral LD_{50}-values range from 5000 to 7500 mg/kg b.w. in rodents and dogs and dermal LD_{50}-values in rabbits range from 9.4 to over 19 g/kg b.w.

The substance is reported not to be a skin irritant following open application. Eye irritation studies in rabbits indicate that the substance is a reversible eye irritant.

109.4.2 Repeated dose toxicity

Nine days inhalation exposure of rats and mice to up to 2033 mg/m^3 DPGME resulted in increased relative liver weights in male rats and female mice, but no pathological changes were found.
Rats exposed for 90 days to up to 1232 mg/m^3 DPGME were not affected by treatment.
Rabbits exposed for 90 days to up to 1232 mg/m^3 of the substance showed increase in relative kidney weight within the range for historic controls and without pathological findings in the organ.
Transient CNS depression was reported and the liver was affected (slight granulation of the cytoplasm in the central area and vacuolisation) in rabbits, guinea pigs and monkeys following inhalation exposure to the maximal attainable vapour concentration of 1848 mg/m^3 for 6 to 8 months.

In a 28-day dermal rat study under both occluded and open application of up to 1000 mg/kg b.w./day, only non-significant effects on haematological parameters were seen. No pathological or histopathological changes were recorded in the liver or on the bone marrow.
In a 90-day dermal rabbit study with application under occluded patch of doses up to 9.4 g/kg b.w./day, the majority of the animals of the high dose group died following symptoms of narcosis and body weight loss. Haemorrhagic areas were observed in the gastric mucosa. There were no other treatment related findings in clinical chemistry or histopathology.

109.4.3 Toxicity to reproduction

No studies on fertility were found. However, a 90-day study by dermal contact in rats and rabbits with up to 1000 mg/kg b.w. and a 2-week inhalation study in rats and mice with up to 200 ppm (1232mg/m^3) showed no effects on testes.

No treatment related effects on development have been reported following inhalation exposure to up to 1756 mg/m^3 of rats from day 6-15, and rabbits day 7-19 of gestation. The low number of malformations observed did not exceed the number observed in the controls.

109.4.4 Mutagenic and genotoxic effects

The substance was negative with and without metabolic activation in an Ames test, a CHO-cell metaphase analysis test and a UDS test in rat hepatocytes. No *in vivo* studies were found.

109.4.5 Carcinogenic effects

No studies were found.

110 Evaluation

The toxicological database on dipropylene glycol monomethyl ether (DPGME) is limited. However, the substance is evaluated from the available data to be of low toxicity, the critical effect being eye and mucous membrane irritation.

Commercial DPGME is a mixture of isomers which only contains small amounts of primary alcohols, which are potentially capable of forming the teratogenic metabolite methoxypropionic acid, MPA. The secondary alcohol isomers are metabolised via dealkylation to carbon dioxide. Metabolism studies on DPGME have not identified MPA in rat urine. The potential of commercial DPGME for causing adverse effect on development is therefore considered to be negligible.

DPGME is of very low acute toxicity to rodents and dogs by inhalation (>3080 mg/m^3), oral (5130-7500 mg/kg b.w.), and dermal (9-19 mg/kg b.w.) exposure. Non-specific CNS depression was seen at sub-lethal doses in rodents.

The substance is a moderate irritant to mucous membranes in humans and in animals. Reversible conjunctivitis and keratitis as well as increase in the intraoccular tension have been reported in humans and in rabbits. DPGME is not a skin irritant in animals or humans. No signs of sensitisation of humans have been reported.

Bone marrow depression has been reported from human exposure to DPGME in a dermal and inhalation exposure with concurrent exposure to other solvents. The finding was not confirmed in a subchronic dermal study in rabbits with exposure levels up to 9400 mg/kg b.w. DPGME. On this background, DPGME is evaluated not to have effect on bone marrow. Subchronic inhalation exposure of rats to the maximum obtainable vapour concentration of DPGME of 1848 mg/m^3 resulted in transient CNS depression. Slight central area cytoplasm granulation and non-fatty vacuolation of the liver was reported in rabbits, guinea pigs and monkeys treated at 1848 mg/m^3 for 6-8 months. No liver effects were seen in other subchronic inhalation studies in rats and rabbits with exposure levels of up to 1232 mg/m^3 DPGME.

No specific studies on fertility effects were found; however, subchronic studies in rodents reported no effect on testes by inhalation to 1232 mg/m^3 or dermal exposure to 1000 mg/kg b.w. Teratogenicity studies in rats and rabbits showed no treatment related developmental toxicity at up to 1756 mg/m^3.

DPGME was negative in *in vitro* genotoxicity tests, but has not been tested *in vivo* for mutagenicity. The subchronic studies indicate no specific concern for a carcinogenic potential of the substance. However, this effect cannot be evaluated conclusively, as no studies were found on carcinogenicity.

In conclusion, the dipropylene glycol monomethyl ether is found to have a low toxicity, the critical effect being irritation of the eye and the mucous membranes.

111 References

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Appendix 15: Polyethylene glycol dodecylether (polyEGDE)

112 General description

112.1 Identity

Polyethylenglycoldodecylether (polyEGDE) is a linear alkyl polyethoxylate. It consists of a dodecyl alcohol attached by an ether linkage to a polyethylene glycol moiety. The number of ethylene oxide molecules can vary, usually being 4, 7, 9 or 23.

Molecular formula: $(C_2H_4O)_nC_{12}H_{26}O$

Structural formula:

Molecular weight: 600 (n = 9)
362 (n = 4)

CAS-no.: 9002-92-0

Synonyms: Polyethylene, dodecyl ether
Ethoxylated dodecyl alcohol
Dodecyl alcohol polyoxyethylene ether
Lauryl alcohol ethylene oxide
Polyoxyethylene lauryl ether
Dodecanol ethylene oxide
Polyethoxylated dodecanol

112.2 Physico / chemical properties

Description: Colourless to yellow liquid with a pleasant odour or solid, depending on chain length.

Melting point: Not applicable

Boiling point: Not applicable

Density: Not applicable

Vapour pressure: Not applicable

Concentration of saturated vapours: Not applicable

Conversion factor: Not applicable

Solubility: Water: = 100 g/l at 20°C (n=4).

References: HSDB (2001), Lægemiddelkataloget (2002), ChemFinder (2002).

113 Toxicokinetics

No data were found.

114 Human toxicity

Application to human skin of unspecified polyEGDE for 3 days was moderately irritating (Drill and Lazar 1977- quoted from RTECS 2001).

No further references on human toxicity were found.

115 Animal toxicity

115.1 Single dose toxicity

115.1.1 Inhalation

Intratracheal instillation to each of 12 male Sprague-Dawley rats of 100 µl of a solution containing 1% polyEGDE (9 ethylene oxide molecules), 1% ethyl alcohol and saline caused irritation of the lungs one day after administration. Histopathology revealed desquamation of bronchial and alveolar epithelium with inflammatory cell infiltration, oedema and haemorrhage. At days 3 and 7 after administration, hyperplasia and squamous metaplasia of bronchial and bronchiolar epithelium and proliferation of fibroblast-like interstitial cells were observed in the alveoli, indicate regenerative process, was reported. No pathological changes were seen in the group of 12 rats treated with saline only. (Suzuki et al. 2000).

Groups of 3 male Wistar rats were dosed with 20, 50 or 100 µl 1% w/v polyEGDE (with 9 ethylene oxides) in saline in one nostril. The epithelium showed cell loss and reduction in epithelium height in the treated side in all groups at 5, 20 and 60 minutes after application, the effect being more pronounced and widespread with dose and time of contact. Thus, 20 µl of the polyEGDE solution only affected a part of the epithelium on the same side of the septum, 50 µl also affected a small area of the untreated side at 5 min. which extended at 20 min. and at 60 min., and 100 µl caused depletion of the epithelium in the whole nasal epithelium including the untreated nostril. (Chandler et al. 1991).

Twenty-five µl of 1% polyEDGE (9 ethylene oxide molecules) in saline were placed into the nostril of Sprague-Dawley rats. Controls only treated with saline were also used. Samples of the nasal epithelium from 2-4 rats were taken at 4, 24 hours, 2, 3, 4, 5, 7 and 10 days after exposure. At 4 hours after exposure, the mucosa was swollen, but no histopathological changes were apparent. Samples, taken 24 and 48 hours after administration, showed necrosis of the epithelium. Regeneration of the epithelium was observed from day 3 including basal cell re-growth and presence of immature cells in a loose and irregular cuboidal to columnar epithelium. Differentiation occurred from day 4. Regeneration, including complete columnar epithelium cells and ciliae, was observed from day 7. (Zhou & Donovan 1995).

115.1.2 Oral intake

An oral LD50-value in rats of 8600 mg/kg was reported for a 4 ethylene-oxides containing polyEGDE. For the mouse, the LD_{50} is reported to be 4940 mg/kg. (RTECS 2001).

A polyEGDE with 7 ethylene oxides was tested in rats and mice for acute oral toxicity. The LD_{50}-values calculated from the results were 4150 mg/kg b.w. in rats and 1170 mg/kg in mice (RTECS 2001).

For polyEGDE with 23 moles ethylene oxide, LD_{50}-values of 8600 mg/kg b.w. in the rat and 3500 mg/kg b.w. in the mouse were reported. (RTECS 2001).

Single oral doses polyEGDE given to dogs (above 2 g/kg b.w.) and monkeys (5 g/kg b.w.) were reported to exert emetic action and central nervous system depression (e.g. ataxia, weakness and sedation) (Gosselin et al. 1984).

115.1.3 Dermal contact

No data on systemic toxicity by skin contact were found.

115.1.4 Irritation

PolyEGDE containing 7 ethylene oxides in the molecule was reported to be irritating to the skin of rabbits following application of 100 mg/kg b.w. No further details were available in RTECS. (RTECS 2001).

Application to rabbit skin of 75 mg/kg b.w. for 24 hours of an unspecified polyEGDE caused mild irritation, while 500 mg/kg b.w. caused moderate irritation. No further details were available in RTECS. (RTECS 2001).

An interlaboratory comparative skin irritation test was conducted with 12 chemicals among which polyEGDE. Each laboratory used eight male albino rabbits for 4 chemicals. The animals were clipped and 0.5 g polyEGDE was applied under non-occlusive 1 square inch patch for 24 hours. Scoring was performed 30 minutes after patch removal and 48 hours later based on a maximum of 8 scoring points for erythema, 8 for oedema and 30 for necrosis. The results ranged from 0 to 28 with a mean of 8.0. Necrosis was reported in 38 out of 183 animals. (Weil & Scala 1971).

A Czech reference is quoted in RTECS to have reported mild irritation in rabbits from a 24-hour skin application of 500 mg polyEGDE of unspecified chain-length (Marhold 1972 – quoted from RTECS 2001).

Ten milligrams polyEGDE (chain of 7 ethylene oxides) instilled in rabbits eye were reported to be irritating. No details are provided in RTECS. (RTECS 2001).

An interlaboratory comparison eye irritation test conducted in 12 laboratories according to the method of Draize with minimum 6 male albino rabbits/laboratory. The maximum possible score was 110 points and consisted of elements: Scoring for effects on cornea, including opacity degree and area affected, contributed to the maximum score with 80 points, effects on iris with a maximum of 10 points and effects on conjuctiva, including redness, chemosis and discharge contributed with a maximum of 20 points. PolyEGDE scored average scores of 28.2 at 24 hours, 29.5 at 72 hours, and 16.0 at 7 days post instillation of 0.1 ml polyEGDE. (Weil & Scala 1971).

Quoting from a Czech reference, RTECS reports that 750 µg polyEGDE applied to rabbits eyes was severely irritating at 24 hours following instillation. (Marhold 1972 – quoted from RTECS 2001).

PolyEGDE was not eye irritating in a Draize test in rabbits. No further details were available. (Conduzorgues et al. 1989 – quoted from Toxline 2001).

Three animals were subject to a low volume eye test (LVET) with polyEGDE's of different chain lengths. Irritation was scored following the Draize scale (0-110) at 1, 24, 48, 72 and 96 hours. Scores varied from 0 to 7.0 for the polyEGDE's, with reversibility within 4 days. The eye irritating potential of the substances was evaluated to be none or low. (Heinze et al. 1999).

115.2 Repeated dose toxicity

No data were found.

115.3 Toxicity to reproduction

No data were found.

115.4 Mutagenic and genotoxic effects

115.4.1 *In vitro* studies

PolyEGDE was negative in a *Salmonella*/microsome assay using 0, 3, 10, 33, 100 and 333 µg/plate in strains TA 98, TA 100, TA 1535 and TA 1537 with and without liver S9 activation (Zeiger 1987, Zeiger et al. 1987 – the latter quoted from HSDB 2001).

PolyEGDE was reported to be negative in a reverse mutation test in *Escherichia col*i WP2uvrA with and without metabolic activation with S9 mix (Suzuki et al. 1989 – quoted from Toxline 2001).

PolyEGDE was reported to induce polyploidy in Chinese hamster lung fibroblast cells without metabolic activation. However, no structural chromosomal aberrations were seen. (Suzuki et al. 1989 – quoted from Toxline).

A sister chromatid exchange (SCE) test was conducted in Chinese Hamster Ovary-cells (CHO) with and without metabolic activation using 5 test concentrations from 0.3 up to 30 µg polyEGDE /ml. The result was negative. A chromosomal aberration test was performed with polyEGDE in CHO cells with and without metabolic activation using 3 test concentrations of 0, 5, 15 and 50 µg/ml. PolyEGDE was also negative in this test.
(Loveday et al. 1990).

An L5178Y mouse lymphoma mutation assay was conducted with 6-7 concentrations from 0.005 to 0.050 ml polyEGDE/l, with and without S9 mix. The assay was conducted in duplicate and included positive and negative controls. The assay was negative, except for one trial without activation where some cultures at 0.020-0.0030 ml/l showed an almost two-fold increase in mutant frequency. Concentrations of 0.030-0.050 ml/l were lethal. (Myhr & Caspary 1991).

115.4.2 *In vivo* studies

A sex linked recessive lethal mutation test was conducted in male *Drosophila melanogaster.* PolyEGDE did not induce mutations in meiotic or post-meitotic germ cells by feeding of 12500 mg/kg food or by injection of 2000 mg/kg food. (Foureman et al. 1994).

Mice were exposed to 3 daily intraperitoneal doses of 0, 31.25, 62.5 or 125 mg/kg b.w. polyEGDE. Bone marrow samples were obtained 24 hours following the final exposure. There was no significant increase in number of micronuclei. (Shelby et al. 1993).

115.5 Carcinogenic effects

The National Toxicology Program (NTP) informs that a carcinogenicity study conducted with polyEGDE in rats and in mice, referred in several references to be negative, was inadequate and that no report would be issued (NTP 2002).

116 Regulations

116.1 Ambient air

-

116.2 Drinking water

-

116.3 Soil

-

116.4 Occupational Exposure Limits

-

116.5 EU-Classification

-

116.6 IARC

-

116.7 US-EPA

-

117 Summary

117.1 Description

Polyethylenglycoldodecylether (polyEGDE) is an alkyl ethoxylate of varying ethylene oxide chain length. It is a colourless to yellow water soluble liquid (100g/l) with a pleasant odour.

117.2 Toxicokinetics

No data were found.

117.3 Human toxicity

The only human toxicity data available report polyEGDE to by moderately skin irritating.

117.4 Animal toxicity

117.4.1 Single dose toxicity

LD50-values of 4150 and 8600 mg/kg b.w. in rats and of 3500, 4940 and 1170 mg/kg b.w. in mice are reported for different chain lengths. In dogs and monkeys, emesis and central nervous system depression was seen following single oral doses of 2000 and 5000 mg/kg. b.w., respectively.

Four different skin irritation studies were performed in rabbits, showing that application of 75-500 mg/kg b.w. polyEGDE caused mild to moderate irritation.

Results of eye irritation tests are contradicting: polyEGDE is reported not to be irritating in a low volume eye test[4] and in a Draize test (no details available), moderately eye irritant in another Draize test with 0.1 g polyEGDE and severely irritating with 0.750 mg in a RTECS quotation of a Czech reference.

Serious inflammation followed by necrosis of the nasal epithelium was seen in rats following single doses of 20-100 µl 1% polyEGDE applied into one nostril. Intratracheal injection of 100µl 1% polyEGDE revealed desquamation of the bronchial epithelium followed by hyperplasia and inflammatory cell infiltration.

117.4.2 Repeated dose toxicity

No data were found.

117.4.3 Toxicity to reproduction

No data were found.

117.4.4 Mutagenic and genotoxic effects

PolyEGDE was reported to be negative in an Ames test and in a reverse mutation assay in *Escherichia coli*. The substance did not induce structural chromosome aberrations in Chinese hamster ovary cells, and the result of a SCE test was negative. A mouse lymphoma cell assay was negative overall, but showed some positive response at cytotoxic levels of 0.030 ml/l.
In vivo, polyEGDE was negative in a sex linked recessive lethal mutation test in *drosophila melanogaster* as well as in a micronucleus test in mice.

117.4.5 Carcinogenic effects

An NTP study was performed in rats and mice, but no report was issued, as the study was considered inadequate (by NTP).

118 Evaluation

PolyEGDE covers several molecules of different ethylene oxides chain-lengths, which have different physical and chemical properties and probably also vary with respect to toxicology. The data found on the toxicity of any of the compounds covered by the name polyEGDE was very scarce. Also, some of the original data were not available and were only very summarily reported in RTECS.

PolyEGDE was of low acute oral toxicity, while toxicity by the dermal and the inhalation routes has not been assessed. A number of studies by dermal and eye application in rats and rabbits indicated that the substance is moderately irritating to skin and eyes. Human data supported that the substance is skin irritating. Studies in rats by direct intratracheal and intranasal application showed that the substance is also severely irritating to mucous membranes. The epithelial damage was however rapidly compensated by proliferation and regeneration. The irritative effect on the mucous membranes is not evaluated to be significant as direct application to the nasal and tracheal membranes would not normally occur.

Mutagenicity data *in vitro* on polyEGDE were overall negative with the indication that polyEGDE is cytotoxic from around 0.030 ng/ml. *In vivo* mutagenicity data on polyEGDE were negative. On this basis, polyEGDE is evaluated not to be genotoxic or mutagenic.

No data were available on repeated dose toxicity, on reproductive toxicity, or on carcinogenicity.

Overall, the critical effect on the scarce information available is evaluated to be irritation to the skin and eyes.

119 References

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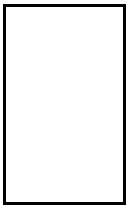
Appendix 16: Cyclohexanone

120 General description

120.1 Identity

Molecular formula: $C_6H_{10}O$

Structural formula:



Molecular weight: 98.14

CAS-no.: 108-94-1

Synonyms: Cyclohexyl ketone
Ketohexamethylene
Pimelic ketone
Ketocyclohexane
Oxocyclohexane
Sextone

120.2 Physical / chemical properties

Description: Cyclohexanone is a colourless to pale yellow oily liquid with peppermint and acetone odour.

Melting point: -32.1°C

Boiling point: 155.6°C

Density: 0.948 g/ml at 20°C

Vapour pressure: 3.38 mmHg (4.5 hPa) at 20°C

Concentration of saturated vapours: (calculated) 4447 ppm (17800 mg/m^3) at 20°C, 1 atm

Conversion factor: 1 ppm = 4.08 mg/m^3 at 20°C and 1 atm
1 mg/m^3 = 0.25 ppm

Solubility: Water: 80 g/l at 20°C

$LogP_{octanol/water}$: 0.81

References: IARC (1989), ACGIH (1991), DECOS (1993), OEL (1993), ChemFinder (2002).

121 Toxicokinetics

121.1 Human data

In humans the inhalation uptake of concentrations of 101, 207 or 406 mg/m^3 cyclohexanone over 8 hours was 57-59%. Skin uptake of liquid cyclohexanone after immersion of the hands of three persons for 30 minutes was estimated to be 0.037- 0.069 mg/cm^2/h corresponding to 1-2% of the dose absorbed by inhalation during 8 hours of exposure to 200 mg/m^3. (Mraz 1994 – quoted from A&H 1999).

Following occupational exposure (8 hours) by inhalation to 1- 40 ppm (4 - 160 mg/m^3, average concentration of 9 ppm, 36 mg/m^3) cyclohexanone, the concentration of cyclohexanone in breath was approximately 4 mg/m^3 and the urinary concentration of cyclohexanol was 9 mg/g creatinine (Ong et al. 1991 – quoted from DECOS 1993 and from IARC 1999).

Analysis of urine collected from three humans exposed for 8 hours to cyclohexanone at a concentration of 415 mg/m^3 showed that 3.5% of the cyclohexanone-dose found in urine was cyclohexanol conjugated to glucuronic acid, with the rest being present as *trans*-1,2-cyclohexanediol glucuronide (68.4%) and *trans*-1,4-cyclohexanediol (28.1%) (Flek et al. 1989 – quoted from OEL 1993).

Four men and four women were exposed to cyclohexanone at concentrations of 101, 207 or 406 mg/m^3 for 8 hours. At 207 mg/m^3, 57% of the inhaled dose was excreted in urine as cyclohexanol (1%), 1,2 and 1,4-cyclohexanediols (39%), and their glucuronide conjugates (18%). Elimination half-lives of the 1,2 and 1,4-diols were 16 and 18 hours, respectively. Following repeated inhalation exposure to 207 mg/m^3 for 5 days, the excretion rate for cylclohexanediol increased, but not for cyclohexanol. (Mráz et al. 1994 – quoted from A&H 1999 and from IARC 1999).

End-of-shift urine samples were collected from 24 factory workers occupationally exposed to up to 9 ppm (37 mg/m^3) of cyclohexanone in combination with toluene and other solvents, and from 10 non-exposed controls. Calculation of relative quantities of the urinary metabolites identified showed that 0.4% of the inhaled dose was excreted in urine as cyclohexanol and 4.4% as *trans*-1,2-cyclohexanediol. Very low levels of cyclohexanone and no *cis*-1,2, cyclohexanediol were detected. (Kawai et al. 1999).

In a case of attempted suicide by ingestion of 720 ml sake, containing 10% ethanol and about 100 ml liquid cement containing 39% cyclohexanone, 28% methyl ethyl ketone, 18% acetone and 15% polyvinyl chloride, the cyclohexanone level in plasma at 5 hours after ingestion was 10 µg/ml. At the same time point, the plasma level of cyclohexanol was about 200 µg/ml. Cyclohexanone excretion in urine was minimal (33 µg/ml at 12 hours after ingestion) with the major metabolite excreted by this route being

cyclohexanol glucuronide (440 µg/ml) and, to a lesser degree, unconjugated cyclohexanol (51 µg/ml). (Sakata et al 1989 – quoted from DECOS 1993 and from IARC 1999).

Accidental exposure of newborn babies to cyclohexanone had occurred in a special care unit from contamination of intravenous nutrient solutions. Isomers of cyclohexanediol (mostly *trans*-1,2-cyclohexanediol) were found in 101 of 584 urine samples. No conjugates of cyclohexanol or cyclohexanediol were detected. (Mills & Walker 1990 – quoted from DECOS 1993 and from IARC 1999).

121.2 Animal data

Results from acute toxicity studies in rodents indicate that cyclohexanone is absorbed after ingestion, skin contact and inhalation in animals. The blood/air distribution coefficient is 2150, implying high uptake via respiratory passages. (OEL 1993 and A&H 1999).

Two rabbits given gavage doses of 890 mg cyclohexanone/kg b.w. excreted glucuronic conjugates in the urine (858 mg at 24 hours, 2632 mg at 48 hours, measured as glucuronic acid), whereas the inorganic sulfate content in urine decreased. This demonstrates, according to the authors, that the metabolism of cyclohexanone includes conjugation to glucuronic acid. (Treon et al. 1943a).

Rabbits dosed orally with 270 mg/kg b.w. excreted 66% cyclohexanol glucuronide conjugate and 6% glucuronide conjugate of *trans*-1,2-cyclohexanediol. Thus, one of the metabolic pathways for cyclohexanone is, according to the authors, P450 dependent. (Elliot et al. 1959 – quoted in OEL 1993 and DECOS 1993).

Groups of 10 Wistar and 10 Gunn rats were dosed intravenously with cyclohexanone (0, 50, or 100 mg /kg b.w.) daily for 28 days. Neither cyclohexanone nor its metabolite cyclohexanol could be detected in plasma at 24 hours. Urine was collected over 24 hours after the last injection and analysed for free cyclohexanone, cyclohexanol, and sulfate and glucuronide conjugates of cyclohexanol. Levels of free cyclohexanone or cyclohexanol in urine were less than 1 %. No sulfate conjugates of cyclohexanol were found. Cyclohexanol-glucuronide conjugate accounted for 15-25% in the low dose group and for 19-34% in the high dose group. No analyses for cyclohexane-diol conjugates in the urine, or of breath or faeces were included in the study. (Greener et al. 1982).

In dogs, approximately 74-100% of an intravenous dose of 285 mg/kg was converted to cyclohexanol. The distribution half-life was 6.6 min. Sixty percent of the dose was excreted in urine as the conjugate of cyclohexanol. (Martis et al. 1980 – quoted from OEL 1993, DECOS 1993, and from A&H 1999).

Overall, the data indicate that both humans and animals rapidly metabolise cyclohexanone to cyclohexanol. Then, the pathway in humans in primarily through cytochrome P-450 oxidation with the formation of diols, which are then conjugated with glucuronic acid before excretion mainly in urine. In animals, it appears that the P-450 system is less important, as the excretion of

cyclohexanol conjugates are more predominant than in humans. The formation of *cis*-2-hydroxylcyclohexylmercapturic acid has also been described. (OEL 1993). The proposed metabolic pathways of cyclohexanone are summarised in Figure 2.

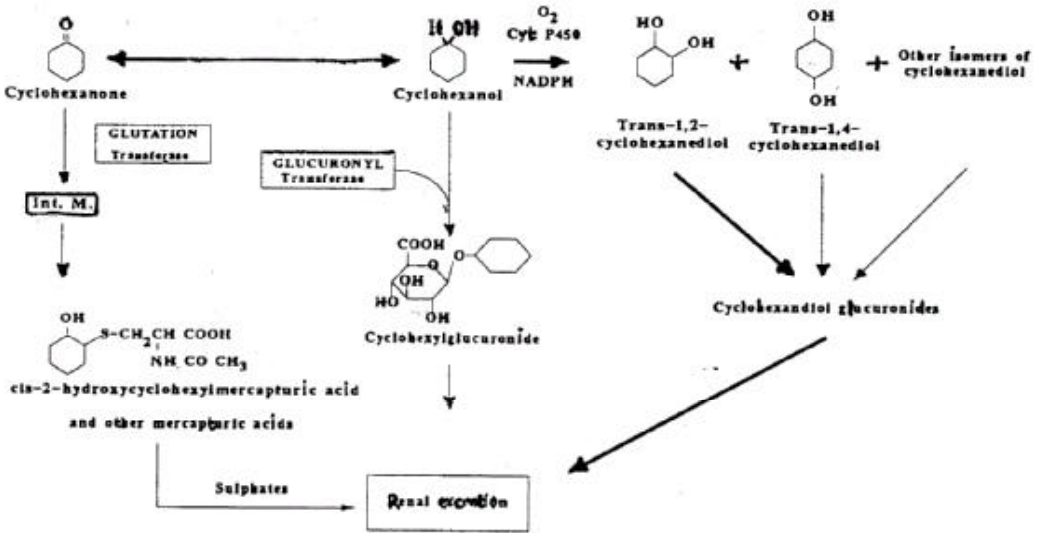


Figure 2. Metabolism of cyclohexanone (modified from OEL 1993).

Dominant pathways in humans is indicated by thick arrows.
Int. M: labile intermediate

121.3 Mode of action

Cyclohexanone is an organic solvent, which causes CNS depression, no details on the mechanism(s) of action have been found.

122 Human toxicity

122.1 Single and repeated dose toxicity

122.1.1 Inhalation

Verbal memory performance and alertness were affected in 23 workers exposed to cyclohexanone at concentrations of 38 to 158 ppm (150 - 630 mg/m^3) as well as to minor amounts of acetone, toluene, and methylethylketone (50-600 ppm) for at least 4 years (Milanovic et al. 1990 – quoted from A&H 1999 and from IUCLID 2000).

Seventy-five workers in a Romanian furniture factory exposed for 14 years to cyclohexanone in a wood coating procedure were monitored over 12 consecutive 8-hour shifts. Exposure levels were measured to be from 162 to 368 mg/m^3 (40-92 ppm). The exposed workers reported the following CNS-symptoms more often than a control group of 85 workers: Mood swings (18% with 7% in controls), irritability (22% with 14% in controls), forgetfulness (22% with 5.8% in controls), insomnia (33% with 12% in controls), and headaches (40% with 21% in controls). Muscle, joint and bone ache was reported for 16-26%. Nerve conduction velocity and latency time in nervi medianus, ulnaris and peroneus were delayed, and amplitude was decreased. Reaction-time to visual and auditory stimuli was reduced. The authors concluded that a number of effects, including effects on the central and the peripheral nervous systems and rheumatic effects, are related to cyclohexanone exposure, but stress that the results on nerve conduction measurements should be challenged further because of limitations in the methodology. (Mitran et al. 1997).

122.1.2 Oral intake

A 15-year old boy had CNS-effect and went into shock after drinking cyclohexanone. Metabolic acidosis, chemical hepatitis, renal insufficiency, muscular degeneration, and myoglobinuria developed. No information is given on the amount ingested or reversibility of the effects. (Zuckerman et al. 1998 - quoted from A&H 1999).

122.1.3 Irritation

10 persons exposed by inhalation for 3-5 minutes to concentrations of 50 ppm (204 mg/m^3) cyclohexanone complained about irritation of the throat. At 75 ppm (306 mg/m^3), irritation of eyes and nose was also reported. Most subjects found 25 ppm (102 mg/m^3) tolerable. (Nelson et al. 1943 – quoted from ACGIH 1991, A&H 1999 and from IUCLID 2000).

Irritation of the eyes, respiratory tract, and skin was observed in 24%, 26%, and 13%, respectively, of 75 furniture factory workers exposed for 14 years to 162-368 mg/m^3 cyclohexanone (Mitran et al. 1997).

122.1.4 Sensitisation

Patch testing of five painters established cyclohexanone resin as the cause of their allergic contact dermatitis. One of the patients was patch-tested with cyclohexanone itself with a negative result. (Bruze et al 1988).

A case report has described allergic contact dermatitis caused by pure cyclohexanone used as PVC adhesive. The woman affected had been working with the substance for 7 years. She did not react to cyclohexanone resin or to colophony. (Sanmartín & de la Cuadra 1992).

122.2 Toxicity to reproduction

No data were found.

122.3 Mutagenic and genotoxic effects

No data were found.

122.4 Carcinogenic effects

No data were found.

123 Animal toxicity

123.1 Single dose toxicity

123.1.1 Inhalation

IUCLID has listed LC_{50}-values in rats ranging from above 6200 to 32500 mg/m^3 for 4-hour exposure periods (IUCLID 2000).

All 6 rats exposed to 4000 ppm (16320 mg/m^3) for 4 hours died. At 2000 ppm (8160 mg/m^3), one out of 6 animals died. (Smyth et al. 1969 - quoted from A&H 1999).

Groups of 5 male ICR-mice were exposed to concentrated vapours of cyclohexanone, measured to 19000 mg/m^3 (4750 ppm) for different periods of time (78, 90, 104 or 120 minutes). The mean time to death in mice was 100 minutes. During exposure, the animals exhibited signs of irritation, laboured respiration and CNS depression. Gross post-mortem examination showed general vascular congestion and haemorrhages of the lungs. Histopathology performed on the survivors 7 days after the 2-hour exposure revealed lung oedema and hyperplasia in the white pulp of the spleen. Findings in the brain, heart, liver adrenals, and gonads were within normal limits. (Gupta et al.1979).

In guinea pigs, concentrations of 4000 ppm (16320 mg/m^3) cyclohexanone for 6 hours caused narcosis and depressed respiration. Three out of 10 animals died within 4 days of exposure. (Specht et al. 1940 - quoted from ACGIH 1991 and from A&H 1999).

123.1.2 Oral intake

LD_{50}-values in the range of 1296 to 3460 mg/kg b.w. in rats and of 1400 to 3200 mg/kg b.w. in mice have been listed in IUCLID (2000). Symptoms of acute toxicity included narcosis and laboured respiration, and the autopsy revealed peritoneal and intestinal congestion. (Gupta et al. 1979).

Minimum lethal doses for rabbits have been reported to be from 1600 up to 1900 mg/kg b.w. Sub-lethal doses of 900 to 1600 mg/kg b.w. caused narcosis, ataxia, pronounced lung oedema, and necrosis of the respiratory epithelium, heart muscle, liver and of the kidneys. (Treon et al. 1943a).

123.1.3 Dermal contact

Dermal LD_{50}-values in rabbits ranged between 794 and 3160 mg/kg b.w. (OEL 1993, A&H 1985, IUCLID 2000). However, minimum lethal doses from 10200 up to 23000 mg/kg after one hour of skin contact have also been reported. Symptoms of toxicity included severe irritation of the application site, convulsive movements, and narcosis. (Treon et al 1943a).

123.1.4 Skin irritation

Concentrations of 0, 12.4, 24.8, 49.5, and 99% cylclohexanone in cottonseed oil were applied to the shaved backs of male rabbits under occlusive patch for 24 hours. The irritation response was concentration dependent with the lowest test concentration producing a minimally detectable, transient response graded 0 on a 3-grade scale. At 24 hours after exposure, the medium test concentrations caused grade 1 and grade 2 irritation, respectively, which disappeared within 72 hours. The highest test concentration caused a grade 3 irritation lasting until day 7 after exposure. (Gupta et al. 1979).

Results in IUCLID vary from reporting cyclohexanone as being non-irritating (in an OECD guideline 404-study) to corrosive to rabbit skin. No further details of these studies are available in IUCLID, and most of the studies are not publicly available. (IUCLID 2000).

123.1.5 Eye irritation

Concentrations of 2.5, 5, 10, 20, 40, 80, or 99% cyclohexanone in cottonseed oil were instilled (volume instilled not indicated) into one eye of New Zealand White rabbits. Concentration dependent eye irritation was observed following instillation from 5% cyclohexanone, reaching a score of 3 on a 3-grade scale from 40% cyclohexanone. (Gupta et al. 1979).

Application of 0.02 ml of undiluted cyclohexanone to the eyes of rabbits caused severe injury of the eye with marked irritation and corneal injury. The authors graded the eye irritation as 5 on a 10-grade scale. (Carpenter et al 1946).

Seven Stauffland Albino rabbits were treated with 0.1 ml of cyclohexanone solutions in distilled water in the lower conjunctival sac of the eye. The concentrations of cyclohexanone tested were 10, 15, 25, 40, 50, 75, and 100%. Measurements of corneal thickness were performed on day 3 and scored in a 5-grade scoring system, including reversibility of effect at 24 hours and thickness ratio between treated and untreated eye. Scoring was also performed according to the EPA scoring criteria over 21 days. The irritation was dose response related, with both systems scoring the 10% solution as a mild eye irritant, and from 40% as corrosive (Morgan et al. 1987).

IUCLID (2000) reports cyclohexanone to be irritating or severely irritating to the rabbits eye.

123.1.6 Sensitisation

In a Guinea Pig Maximisation Test, groups of 25 guinea pigs were exposed to one of 2 batches of cyclohexanone resin or to cyclohexanone and challenged with 5, 10 or 20% at 24 and 48 hours. Nine, 13 and 8 animals, respectively, reacted in the re-challenge test to the first batch of cyclohexanone resin, while 2, 1 and 0, respectively, reacted to the second batch. No animals reacted at any concentration at challenge to cyclohexanone. (Bruze et al. 1988).

Another Guinea Pig Maximisation Test was performed with cyclohexanone in a validation procedure of the Mouse Ear Swelling test. The GPMT test was negative (no details on the protocol are given in the reference). The Mouse Ear Swelling Test was also negative with topical application of undiluted cyclohexanone to the left ear, while the right ear only had the vehicle applied. The swelling percentage (left ear thickness / left ear thickness · 100) was 102%. Cyclohexanone was also reported to be negative in a closed patch test in guinea pigs (no details on the protocol are given in the reference). (Gad et al. 1986).

123.2 Repeated dose toxicity

123.2.1 Inhalation

Continuous exposure of young rats to 2 ppm (8.2 mg/m^3) cyclohexanone for 7 weeks and of adult rats to 8 ppm (32.6 mg/m^3) for 10 weeks resulted in morphological changes in cells of the olfactory bulbs (Panhuber et al. 1987, Rehn et al. 1988 – both quoted from OEL 1993 and from A&H 1999).

Rabbits exposed to 12120 mg/m^3 (corresponding to 3082 ppm), 6 hours a day, 5 days per week for 3 weeks showed weight loss, symptoms of CNS depression (narcosis, laboured breathing, ataxia, and increased salivation), and eye irritation; two of the 4 rabbits died. When exposed to lower concentrations (190, 309, 608, 773, or 1414 ppm - corresponding to 750, 1210, 2390, 3040, or 5560 mg/m^3) for 10 weeks, rabbits showed evidence of eye irritation from 309 ppm (1210 mg/m^3). At concentrations of 773-3082 ppm (3040-12120 mg/m^3), irritation symptoms were more marked and included lachrymation, salivation and watery discharge from the nose. Narcotic effects were reported at the highest concentration. No effects were seen at any dose level on haematology. No control group was included in the study. (Treon et al. 1943b).

One rhesus monkey exposed to 2390 mg/m^3 cyclohexanone (6 hours/day, 5 day/week for 10 weeks) was reported to have extensive injury in the heart muscle, the lungs, the liver, and the kidneys (not specified further). The monkey had also contracted a bronchopulmonary infection. (Treon et al. 1943b).

123.2.2 Oral intake

In a range-finding study preceding a chronic study, five F-344 rats/sex/group were given 0, 190, 400, 800, 1600, 3300, 4700, or 6500 mg/l cyclohexanone in the drinking water for 6 months (the top-dose corresponded to approximately 1000 mg/kg b.w./day). No death occurred during the study, but weight gain was depressed approximately 10% in both males and females of the highest dose group. A mild degenerative change in the thyroid gland (no details in the reference) was observed at pathological examination in 2 males administered 4700 mg/l. There were no significant histopathological changes (the changes are not specified by the authors). The authors considered a maximum tolerated concentration of 6500 mg/l for the chronic study (see 4.5.2). (Lijinsky & Kovatch 1986).

A range-study was also performed in mice. Ten (C57BL/6 x C3H)F_1 mice/sex/group were given 0, 400, 2300, 6500, 13000, 25000, 34000, or

47000 mg/l cyclohexanone in the drinking water for 13 weeks. In the high-dose group, 1/3 of the females and 2/3 of the males died during treatment. One male of the 34000 mg/l group also died. Body weights were reduced 15% in females and 24% in males at this dose, and 19% in males of the 25000 mg/l group. At pathological examination, some of the animals of the high dose group had focal liver necrosis, and 2 high-dose females had thymus hyperplasia, whereas changes at the lower doses were minimal. A maximum tolerated concentration of 25000 mg/l for female and 13000 mg/l for male mice was considered for the chronic study (see 4.5.2). (Lijinsky & Kovatch 1986).

123.2.3 Dermal contact

Repeated cutaneous applications of cyclohexanone (dose not specified) to 120 guinea pigs for 3-8 weeks resulted in cataract with subcapsular focal vacuolated areas in 29 animals. No effects were seen in the control animals. (Rengstorff et al. 1972 – quoted from DECOS 1993, Henschler 1994, and from A&H 1999).

In another study including 12 New Zealand White rabbits and 12 Harley albino guinea pigs of both sexes treated on a 3- inch shaved area of their backs with 0.5 ml (470 mg) undiluted cyclohexanone 3 times/week for 3 weeks, ophthalmological examination 6 months after treatment showed subcapsular vacuolisation and opacification of the lenses due to fiber degeneration both in the treated and the control group guinea pigs. No lenticular effects were recorded in the rabbits. The authors concluded that the lens changes are natural for guinea pigs thus making this species unsuited for studying this effect. (Greener and Youkilis 1984 – quoted from DECOS 1993 and from A&H 1999).

In a study in CD Sprague Dawley rats and Hartley guinea pigs treated on the back with 0.5 ml (470 mg) of undiluted or a 2% solution of cyclohexanone 3 times/week for 3 weeks, the guinea pigs of the treated as well as of the positive and the negative control groups had lenticular vacuolisation. No eye effects were seen in the rats even after prolongation of the dosing period to 13 weeks. IUCLID comments that the guinea pig is not a suitable animal model for investigation of ophthalmological effects. (Mayhew 1984 – quoted from DECOS 1993 and from IUCLID 2000).

123.2.4 Other routes

Dogs exposed by intravenous administration to 284 mg/kg b.w. cyclohexanone for 18-21 days showed lachrymation, mydriasis, salivation, ataxia, occasional convulsive movements, stupo, and/or dyspnoea/hypernoea. Absolute and relative liver weights were affected. Pathological examination of the organ showed glycogen depletion, plasma cell infiltrates round the hepatic veins, and haemosiderin deposits. Haemolysis, bone marrow hyperplasia, and extramedullary haematopoiesis were also reported. (Koeferl et al. 1981- quoted from DECOS 1993).

123.3 Toxicity to reproduction

123.3.1 Inhalation

In a two-generation study, 30 Sprague Dawley rats/sex were exposed (6hours/day, 5 days/week) to concentrations of 0, 250, 500, or 1000 ppm (0, 1020, 2040, or 4080 mg/m^3) in the F_0-generation and to 0, 250, 500, or 1400 ppm (5712 mg/m^3) in the F_1-generation. No adverse effects were seen in the F_0-generation. Rats in the high-dose group of the F_1-generation showed increased mortality, lachrymation, ataxia, irregular breathing, and body weight gain depression in males. The fertility of the males was reduced (no details on which parameters were examined), and the offspring of this dose group had body weight gain depression and reduced survival. No changes in reproductive organs were seen at histopathological examination of animals of the F_1 and F_2 generation. (American Biogenics Corporation 1986 – quoted from DECOS 1993, BUA 1997, and IUCLID from 2000).

CD rats (26 animals per group) were exposed by whole body inhalation to 0, 300, 650 or 1400 ppm (0, 1224, 2652 or 5712 mg/m^3) cyclohexanone, 6 hours a day on gestational days 6-19. The highest concentration caused lachrymation, nasal discharge and lethargy as well as significantly reduced maternal and foetal body weights. Effects on pregnancy rate, pre-implantation loss, number of resorptions, or number of live foetuses per litter were non significant. There was a sporadic increase in skeletal and visceral variations, but no malformations were seen. The authors concluded that there was no evidence of teratogenicity. (Homan & Schroeder 1984 and Bio/dynamics 1984– quoted from DECOS 1993 and from IUCLID 2000).

Groups of 5-9 Sprague-Dawley rats were exposed to cyclohexanone at gestation days 5-20 to concentrations of 100, 250, or 500 ppm (408, 1020, or 2040 mg/m^3) for 7 hours a day. Positive and negative controls were included. Only slightly lower weight gains were recorded in the dams of all treated groups. In the two highest dose-groups, a grey mottling of the lungs were observed in the dams. There was no treatment related effects on mean number of corpora lutea, implantation, foetal death, foetal weights, resorptions, or sex-ratio. There was a weak, non-significant increase in the mean percent of rudimentary ribs. In the two highest dose groups, three foetuses had heart artery malformations and 2 foetuses had skeletal malformations, but the findings were not statistically significant. The authors concluded that the substance was unlikely to be a developmental toxicant by inhalation exposure. (Samimi et al. 1989).

Mice exposed to cyclohexanone at concentrations of 300, 650 ,or 1400 ppm (1224, 2652, or 5712 mg/m^3), 6 hours a day from gestation day 6 to 17 had reduction of the maternal body weight, and of the number of corpora lutea and live foetuses at the highest exposure level. There was no significant increase of external malformations. No effects were reported at lower exposure levels. (Homan & Schroeder 1984 – quoted from DECOS 1993).

A study with CD-1 mice exposed to concentrations of 0 or 1400 ppm (5712 mg/m^3) cyclohexanone, 6 hours a day from day 6 to 17 of pregnancy showed decreased body weight gain in the treated group. Clinical symptoms included lethargy, lachrymation, and white ocular discharge. The number of resorptions was significantly increased and some dams resorbed their litter

totally. The number of live foetuses was significantly decreased, and there was an increase in skeletal variations, a weak significant trend of dilated renal pelvis, and one case of cleft palate. The authors concluded that 1400 ppm was toxic to the dams and the foetuses, but not teratogenic. (Biodynamics 1984 – quoted from IUCLID).

123.3.2 Oral intake

No maternal or developmental effects were seen in a study with CD-1 mice dosed with 0 or 800 mg/kg b.w./day cyclohexanone by gavage on days 8-12 of gestation, with a post-treatment period of 250 days. No effects of treatment on body weights or reproductive parameters were recorded in the treated dams. The offspring were monitored for postnatal viability, growth, morphology, locomotor activity in a figure eight maze, and reproductive function. No differences between treated and control animals were recorded for these parameters. (Gray & Kavlock 1984 – quoted from IARC 1989 and from IUCLID 2000; Gray et al 1986).

In a another study where groups of pregnant ICR/SIM were treated by gavage with 0 or 2200 mg/kg b.w. cyclohexanone from day 8-12 of gestation, 6 of 28 treated mice died while no mice of the control group died; 2 dams resorbed their litters completely. Maternal body weight gains were reduced in the treated group. Birth weights and body weight gains of the pups were depressed, but their viability was normal. (Seidenberg 1986 – quoted from IARC 1989, IUCLID 2000, and from A&H 1999).

Cyclohexanol, the major metabolite of cyclohexanone, has been reported to affect the testes of rabbits dosed with 25 mg/kg b.w./day for 40 days (Dixit et al. 1979 – quoted from OEL 1993).

123.3.3 Dermal contact

No data were found.

123.4 Mutagenic and genotoxic effects

123.4.1 *In vitro* studies

A number of *in vitro* mutagenicity assays on cyclohexanone have been performed, the studies are summarised in Table 4.

Table 4. *In vitro* mutagenicity studies.

Test system	Type of test / test parameters	Highest test concentration	Metabolic activation	Results	References
Salmonella Typhimurium: TA 98, 100, 1535, 1537	Ames test / gene mutation	10000 µg/plate	yes/no	Negative.	Haworth et al. 1983 BUA; IUCLID
Salmonella Typhimurium: TA 98, 100, 1535, 1537	Ames test / gene mutation	30 µg/plate	yes/no	Negative.	Florin et al. 1980 BUA; IUCLID
Salmonella Typhimurium: TA 98, 100, 1530, 1535, 1537, 1538, G-46	Ames test / gene mutation	1000 µg/plate	yes	Negative.	NCI 1979 BUA; IUCLID
Salmonella Typhimurium: TA 98, 100, 1530, 1535, 1537, 1538	Ames test / gene mutation	1000 µg/plate	no	Ambiguous: reversions in controls. No dose-dependency	Massoud et al. 1980 and 1983
Bacillus subtilis	Forward gene mutation	300 µl/1.5 ml cell suspension	no	Positive result, but no dose-dependency. High sponta-neous mutation frequency	Massoud et al. 1980; A&H; BUA; IUCLID
Mouse lymphoma test L5178 Y cells	Gene mutation	5000 µg/ml	yes/no	Negative.	Mc Gregor et al. 1988 BUA; IUCLID
HGPRT Assay CHO cells	Cytogenetic assay / gene mutation	12.5 µl/ml	yes no	Negative. Positive.	Aaron et al. 1984; BUA; IUCLID
CHO cells	Chromosome aberrations	10µl/ml	yes/no	Negative.	Aaron et al. 1984; BUA; IUCLID
CHO cells	Sister chromatid exchange DNA repair	12.5 µg/ml	yes no	Negative. Positive, but ambiguous because of weak increase in SCE rate and of use of toxic conc.	Aaron et al. 1984 BUA
Human lymphocytes	DNA repair UDS test Chromosomal aberration	10 µg/ml	no	negative The study is criticised by IUCLID and BUA for too low number of cells (12), records of gaps, not breaks, and no controls	Lederer et al. 1971; BUA; IUCLID; IARC 1999
Human lymphocytes	UDS test	not given	yes/no	negative- possible cytotoxicity	Perocco et al. 1983; BUA; IUCLID
Human diploid fibroblasts	UDS test DNA repair	50 µl (9.5 µg/ml culture)	yes/no	negative	NIOSH 1980; BUA; DECOS; IUCLID

123.4.2 *In vivo* studies

A sex-linked recessive lethal (SLRL) test, where male Drosophila melanogaster were exposed by inhalation to 50 ppm cyclohexanone for 7 hours (204 mg/m^3) or to 400 ppm (1632 mg/m^3) for 40 minutes, showed lethality rates of 0.25% and 1%, respectively. However, because of the non-reproducible result, the authors concluded that cyclohexanone is not mutagenic in Drosophila. (NIOSH 1980 / McGregor 1980 – quoted from DECOS 1993 and from IUCLID 2000).

Inhalation exposure of Drosophila melanogaster for 4 hours to 1900 ppm cyclohexanone did not induce gene mutation (Unpublished report from Nipro Inc. 1986 – quoted from IUCLID 2000).

Another Drosophila melanogaster gene mutation test with oral exposure to 0.1 or 100 ml/day for 3 days was negative (Goncharova 1970 citation in Chem Abstr 1972 - quoted from BUA 1993 and from IUCLID 2000).

A dominant lethal test where male rats were exposed to 50 or 400 ppm (204 or 1632 mg/m^3) cyclohexanone 7 hours a day for 5 consecutive days did not induce chromosomal aberrations. Pregnancy frequency, numbers of corpora lutea and implantations sites, and survival of the embryos were not affected. (NIOSH / McGregor 1980 – quoted from DECOS 1993, BUA 1993, and from IUCLID 2000).

Cyclohexanone has been reported to induce chromosomal aberrations, including chromatid gaps, breaks, centric fusions, chromatid exchanges, and polyploidy, in bone marrow cells of rats treated subcutaneously with one or five doses of 0.1, 0.5 and 1.0 g/kg b.w. However, the test quality was criticised in BUA and in IUCLID because of lack of a control group, lack of dose-dependency, and cytotoxic effect of the doses. (De Hondt et al. 1983 - quoted from IARC 1989, DECOS 1993, BUA 1993, and from IUCLID 2000.)

In a micronucleus test in bone marrow cells of mice exposed to 204 or 1632 mg/m^3 cyclohexanone in one single 7-hour-exposure or at 7 hours a day for 5 days, the frequencies of chromosomal aberrations were not increased significantly (NIOSH / McGregor 1980 – quoted from DECOS 1993, BUA 1993, and from IUCLID 2000).

123.5 Carcinogenic effects

123.5.1 Inhalation

No data were found.

123.5.2 Oral intake

In a 2-year toxicity assay, groups of 52 F344 rats/sex were exposed to 0, 3300, or 6500 mg/l in the drinking water (according to DECOS corresponding to 500, or 1000 mg/kg b.w./day). Survival was comparable in treated and control groups except for a non-significant decrease in the high-dose group. Body weight gain was significantly depressed in a dose-related way. The incidence of adrenocortical adenomas was statistically significantly increased in male rats of the low-dose group, but not at the high dose and not in females. The number of fibroadenomas in the mammary glands in females of the high dose was reduced compared to controls. The incidence of thyroid follicular-cell adenomas/carcinomas was increased with statistical significance in the high-dose group males (6/51, compared to 1/52 in controls, but this finding was not commented by the authors, thus probably not regarded as a carcinogenic effect. (Lijinsky & Kovatch 1986).

The study by Linjinsky & Kovatch included also groups of 50 or 52 (C57BL/6 x C3H)F_1 mice/sex exposed to 0, 6500, or 13000 mg/l with an

additional group of 41 female mice receiving 25000 mg/l in the drinking water. Survival was depressed to 40% in females and to 70% in males at 13000 mg/l and dropped to 15% in the 25000 mg/l group of females. Body weight gains were affected resulting in 15-20% lower body weights of males at 13000 and females at 25000 mg/l from week 15 through most of the study when compared to those of the controls. Histopathology revealed hepatocellular adenomas/carcinomas in male mice in 25/51 males at 6500 mg/l and 13/46 at 13000 mg/l, while the controls had 16/52 liver tumours. Thus, no dose-relationship was seen and only the incidence of the tumours in the low-dose group was statistically significant. Alveolar-bronchiolar adenomas/carcinomas were found in male mice in a negative trend, with significance in the high dose group. The number of lymphosarcomas was statistically elevated in female mice of the 6500 mg/l group (17/50, compared to 8/52 in controls, 4/50 at 13000 mg/l and 0/41 at 25000 mg/l). The authors found this effect at only one dose level only suggestive of a weak carcinogenic effect. IUCLID finds the relevance of the finding questionable. (Lijinsky & Kovatch 1986, IUCLID 2000).

123.5.3 Dermal contact

No data were found.

124 Regulations

124.1 Ambient air

Denmark (C-value): 0.1 mg/m^3 (MST 2002)

124.2 Drinking water

Denmark: -

124.3 Soil

Denmark: -

The Netherlands: 270 mg/kg (Van den Berg et al. 1993)

124.4 Occupational Exposure Limits

Denmark: 10 ppm – skin notation (At 2002)

ACGIH: 25 ppm – skin notation (ACGIH 1991)

Germany: 50 ppm (MAK 1998)

124.5 EU Classification

Cyclohexanone is classified as flammable (R10) and for acute toxicity (Xn;R20 - harmful by inhalation) (MM 2002).

124.6 IARC

Not classifiable as to its carcinogenicity to humans (Group 3) (IARC 1989, 1999).

124.7 US-EPA

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125 Summary

125.1 Description

Cyclohexanone is a colourless or pale yellow liquid. It is has a vapour pressure of 3.38 mmHg and is moderately soluble in water (80 g/l at 20°C).

125.2 Toxicokinetics

Cyclohexanone is absorbed by all three routes of exposure. The substance is rapidly metabolised to cyclohexanol by alcohol dehydrogenase. In humans, the main part is then hydroxylated through the mixed microsomal P-450 function, the resulting cyclohexanedioles conjugated with glucuronic acid. Cyclohexanol may also be conjugated directly and excreted as cyclohexanol glucuronide. Excretion is mainly in urine, with biliary and lung excretion also playing a role.
In a case of acute poisoning, no urinary diol-conjugates, but large amounts of cyclohexanol glucuronides were found. In babies, cyclohexanol was excreted and no conjugates were found in urine.
Animals also metabolise cyclohexanone to cyclohexanol, but they form less diols and thus primarily excrete cyclohexanol glucuronides.

125.3 Human toxicity

CNS effects and metabolic acidosis have been recorded after accidental ingestion of cyclohexanone.

Exposure of workers to 150 - 630 mg/m^3 cyclohexanone for 4 years in mixed exposure with other solvents affected the memory and alertness of the workers. Workers in a furniture factory exposed to 162 - 368 mg/m^3 cyclohexanone for 14 years reported CNS symptoms (memory and personality alterations) and measurements of periferic nerve conductivity indicated decreased function.

Cyclohexanone is an eye, nose and throat irritant following inhalation of 306 mg/m^3 for a few minutes; a concentration of 102 mg/m^3 was reported as being tolerable. Cyclohexanone was also reported to be irritating to the eyes, respiratory tract and the skin following occupational exposure to 162 - 368 mg/m^3 for 14 years.

One reference has reported a case of sensitisation from occupational exposure to pure cyclohexanone. In another reference, it was reported that cyclohexanone is not sensitising to man, whereas resin of cyclohexanone caused allergic contact dermatitis in 5 painters.

No data on toxicity to reproduction, mutagenicity and genotoxicity, or carcinogenicity were found.

125.4 Animal toxicity

125.4.1 Single dose toxicity

LC_{50}-values reported for rats range from above 6200 to 32500 mg/m^3 for 4 hours of exposure. Symptoms of irritation and narcosis were recorded at sub-lethal concentrations.
Oral LD_{50}-values are reported to be between 1296 and 3460 mg/kg b.w. in rats and between 1400 to 3200 mg/kg b.w. in mice. Minimal lethal doses in rabbits were reported to be 1600-1900 mg/kg b.w. Narcosis was reported in rats, mice and rabbits, and damage in the major organs were reported in rabbits as well.
Reported dermal LD_{50}-values for rabbits range from 794 to 3160 mg/kg b.w.

Reports on the skin irritative potential of cyclohexanone vary from non-irritating to corrosive. One study including more details reports cyclohexanone to be a skin irritant with a dose-response relationship in an occlusive test in rabbits.
Eye irritation is reported to be moderate to severe in rabbits eye; concentration dependency of the irritation was demonstrated in a study resulting in severe injury from instillation of 0.02 ml.

Cyclohexanone was not sensitising in the Guinea Pig Maximisation Test and in the Mouse Ear Swelling Test.

125.4.2 Repeated dose toxicity

Continous inhalation exposure of young rats to 8.2 mg/m^3 and of adult rats to 32.6 mg/m^3 cyclohexanone for 7 or 10 weeks, respectively, resulted in morphological changes of cells in the olfactory bulbs. Rabbits exposed by inhalation to 12120 mg/m^3 cyclohexanone for 3 weeks had CNS depression; 2 of the 4 animals died. Irritation of the eyes and respiratory tract were reported from exposure levels of 1210 mg/m^3 and the effect was marked from 3082 mg/m^3.

Exposure of rats for 6 months in the drinking water at doses up to 6500 mg/l/day (1000 mg/kg b.w./day) caused weight gain depression at the top dose and mild thyroid gland degeneration at 4700 mg/l (660 mg/kg b.w./day). In a 13-week mice study with concentrations up to 47000 mg/l in the drinking water, 1/3 of the female mice and 2/3 of the male mice of the high dose died. Pathological examination revealed liver necrosis in both sexes and thymus hyperplasia in 2 females of the high dose group. The body weights were reduced in both sexes at 34000 mg/l and at 25000 mg/l in males, but there were only minimal pathological changes at these doses.

Three studies with dermal applications of 0.5 ml cyclohexanone 3 times a day over 3 to 8 weeks resulted in lens clouding in guinea pigs. However, also guinea pigs of the control groups showed the effect, and no effects were seen in rabbits or rats in these studies.

No adverse effects were reported from a 28 days intravenous test in rats with doses up to 100 mg/kg b.w./day of cyclohexanone. In dogs exposed to 284 mg/kg b.w./day of cyclohexanone for 18-21 days, a large number of irritative,

CNS, and general intoxication symptoms were recorded. Pathology showed liver insufficiency and haemolysis.

Dose-dependent eye irritation was recorded in rabbits exposed for 10 weeks to cyclohexanone concentrations of 1224 to 16320 mg/m^3.

125.4.3 Toxicity to reproduction

A two-generation study in rats exposed by inhalation to cyclohexanone at concentrations up to 5712 mg/m^3 did not show any adverse effects in the F_0-generation, but the high dose caused lachrymation, ataxia and body weight depression in the F_1-generation. The fertility of the males in this group was reduced, and their offspring had reduced survival and body weight depression.
An oral multigeneration study in mice dosed with 2000 mg/kg b.w./day showed reduced viability of pups in both treated and control groups in the first, but not in the second generation.

Two inhalation studies in rats exposed on gestation days 6-19 by inhalation to cyclohexanone at concentrations ranging from 1224 to 5712 mg/m^3 showed maternal toxicity (reduced maternal body weights, lachrymation, nasal discharge, lethargy) at the highest dose level, but there was no or only slight effects on reproductive parameters (pregnancy rate, uterine implant data, and number of live foetuses). Foetuses from dams at the highest exposure level had significantly reduced body weights and increased number of skeletal variations, but no malformations. In another inhalation study, where the rats were exposed to up to 2040 mg/m^3 from day 5-20 of gestation, three foetuses had heart artery malformations and 2 had skeletal malformations at the two highest exposure levels, which were slightly maternally toxic; however, the findings were not statistically significant. Mice exposed at concentrations up to 5712 mg/m^3 from gestation day 6-17 had reduced maternal body weights at the high dose; the number of corpora lutea and live foetuses was also reduced in that dose group whereas no effects were reported at lower dose levels. Oral dosing of mice on gestation days 8-12 with 0, 800 or 2200 mg/kg b.w./day resulted in high mortality of the dams, 2/28 litter resorptions, and birth weight depression in the high dose group; no effects were seen on viability of the pups at this dose level, and no effects in the dams or the pups were seen at the lower dose levels.

125.4.4 Mutagenic and genotoxic effects

Cyclohexanone was negative in three Ames tests with *Salmonella typhimurium* and in a mouse lymphoma assay. Ambiguous results were reported in one test in Salmonella typhimurium and a positive result in *Bacillus subtilis* in a forward mutation assay. Mutations were also induced in one test (HGPRT) in CHO cells in vitro without metabolic activation, but not when a metabolic activator was added.

No chromosome aberrations were induced in CHO cells whereas, in another assay in CHO cells, a weak increase in sister chromatid exchanges was seen without metabolic activation, but not with metabolic activation. Chromosome aberrations, DNA repair and unscheduled DNA-synthesis was not observed in human lymphocytes or human diploid fibroblast *in vitro*.

Three gene mutation tests in Drosophila melanogaster were negative.
In a dominant lethal test in rats, cyclohexanone did not induce chromosomal aberrations in bone marrow cells. Another test of chromosome aberrations in rat bone marrow cells was reported to be positive; however, the test quality was low.
A micronucleus test in mouse bone marrow cells was reported to be negative.

125.4.5 Carcinogenic effects

In a 2-year oral rat study with doses in the drinking water of approximately 0, 330, or 650 mg/kg b.w./day, body weight gain was decreased in a dose-related way and survival was slightly lower in the high dose group. Incidences of adrenocortical adenomas in low-dose males and of thyroid follicular-cell adenomas/carcinomas in high-dose males were significantly increased.

A 2-year oral mice study with concentrations in the drinking water of 0, 6500, 13000 mg/l to both sexes with an extra 25000 mg/l-group in females resulted in a marked decrease in survival rates in the 25000 mg/l and in the 6500 mg/l groups. Body weights were also affected at these two dose levels. The incidence of hepatocellular adenomas/carcinomas was increased in male mice, with statistical significance at the low dose only, and a not dose-related response. Alveolar-bronchiolar tumour incidence was significantly lower in high dose males compared with controls. The incidence of lymphomas was statistically elevated in female mice of the low-dose group.

126 Evaluation

The available toxicological data on cyclohexanone suggest that its critical effects are the irritant effect to the skin, the eyes and the respiratory tract, and the depressant effect on the central nervous system.

Cyclohexanone is absorbed by all routes of exposure. It is rapidly metabolised to cyclohexanol, which is either excreted in urine following conjugation with glucuronic acid or metabolised through the P450 oxidation system to cyclohexanediols, and then conjugated to their glucuronides and excreted in urine. The P450-oxidase function appears to be more important in humans than in animals.

Acute toxicity by inhalation is moderate, with LC_{50}-values ranging from 6200-32500 mg/m^3 in rats after 4 hours of exposure. CNS depression has been reported in both humans and animals. The substance is classified in the EU as 'Harmful by inhalation' (Xn;R20). The acute oral and dermal toxicity of cyclohexanone are in a similar range, with oral LD_{50}-values in rats between 1296 and 3460 mg/kg b.w and dermal LD_{50}-values in rabbits between 794 and 3160 mg/kg b.w. Cases of oral poisoning of humans have been reported.

The substance is irritating to eyes, nose and throat in humans from short-time exposure to 306 mg/m^3. Skin irritation is also reported from repeated occupational exposure to 162-368 mg/m^3 cyclohexanone. Results form animal studies vary from non-irritating to corrosive, but overall confirm that the substance is a skin and eye irritant following short term and long-term exposure.

Only one case of occupational allergic dermatitis has been reported. Sensitisation studies in guinea pigs and mice are negative. The sensitising potential of cyclohexanone is considered to be negligible.

Neurological symptoms in the central nervous system, including cognitive changes, were reported from long-term occupational exposure to cyclohexanone levels of 162-368 mg/m^3. Impairment of the peripheric nervous system was also reported, but the robustness of the methodology was questioned, and the findings were not confirmed in animals. In animals, symptoms of CNS-depression were reported in studies in rats and in rabbits after short time and prolonged exposure.

No information on toxicity to reproduction in humans exposed to cyclohexanone was available.
In a two-generation study in rats, fertility of male rats was reported to be reduced at concentrations above 2040 mg/m^3, but without details on the parameters leading to this evaluation. No other studies regarding fertility are available and thus, an evaluation of the effect of cyclohexanone on fertility is not possible on this basis.
In rats and mice, developmental toxicity (slight increase in the number of resorptions, skeletal and visceral malformations) was recorded to occur, but only at maternally toxic levels (irritation, CNS effects, reduced body

weights). On the basis of the available database, it is considered that cyclohexanone is not a developmental toxicant.

A number of mutagenicity and genotoxicity tests on cyclohexanone have been performed. Most of the *in vitro* tests on cyclohexanone are negative. A few *in vitro* tests indicate that the substance may induce cytogenetic effects; these effects have only been reported in assays performed without an exogenous metabolic activation system suggesting that if cyclohexanone has a cytogenetic potential itself, this property disappears following metabolic degradation.
All *in vivo* studies but one, of poor quality, are negative.
Overall, the available data indicate that cyclohexanone is not a genotoxic or mutagenic substance.

In chronic studies in rats and in mice exposed orally to cyclohexanone in the drinking water, tumours were reported in the lymphatic tissue, the liver, and in the lungs in mice, and in the adrenals and in the thyroid in rats. Thyroid tumours were not considered relevant by the authors; thyroid tumours in rats may, in some cases, not be relevant for humans; however, as the tumourigenic mechanism for cyclohexanone is not elucidated, no conclusion can be drawn on this effect. Some of the other tumour-types found are also of questionable relevance for humans, and the lack of dose-response indicate that the substance is not carcinogenic in these studies. However, no conclusive evaluation on the carcinogenic effect of cyclohexanone can be performed on basis of the available data.

In conclusion, the available data on cyclohexanone indicate that the critical effects of cyclohexanone are CNS depression and irritation of the skin, the eyes and the respiratory tract.

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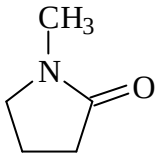
Appendix 17: 1-Methylpyrrolidone (NMP)

128 General description

128.1 Identity

Molecular formula: C_5H_9NO

Structural formula:



Molecular weight: 99.13

CAS-no.: 872-50-4

Synonyms: N-methyl-2-pyrrolidone
Methylpyrrolidone
N-Methyl-2-pyrrolidinone
1-Methyl-5-pyrrolidinone
N-Methyl-2-oxypyrrolidine
N-Methyl-2-ketopyrrolidine
NMP
Butyrolactam
N-Methyl-gamma-butyrolactone
1-Methylazacyclopentan-2-one

128.2 Physical / chemical properties

Description: NMP is a colourless, hygroscopic liquid with a mild amine odour.

Melting point: -23 to -24.4 °C

Boiling point: 202 °C (at 760 mmHg)

Density: 1.034 g/ml (at 20°C)

Vapour pressure: 0.29 mmHg (39 Pa) (at 20°C)

Concentration of saturated vapours: 1400 mg/m^3 (Åkesson 1994)
1600 mg/m^3 (calculated)

Conversion factor: 1 ppm = 4.12 mg/m^3 (at 20°C and 760 mmHg)
1 mg/m^3 = 0.243 ppm

Solubility: Water: Miscible with water.

	Highly soluble in lower alcohols, lower ketones, ether, ethyl acetate, chloroform and benzene. Moderately soluble in aliphatic hydrocarbons.
$\log P_{octanol/water}$:	-0.46 - 0.42
References:	Merck Index (1996), Åkesson (1994), IUCLID (2000), HSDB (2001), Trochimowicz et al. (1994).

129 Toxicokinetics

129.1 Absorption and distribution

129.1.1 Inhalation

In male volunteers (six individuals) exposed for 8 hours on four different days to NMP at levels of 0, 10, 25, or 50 mg/m^3, NMP was readily absorbed from the respiratory tract; a plasma half-life of about 4 (2.9-5.8) hours was found. At the end of the exposure there was a close correlation between exposures and the plasma concentration of NMP. (Åkesson & Paulsson 1997).

Rats exposed by inhalation to 620 mg/m^3 of NMP for 6 hours showed an increase in the concentration of NMP in the blood from 0 to 4 hours after termination of the exposure. (Ravn-Jonsen et al. 1992 - quoted from Åkesson 1994 and from IUCLID 2000).

129.1.2 Oral intake

In rats, NMP is readily absorbed from the gastrointestinal tract. The plasma radioactivity concentration reached peak values at 2 hours after oral administration (gavage, in water) of 22.5 mg ^{14}C-NMP (112 mg/kg b.w.). Radioactivity concentrations declined thereafter with a terminal half-life of 27 and 29 hours for females and males, respectively, and concentrations were still measurable 5 days after dosing. The parent compound accounted for more than 80% of the plasma radioactivity until 8 hours post-administration, indicating little first-pass metabolism. Biotransformation rapidly became more evident thereafter, and by 12 hours virtually all of the plasma radioactivity was in the form of polar metabolites. (Midgley et al. 1992).

129.1.3 Dermal contact

After application of ^{14}C-NMP to the skin of rats (2.5 mg in isopropanol to an area of 9 cm^2 of shaved skin on the back), the plasma radioactivity levels changed little during 1 to 6 hours and reached peak values at 6 and 2 hours for males and females, respectively. Radioactivity concentrations declined thereafter and were still measurable 5 days after dosing. The urinary excretion profiles suggested a percutaneous uptake of about three-quarters of the applied dose. The parent compound accounted for more than 80% of the plasma radioactivity until 8 hours post-administration, thereafter biotransformation rapidly became more evident and at 12 hours virtually all of the plasma radioactivity was in the form of polar metabolites. (Midgley et al. 1992).

In male rats administered ^{14}C-NMP dermally at doses of 0.2, 2.0, or 20 mg/cm^2, 50% of the 0.2 or 2.0 mg/cm^2 doses and 75% of the 20 mg/cm^2 dose was absorbed (Research Triangle Institute 1991 - quoted from IUCLID 2000).

The uptake of NMP through human skin *in vitro* is about 4 times lower than through rat skin (Priborsky & Mühlbachova 1990 - quoted from Åkesson 1994).

129.1.4 Other routes

The disposition of NMP was studied in male rats given a single intravenous dose (45 mg/kg b.w.) of [4-^{3}H]-NMP, [*methyl*-^{14}C]-NMP and [*ring*-^{14}C]-NMP in single-labelled studies or double-labelled studies. Plasma levels of NMP measured at 6 hours after dosing suggest a rapid distribution phase followed by a slow elimination phase; the half-life for the elimination from plasma was about 7 hours for both ^{14}C-isomers and about 10 hours for the ^{3}H-isomer. Metabolites were not detected in plasma in any appreciable quantities until 4 hours after administration and unmetabolised NMP still accounted for more than 80% of the radioactivity in plasma after 6 hours. Six hours after administration of NMP, the highest concentration of radioactivity occurred in the liver, small and large intestine, testes, stomach, and kidneys. After 24 hours, only the liver and intestines contained appreciable amounts of radioactivity. (Wells & Digenis 1988).

NMP passes the placenta and after exposure for 6 hours (by inhalation to 620 mg/m^{3}) foetal blood concentrations were similar to maternal blood concentrations (Ravn-Jonsen et al. 1992 - quoted from Åkesson 1994 and from IUCLID 2000).

129.2 Elimination

129.2.1 Inhalation

In male volunteers (six individuals) exposed for 8 hours on four different days to NMP at levels of 0, 10, 25, or 50 mg/m^{3}, NMP was readily eliminated from the body, mainly by biotransformation to other compounds. The elimination curve suggested a non-linear pattern and at the end of exposure showed a mean (range) half-life of NMP in the urine of about 4.5 (3.5-6.6) hours. The parent compound found in urine samples collected during exposure and at the subsequent 44 hours corresponded to about 2% of the calculated absorbed dose. At the end of the exposure, there was a close correlation between exposures and the urinary excretion of NMP. (Åkesson & Paulsson 1997).

129.2.2 Oral intake

Three healthy male volunteers were administered 100 mg NMP orally and all urine was collected during 9 consecutive days. The mean excreted fractions in the urine were 0.8% for NMP, 44% for 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP), 0.4% for N-methylsuccinimide (MSI), and 20% for 2-hydroxy-N-methylsuccinimide (2-HMSI); one-third of the dose was not recovered in urine as any of these four metabolites. There was no conjugation with glucuronic acid or sulphate. The half-lives for 5-NHMP, MSI, and 2-HMSI in urine was approximately 4, 8, and 17 hours, respectively. (Åkesson & Jonsson 1997 - quoted from TOXLINE PLUS 1999-2000/01).

Following oral administration (gavage, in water) of 22.5 mg ^{14}C-NMP (112 mg/kg b.w.) to rats, about 95% of the radioactivity was excreted during 5

days mainly through the urine (85-88% of the dose within 24 hours after dosing). Faecal excretion and exhalation were of minor importance accounting for 1-2% and 6-7% of the dose in the faeces and in exhaled air (as [^{14}C]CO_2), respectively. (Midgley et al. 1992).

In male rats given ^{14}C-NMP orally at doses of 5 or 500 mg/kg b.w., 84 and 75% of the dose, respectively, was excreted in the urine within 24 hours. In the urine, 4 metabolites were found with 5-hydroxy-N-methyl-2-pyrrolidone accounting for 60% of the administered dose; NMP was not identified in the urine. (Research Triangle Institute 1991 - quoted from IUCLID 2000).

129.2.3 Dermal contact

Following topical administration of ^{14}C-NMP to the skin of rats (2.5 mg in isopropanol to an area of 9 cm^2 of shaved skin on the back), 69 to 78% of the radioactivity was excreted during 5 days mainly through the urine (61-70% of the dose within 24 hours after dosing). Faecal excretion and exhalation were of minor importance accounting for 1-2% and 6-7% of the dose in the faeces and in exhaled air (as [^{14}C]CO_2), respectively. (Midgley et al. 1992).

Following dermal application (doses of 0.2, 2.0, or 20 mg/cm^2), the primary route of excretion was in the urine; four metabolites were found with 5-hydroxy-N-methyl-2-pyrrolidone accounting for 60% of the administered dose, NMP was not identified in the urine (Research Triangle Institute 1991 - quoted from IUCLID 2000).

129.2.4 Other routes

The disposition of NMP was studied in male rats given a single intravenous dose (45 mg/kg b.w.) of [4-^{3}H]-NMP, [*methyl*-^{14}C]-NMP and [*ring*-^{14}C]-NMP in single-labelled studies or double-labelled studies. The major route of excretion of radioactivity was via the urine and accounted for about 70% of the dose within 12 hours. After 24 hours, cumulative excretion in the urine represented about 80% of the dose. Cumulative excretion of total radioactivity into faeces and exhaled air was minor, with only 2.4% of the dose recovered in faeces after 72 hours and about 1% (as [^{14}C]CO_2) in exhaled air after 24 hours. There was a minor biliary excretion of about 2% of the administered dose.
Analyses of urine revealed the presence of one major metabolite accounting for 72% of the excreted radioactivity and two minor metabolites accounting for 9 to 21% and 9-15% of the excreted radioactivity, respectively. Acidic hydrolysis of the major metabolite yielded a substance, which was identified as 4-(methylamino)-butenoic acid; the identity of the intact major metabolite remained to be identified. Only a minor part (<1%) was excreted in the urine as the parent compound. (Wells & Digenis 1988).

129.3 Mode of action

No data have been found.

130 Human toxicity

130.1 Single dose toxicity

No data have been found.

130.2 Repeated dose toxicity

130.2.1 Inhalation

In male volunteers (six individuals) exposed for 8 hours on four different days to NMP at levels of 0, 10, 25, or 50 mg/m^3, no discomfort to eyes or upper airways was reported. Acute changes in nasal volume were not found, and no changes in the spirometric data (FEV_1, VC or FVC) could be registered. Two individuals reported an odour of acetone at 50 mg/m^3; the odour was reported as "not uncomfortable". (Åkesson & Paulsson 1997).

A study of workers employed in the microelectronic (semiconductor) fabrication industry indicated that typical exposures to NMP as the vapour ranged from 0.02 to 1.5 ppm (0.08-6.2 mg/m^3). All samples taken consisted of approximately 8-hour time-weighted average (TWA) samples (area and personal breathing zone) and were taken from 5 different work areas: 1) application of a liquid "protective coating" onto the surface of the microelectronic devices with NMP being the primary vehicle (automated process at room temperature); 2) use of pure NMP liquid for the flushing of equipment tubing during routine cleaning operations (room temperature); 3) "assembly die-coat area" where an oven door (oven temperature 140°C) is opened and the warm product is removed and loaded onto a transfer cart (local exhaust ventilation along with general ventilation from ceiling to floor); 4) "T/A die-coat area" with same procedure as 3) but without the general ventilation system; 5) "NMP dip tank cleaning area" where products are immersed in NMP baths kept at 71-82°C.
Workers reported exposures from 49 to 83 ppm (202-342 mg/m^3) as being unbearable for even a minimal amount of time. At lower concentrations of about 16 ppm (66 mg/m^3), the workers reported an immediate perception, immediately uncomfortable (within 30 seconds) with minor eye irritation for this short exposure time. At concentrations from 0.72 to 1.5 ppm (3.0-6.2 mg/m^3, 8-hour TWA), the workers reported immediate perception as a mild, yet pungent odour, uncomfortable (severe eye irritation) after about 30 minutes, and some workers with full-shift exposures complained of chronic headaches. At exposures below 0.03 ppm (0.1 mg/m^3), no sensation and no problems or concerns were reported.
(Beaulieu & Schmerber 1991).

Exposures to NMP levels of 280 mg/m^3 or higher were unbearable to exposed workers even for a few seconds (Åkesson 1994).

130.2.2 Oral intake

No data have been found.

130.2.3 Dermal contact

Several workers in an electrotechnical company experienced skin irritation after a few days of working with NMP. Ten out of 12 exposed workers displayed acute irritant contact dermatitis of the hands. The severity of the reactions seemed to reflect the degree and duration of contact with NMP and the effects cleared within 3 weeks after termination of exposure. (Leira et al. 1992 - quoted from Åkesson 1994 and from Toxline 1991-1994).

A total of fifteen 24-hour exposures of NMP to the skin every other day in 50 human subjects caused various minor to moderate transient irritations; no signs of contact sensitisation were observed (International Speciality Products - quoted from Åkesson 1994; GAF Corp. 1974 - quoted from Trochimowicz et al. 1994 and from IUCLID 2000).

Evidence has shown the product (no further details are given) to be moderately irritating to human skin with prolonged or repeated exposure. Dermatitis, oedema, redness, blisters or cracking can occur. No further details are given. (IUCLID 2000).

130.3 Toxicity to reproduction

A case of intrauterine growth retardation followed by foetal demise at 31 weeks gestation has been reported; the mother was a laboratory worker with no other apparent risk factors, who sustained occupational exposure to NMP throughout the first trimester of pregnancy (Solomon et al. 1996).

130.4 Mutagenic and genotoxic effects

No data have been found.

130.5 Carcinogenic effects

No data have been found.

131 Animal toxicity

131.1 Single dose toxicity

131.1.1 Inhalation

In a study (OECD Guideline 403), the LC_{50}-value (4-hour exposure) was reported as being greater than 5.1 mg/l (>5100 mg/m^3) in the rat (BASF 1988 - quoted from IUCLID 2000). In another study in the rat, the LC_{50}-value (4-hour exposure) was reported as being in the range of 3.1 to 8.8 mg/l (3100-8800 mg/m^3) (Du Pont 1988 - quoted from IUCLID 2000).

In rats exposed to a saturated atmosphere (NMP heated to 110°C) for 6 hours, no mortality was observed and the animals did not show any signs of toxicity during the 2-week observation period (GAF 1986 - quoted from IUCLID 2000).

When rats were exposed to a saturated atmosphere (NMP at 20°C) for 8 hours, no mortality occurred but animals showed a mild irritation of mucous membranes (BASF 1963 - quoted from IUCLID 2000).

In mice exposed to NMP at concentrations around 0.2 mg/l (200 mg/m^3) (NMP heated to 100-120°C) for 2 hours, no mortality was observed, but irritation of eyes and the upper respiratory tract was observed. No histopathological changes were observed. (Stasenkow & Kotschekov 1965 - quoted from IUCLID 2000).

131.1.2 Oral intake

The reported oral LD_{50}-values for NMP ranged from 3600 to 7900 mg/kg in rats (13 values reported), from 4100 to 7700 mg/kg in mice (7 values reported), and of 3500 mg/kg and 4400 mg/kg in rabbits and guinea pigs, respectively (2 values reported for each species) (Studies quoted in IUCLID 2000).

131.1.3 Dermal contact

The reported dermal LD_{50}-values for NMP ranged from 2500 to 10000 mg/kg in rats (4 values reported), and from 2000 to 8000 mg/kg in rabbits (5 values reported) (Studies quoted in IUCLID 2000).

Skin irritation tests in rabbits using a modified Draize procedure indicated a low potential for skin irritation. (International Speciality Products - quoted from Åkesson 1994).

Skin irritation tests in rabbits (7 references) indicate that NMP is slightly irritating in this species; no irritation was reported in 2 other references (Studies quoted in IUCLID 2000).

Aqueous solutions of NMP caused skin irritation in guinea pigs at 50% but not at 5% (International Speciality Products - quoted from Åkesson 1994; GAF 1974 - quoted from IUCLID 2000).

131.1.4 Other routes

Eye irritation tests in rabbits (8 references) indicate that NMP is an eye irritant in this species; no eye irritation was noted (one reference) when NMP was tested as a 10 or 25% aqueous solution (Studies quoted in IUCLID 2000).

131.2 Repeated dose toxicity

131.2.1 Inhalation

In Wistar rats (10 animals) exposed (head-nose only) to NMP (mixture of vapour and aerosol) at 1000 mg/m^3 8 hours a day, 5 days per week for 2 weeks, no pathological lesions were observed. The urine was dark yellow. (BASF 1989 - quoted from IUCLID 2000).

Wistar rats (5 animals of each sex per group) were exposed (head-nose only, OECD-guideline 412) to NMP aerosol at 4000, 7000, or 10000 mg/m^3 6 hours a day, 5 days per week for 14 days. High-dose animals as well as some mid-dose animals died within 5 days. The urine was dark yellow in all animals. A dose-related reduced body weight gain and respiratory tract irritation were observed. Pathological lesions were observed in the lungs of mid- and high-dose males and in the glandular stomach in all high-dose males and in all exposed females. (BASF 1993 - quoted from IUCLID 2000).

Rats (Charles River CD, 15 animals of each sex per group) were exposed to an aerosol-vapour mixture of NMP at levels of 100, 500, or 1000 mg/m^3 for 6 hours a day, 5 days per week for 4 weeks. The control group was exposed to air only. Each test level of NMP was generated as a respirable aerosol (>95% of the droplets below 10 µm in diameter). (Lee et al. 1987).
All exposed rats showed signs of lethargy and irregular respiration after about 3 to 4 hours of exposure; these signs usually persisted until the end of each exposure. Low- and mid-dose rats recovered within 30 to 45 minutes post-exposure whereas high-dose rats generally did not recover by 18 hours post-exposure.
In high-dose rats, excessive mortality occurred (8 died and 5 were sacrificed of 30 animals) within the first 9 days of exposure and exposure was discontinued after 10 days; the surviving rats were placed on 2 weeks of recovery. In dead rats, marked pulmonary oedema and congestion were observed; the bone marrow showed haemorrhage, hypoplasia, and necrosis in the haemopoietic cells; and prominent atrophy and necrosis were observed in the lymphoid tissue in the thymus, spleen, and lymph nodes. Rats sacrificed after 10 days of exposure revealed slight bone marrow hypoplasia and atrophy of lymphoid tissue in the thymus, spleen, and lymph nodes; minimal necrotic changes were observed in the haemopoietic cells of the bone marrow and lymphoid cells in the thymus, spleen, and lymph nodes. The histopathological changes were almost reversible within the 2-week recovery period.

No significant histopathological lesions were observed in low- and mid-dose animals.

In Wistar rats exposed (head-nose only, OECD-guideline 412) to NMP aerosol at 10, 30, or 100 mg/m^3 6 hours a day, 5 days per week for 4 weeks, no pathological lesions were observed. In high-dose animals, the urine was dark yellow. (BASF 1993 - quoted from IUCLID 2000).

Sprague-Dawley rats (10 animals of each sex) were exposed to NMP vapour at 1750 mg/m^3 6 hours a day, 5 days per week for 6 weeks. From the 8th day of exposure, rats exhibited a mild secretion from the nose. The urine was dark yellow in all animals. No pathological lesions were observed. (BASF 1983 - quoted from IUCLID 2000).

Wistar rats were exposed (head-nose only, OECD-guideline 413) to NMP (aerosol or vapour not stated) at 500, 1000, or 3000 mg/m^3 6 hours a day, 5 days per week for 90 days. The urine was dark yellow in all animals. Irritation of the respiratory tract and reduced body weight (males) were observed in mid- and high-dose animals. Signs of mild hepatic effects were observed in high-dose animals. No histopathological lesions or signs of toxicity were observed in the mid- and low-dose groups. Testicular effects are given in 4.3. (BASF 1994 - quoted from IUCLID 2000).

In Wistar rats exposed to 150 ppm (620 mg/m^3, the highest possible concentrations of NMP vapour) NMP 6 hours a day, 7 days per week for 90 days, examination of evoked potentials in the brain at the termination of exposure showed no indications of neurotoxic effects in the central nervous system (Fries et al. 1992).

Rats (Charles River CD, 120 animals of each sex per group) were exposed to a mixture of NMP aerosol and vapour (only trace amounts of aerosol was detected when chamber atmosphere was analysed) at levels of 40 or 400 mg/m^3 for 6 hours a day, 5 days per week for 2 years. The control group (120 animals of each sex) was exposed to air only. (Lee et al. 1987).
No significant differences in either morbidity or mortality were observed between exposed and control rats. Low-dose females and high-dose rats showed a greater incidence of stained wet perinea than the respective controls. High-dose rats discharged dark yellow urine and the male rats had a greater urine volume. High-dose male rats had after 2 years exposure gained approximately 6% less body weight than the controls.
Histopathological examinations were performed on most organs and tissues. There were no differences in either the incidence or severity of neoplastic or nonneoplastic lesions between exposed and control rats except for a slightly higher incidence of chronic progressive nephropathy in low-dose male rats at 12 months.

131.2.2 Oral intake

NMP was administered in the diet to rats (Crl:CD BR; 5 animals of each sex per group) at dose levels of 0, 2000, 6000, 18000, or 30000 ppm (equivalent to 0, 200, 600, 1800, 3000 mg/kg b.w. per day according to WHO standard assumptions) for 28 days. Abnormal urine coloration was observed at dose levels from 18000 ppm. Reduced body weight gain and food consumption were observed in the 18000 ppm (males) and 30000 ppm dose groups.

Histopathological alterations (hypocellular bone marrow, testicular degeneration and atrophy, and thymic atrophy) observed in high-dose animals were judged to be secondary to nutritional and body weight effects in these rats. (Malek et al. 1997 - quoted fromTOXLINE PLUS 1999-2000/01).

Sprague-Dawley rats (10 animals of each sex per group) were given NMP by gavage at 0, 258, 517, 1033, or 2066 mg/kg b.w. 5 days per week for 4 weeks. High-dose animals exhibited tremor, restlessness, piloerection, and defensive reactions after two weeks of exposure. The urine was dark yellow in all animals after one week of exposure. A reduced body weight gain was observed in male rats of the three highest dose groups (from 517 mg/kg b.w.). (BASF 1978 - quoted from IUCLID 2000).

In Wistar rats (25 animals of each sex per group) given NMP in the diet at 0, 800, 2000, or 5000 ppm (NMP levels in diet equivalent to 70, 170, 420 mg/kg b.w. according to IUCLID) for 90 days, no pathological lesions were observed. In high-dose males, the thyroid weight was increased when compared to the control group. (GAF Corp. 1976 - quoted from IUCLID 2000).

Rats (20-26 male and females per group) were administered NMP at dietary levels of 0, 3000, 7500, or 18000 ppm (equal to 0, 169/217, 433/565, or 1057/1344 mg/kg b.w./day for males/females, respectively) for 90 days. A change in urine coloration was observed at all dose levels in both sexes. Decreased body weight and body weight gain were observed in mid- and high-dose groups. Increased absolute and relative kidney weights were observed in high-dose animals. In high-dose females, absolute and relative liver weights were increased and were associated with an increased incidence of centrilobular hepatocellular hypertrophy. Of 36 neurobehavioral parameters investigated, mid- and high-dose male rats showed increases in mean foot splay values and high-dose males had a higher incidence of low arousal and slight palpebral closure. There were no compound-related gross or microscopic lesions in neural or muscular tissues at any dietary concentration. The NOAEL was considered to be 3000 ppm (equal to 169/217 mg/kg b.w./day for males/females, respectively). (Malley et al. 1999).

NMP was administered for 28 days in the diet to mice (B6C3F1; 5 animals of each sex per group) at dose levels of 0, 500, 2500, 7500, or 10000 ppm (equivalent to 0, 75, 375, 1125, 1500 mg/kg b.w. per day according to WHO standard assumptions). Abnormal urine coloration was observed at dose levels from 2500 ppm. Histopathological alterations (cloudy swelling of the epithelia of the distal parts of the renal tubuli) were observed in some animals of the two highest dose levels. (Malek et al. 1997 - quoted from Toxline 1995-1998).

CD-1 mice (30 animals of each sex per group) were given NMP in the diet at 0, 400, 1000, or 2500 ppm (NMP levels in diet equivalent to 80, 200, 500 mg/kg b.w. according to IUCLID) for 90 days. A dose-related decreased body weight gain and increased relative liver weight, kidney weight, thyroid weight and pituitary weight were observed in male animals, a NOAEL of 400 ppm was stated. No effects were noted in female animals. (GAF Corp. 1977 - quoted from IUCLID 2000).

Mice (10 male and females per group) were administered NMP at dietary levels of 0, 1000, 2500, or 7500 ppm (equal to 0, 277, 619, or 1931 mg/kg b.w./day over the 90-day period) for 28 or 90 days. A change in urine coloration was observed in mid- and high-dose animals. Absolute and relative liver weights were elevated in mid- and high-dose males and centrilobular hepatocellular hypertrophy was seen in both sexes fed 7500 ppm. The NOAEL was considered to be 1000 ppm (equal to 1931 mg/kg b.w./day). (Malley et al. 1999).

In Beagle dogs given NMP in the diet at 0, 25, 79 or 250 mg/kg b.w. per day for 90 days, a dose-dependent decrease in body weight gain and a reduced serum cholesterol level in high-dose males were observed (Becci et al. 1983 - quoted from IUCLID 2000 and from Åkesson 1994).

131.2.3 Dermal contact

When NMP was applied to intact or damage skin of rabbits at concentrations of 0.4, 0.8, or 1.6 ml/kg (411, 822, 1645 mg/kg b.w.) once daily for 20 days, mild irritation of the skin but no systemic effects were noted in low- and mid-dose animals. Among high-dose animals, 1/4 died when NMP was applied to damaged skin. (GAF 1986 - quoted from IUCLID 2000).

NMP did not show any skin sensitisation when tested in guinea pig using the Draize test or an intracutaneous test (Studies quoted in IUCLID 2000).

In the intracutaneous test, male guinea pigs received 4 injections (one per week, 0.1 ml 1% v/v aqueous solution); after a 2-week rest period they were challenged by dermal application of a 5 or 50% solution. No sensitisation was observed. (DuPont 1976 - quoted from IUCLID 2000).

131.2.4 Other routes

No data have been found.

131.3 Toxicity to reproduction

131.3.1 Inhalation

In Wistar rats exposed (head-nose only, OECD-guideline 413) to NMP (aerosol or vapour not stated) at 500, 1000, or 3000 mg/m^3 6 hours a day, 5 days per week for 90 days, reduced testicular weight and testicular damage were observed in high-dose animals; the testicular damage was not reversible within a 4-week post-treatment period. Non-testicular effects are given in 4.2. (BASF 1994 - quoted from IUCLID 2000).

In Wistar rats exposed to 150 ppm (620 mg/m^3, the highest possible concentrations of NMP vapour) NMP 6 hours a day, 7 days per week for 90 days, examination at the termination of exposure and 90 days later showed no macroscopically pathological findings and no differences in testes weights between the control and exposed group. By histopathological examination of the testes no abnormal changes were found and by the examination of the semen, sperm cell morphology and sperm cell concentration, no abnormalities were observed. (Fries et al. 1992).

A two-generation reproduction study with a developmental toxicity component was conducted on rats. For the reproduction phase, male and female rats were exposed to NMP at levels of 0, 10, 51, or 116 ppm (0, 42, 210, or 480 mg/m^3) 6 hours a day, 7 days per week from 34 days of age to the end of the mating period for the males (100 exposure days) and till weaning for the females (about 143 exposure days, but interrupted from day 20 of gestation to day 4 postpartum). For the developmental phase, rats of both sexes were exposed to 0 or 116 ppm. The indices of reproductive performance for exposed rats did not differ significantly from those obtained for the control rats. High-dose rats had a detectable decrease in response to sound; no other signs of NMP-related toxicity were detected among the parental rats. An exposure-related but slight decrease in foetal weight was detected only among the F1 offspring whose parents were exposed to 116 ppm; this slight effect also appeared at birth among the pups of the reproductive phase where it persisted till 21 days after birth when NMP inhalation by the mother ceased. Thereafter, the body weight of the offspring was comparable to the control values. No developmental effects appeared in the 10 or 51 ppm groups. (Solomon et al. 1995 - quoted from Toxline 1995-1998).

Female rats (Charles River CD, 25 animals per group) were exposed to NMP (aerosol) at levels of 100 or 350 mg/m^3 for 6 hours a day on gestation days 6 to 15. The control group was exposed to air only. Sporadic lethargy and irregular respiration were observed in several animals at both exposure levels during the first 3 days of exposure. Exposed rats did not show any significant pathological changes in any vital organs or tissues. Exposure did not affect the outcome of pregnancy or embryonal growth rate. No abnormal development was detected in vital organs and skeletons of the foetuses. (Lee et al. 1987).

In Wistar rats exposed to 150 ppm (620 mg/m^3) NMP 6 hours a day, 7 days per week on gestation days 4 to 20, increased preimplantation loss, lower foetal body weights, and delayed ossification were observed; these effects were induced at a dose that did not induce maternal toxicity. No increase in malformations was found. (Hass et al. 1995).

In a behavioural teratology study, Wistar rats were exposed to 150 ppm (620 mg/m^3) NMP 6 hours a day on gestation days 7 to 20. No clinical signs of maternal toxicity were observed and no differences between the exposed and the control group concerning maternal weight gain during the gestation period, pregnancy length, number of implants per dam, number of pups and sex distribution in the litters, and neonatal deaths were found. The only difference observed in the gestation period was that the urine of the females in the exposed group was coloured bright yellow. In the pre-weaning period, the exposed offspring had a lower body weight and their physical development was delayed. Neurobehavioral evaluation of the male pups revealed no effects on basal functions of the central nervous system. The animals appeared normal and motor function (rotarod), activity level (open field), and performance in learning tasks with a low grade of complexity were similar in the two groups. However, in more difficult tasks such as the reversal procedure in Morris water maze and operant delayed spatial alternation (Skinner boxes), performance was impaired in exposed offspring. (Hass et al. 1994).

Female rats (Wistar, 9-10 animals per group) were exposed (head-nose only) to NMP (aerosol) at levels of 1000, 2000, or 3000 mg/m^3 for 6 hours a day, 5 days per week on gestation days 5 to 16. In maternal animals, body weight and uterine weight were reduced in a dose-related manner and the urine was yellow to red coloured in all exposed groups. In the mid- and high-dose groups, vaginal bleeding at days 14 and 15 post-conception, increased post-implantation loss, and reduced percentage of live foetuses per litter were observed. (BASF 1992 - quoted from IUCLID 2000).

In Sprague-Dawley rats (20 animals) exposed to 800 ppm (3300 mg/m^3) NMP for 6 hours a day on gestation days 4 to 8, or on gestation days 11 to 15, no differences were observed between exposed animals and controls in the outcome of pregnancy, embryonal growth rate, weight of placenta, and number of malformations. No maternal toxicity was observed. (BASF 1976 - quoted from IUCLID 2000). However, according to Trochimowicz et al. (1994) “slight embryo and maternal toxicity was observed”.

Sprague-Dawley rats (20-25 pregnant rats per group) were exposed whole-body to NMP vapours at concentrations of 0, 30, 60, or 120 ppm (0, 124, 248, or 495 mg/m^3) 6 hours/day on gestational days (GD) 6 through 20. Maternal body weight gain was significantly decreased in the mid- and high-dose groups on GD 6 to 13 and maternal food consumption was reduced in the high-dose group on GD 13 to 21. No significant difference in the gestational weight change corrected for the weight of the gravid uterus was observed. There were no adverse effects on embryo/foetal viability as the mean numbers of implantation sites and of live foetuses and the incidences of non-live implants and resorptions were comparable across groups. Foetal toxicity indicated by reduced foetal weight was observed at 120 ppm. There was no evidence of teratogenicity at any concentration tested. (Saillenfait et al. 2003).

In rabbits (5 animals per group) exposed to NMP at levels of 300, 1000, or 2000 mg/m^3 for 6 hours a day on gestation days 7 to 19, maternal toxicity (increased liver weight, decreased uterine weight, and a number of haematological parameters) were observed in mid- and high-dose animals and the number of resorptions was increased in the high-dose group (BASF 1991 - quoted from IUCLID 2000).

Rabbits (15 animals per group) were exposed to NMP at levels of 200 (vapour), and 500 or 1000 (mixture of vapour and aerosol) mg/m^3 for 6 hours a day on gestation days 7 to 19 (OECD-guideline 414). No signs of maternal toxicity were observed; the urine was dark yellow to orange coloured in all exposed groups. The only effect observed in the foetuses was a skeletal variation in rib number 13 in the high-dose group. (BASF 1993 - quoted from IUCLID 2000).

131.3.2 Oral intake

In Sprague-Dawley rats (10 animals of each sex per group) given NMP by gavage at 0, 258, 517, 1033, or 2066 mg/kg b.w. 5 days per week for 4 weeks, the absolute and relative testicular weights were significantly reduced and testicular damage was observed in high-dose male rats. (BASF 1978 - quoted from IUCLID 2000).

In a multi-generation study (OECD-guideline 416), Sprague-Dawley rats (30 animals of each sex per group) were given NMP at levels of 0, 50, 160, or 500 mg/kg b.w. per day in the diet for 13 months. The highest dose level caused reduced parental body weight and feed consumption, and affected reproduction with a concomitant reduction in survival and growth rates in the offspring. (Exxon 1991 - quoted from IUCLID 2000; EPA - quoted from Åkesson 1994). It is not clear from the two citations whether effects were observed in low- and mid-dose groups. In the IUCLID citation it is stated that "no effects were observed in low- and mid-dose groups", whereas in the citation by Åkesson it is stated that "the data from the 50 and 160 mg/kg b.w. per day experiments do not clearly demonstrate a NOAEL.

In Sprague-Dawley rats given NMP by gavage at 0, 40, 125, or 400 mg/kg b.w. on gestation days 6 to 15, a reduced body weight gain was observed in the high-dose group; however, when correction for the gravid uterine weight was done, no difference in body weight gain was observed between exposed animals and the controls. The foetal body weight was significantly reduced in the high-dose group and an increased number of stunted foetuses was reported;the incidence of foetal variations and malformations was not increased in offspring of exposed dams when compared to the control group. (TSCAT 1992 - quoted from IUCLID 2000; EPA - quoted from Åkesson 1994).

Repeated oral doses of 1000 mg/kg b.w. per day to Sprague-Dawley rats through gestation days 6 to 15 caused a very high resorption rate (95%) and malformations in 8 out of 15 surviving developed foetuses; reduction in body weight (not further specified) of the dams was observed. No adverse effects were observed at doses of 330 mg/kg b.w. per day. (BASF 1971 - quoted from IUCLID 2000; EPA 1991 - quoted from Åkesson 1994 and from Toxline 1991-1994; BASF 1987 - quoted from Trochimowicz et al. 1994).

Repeated oral doses of 2640 mg/kg b.w. per day to NMRI mice through gestation days 11 to 15 caused increased resorption rate, increased incidence of runts, decreased foetal weight and length, and increased incidence of malformations such as cleft palate; no maternal toxicity was observed. Doses of 1060 mg/kg b.w. per day caused no observable embryotoxicity. (BASF 1970 - quoted from IUCLID 2000; EPA - quoted from Åkesson 1994; Schmidt 1976 - quoted from Trochimowicz et al. 1994).

In rabbits given NMP by gavage at 0, 55, 175, or 540 mg/kg b.w. on gestation days 6 to 18 (OECD-guideline 414), maternal toxicity (reduced body weight gain and feed consumption) was evident in the high-dose group with only trends being observed in the mid-dose group. Developmental toxicity in form of increased post-implantation loss, altered foetal morphology and increased incidences of cardio-vascular and skull malformations was observed in the high-dose group; no developmental toxicity were detected in the lower dose groups. (GAF Corp. 1991 - quoted from IUCLID 2000; ISP - quoted from Åkesson 1994).

131.3.3 Dermal contact

In Sprague-Dawley rats given NMP (undiluted) topically at 0, 75, 237, or 750 mg/kg b.w. on gestation days 6 to 15, a reduced body weight gain was observed in the high-dose group; the resorption of foetuses was increased, the

foetal body weight decreased, and skeletal anomalies were observed in this dose group (no correction for gravid uterine weight was done). No effects in dams or foetuses were observed in the lower dose groups.
In a preceding dose range-finding study, a dermal dose of 1100 mg/kg b.w. per day during days 6 to 15 of gestation was embryolethal (65/66 foetuses absorbed) and caused decreased body weight gain in dams; a dose of 500 mg/kg b.w. per day had no adverse effect on pregnancy, dam body weights, implantations or gestation.
(Becci et al. 1982 - quoted from Åkesson 1994, IUCLID 2000, and from Trochimowicz et al. 1994).

Rabbits (15 animals per dose group) were given NMP (40% solution in water) topically at 0, 100, 300, or 1000 mg/kg b.w. for 6 hours a day on gestation days 7 to 19 (OECD-guideline 414). No signs of maternal toxicity were observed in any dose-group; the urine was red-brown coloured in the second half of the gestation period in mid- and high-dose groups. The only effect observed in the foetuses was a skeletal variation in rib number 13 in the high-dose group. (BASF 1993 - quoted from IUCLID 2000).

131.3.4 Other routes

Various intraperitoneally administered NMP doses (14-166 mg/kg b.w.; single or repeatedly) to mice (two strains) during various phases of pregnancy, caused increased post-implantation loss and a reduced body weight of the foetuses; and morphological malformations were observed in the foetuses. No information on maternal toxicity is given in the study. (Schmidt 1976 - quoted from Åkesson 1994 and from IUCLID 2000).

Repeated intraperitoneal doses of 1570 mg/kg b.w. per day to NMRI mice through gestation days 11 to 15 caused increased resorption rate, increased incidence of runts, decreased foetal weight and length, and increased incidence of malformations such as cleft palate; no maternal toxicity was observed. Doses of 630 mg/kg b.w. per day caused no observable embryotoxicity. (BASF 1970 - quoted from IUCLID 2000; EPA - quoted from Åkesson 1994).

131.4 Mutagenic and genotoxic effects

131.4.1 *In vitro* studies

NMP has been tested in different test systems, the results of these tests as given below are quoted from Åkesson (1994), Trochimowicz et al. (1994), and from IUCLID (2000).

In tests for point mutations, NMP was negative in several (10) Ames tests using various strains of *Salmonella typhimurium* when tested up to cytotoxic concentrations both with and without metabolic activation systems.

NMP showed positive results for aneuploidy in yeast (*Saccharomyces cerevisiae*) in combination with cold shock (4°C or ice-bath), but not with continuous incubation at 20°C.

When tested in mammalian cells *in vitro*, NMP was negative for mutations in HGPRT-locus in CHO cells, in the mouse lymphoma assay, and for unscheduled DNA synthesis in rat primary hepatocytes.

131.4.2 *In vivo* studies

When tested in mammalian cells *in vivo*, NMP was negative in a cytogenetic assay (bone marrow test for structural and numerical chromosomal aberrations) in Chinese hamsters (exposed to 800 ppm (3300 mg/m^3) NMP 6 hours a day, 5 days per week for 6 weeks), in the dominant lethal assay in NMRI mice (single intraperitoneal injection of 393 mg/kg b.w.), and in the micronucleus assay in NMRI mice (single oral dose of 950, 1900, or 3800 mg/kg b.w.).

131.5 Carcinogenic effects

Rats (Charles River CD, 120 animals of each sex per group) were exposed to a mixture of NMP aerosol and vapour (only trace amounts of aerosol was detected when chamber atmosphere was analysed) at levels of 40 or 400 mg/m^3 for 6 hours a day, 5 days per week for 2 years. The control group (120 animals of each sex) was exposed to air only. (Lee et al. 1987).
There were no differences in the incidence or severity of neoplastic lesions between exposed and control rats. High-dose female rats exhibited a decreased incidence of mammary gland tumours and increased incidence of mammary gland hyperplasia. A slightly greater incidence of pituitary tumours was observed in low-dose but not in high-dose rats. Nonneoplastic findings are given in 4.2.

A two-year carcinogenicity study (feeding) has been performed in rats (Dupont Haskell Laboratory 1998 – cited in Toxline 2001); however, the results of the study have apparently not been published.

132 Regulations

132.1 Ambient air

Denmark (C-value): -

132.2 Drinking water

Denmark: -

132.3 Soil

Denmark: -

132.4 Occupational Exposure Limits

Denmark: 5 ppm (20 mg/m^3) (At 2002)

132.5 Classification

NMP is classified for irritative effects (Xi;R36/38 - irritating to eyes and skin) (MM 2002).

132.6 IARC

-

132.7 US-EPA

-

133 Summary

133.1 Description

NMP is a colourless, hygroscopic liquid with a mild amine odour. NMP is miscible with water. Air saturated with NMP at 20°C has an NMP concentration of around 1400 to 1600 mg/m^3; higher airborne exposure levels will thus consist of a mixture of vapour and aerosol.

133.2 Toxicokinetics

In humans, a close correlation between inhalation exposures and the plasma concentration of NMP was observed; a plasma half-life of about 4 hours was found. NMP was readily eliminated, mainly by biotransformation to other compounds (major metabolites in urine being 5-hydroxy-N-methyl-2-pyrrolidone and 2-hydroxy-N-methylsuccinimide); the major route of excretion being the urine. The elimination curve suggested a non-linear pattern and at the end of exposure showed a main (range) half-life of NMP in the urine of about 4.5 (3.5-6.6) hours.

In the rat, NMP is readily absorbed through the respiratory and gastrointestinal tracts and through the skin. The plasma radioactivity concentration peaked at 2 or 2-6 hours following oral or topical administration, respectively. Following absorption, NMP is distributed widely to organs and tissues with the highest concentrations occurring in the liver, small and large intestine, testes, stomach, and kidneys. NMP is rapidly metabolised and excreted, the major route of excretion being the urine (85-88%). Four metabolites have been found in the urine with 5-hydroxy-N-methyl-2-pyrrolidone being the major one.

133.3 Human toxicity

133.3.1 Single dose toxicity

No data have been found.

133.3.2 Repeated dose toxicity

Volunteers exposed to NMP at levels up to 50 mg/m^3 did not report any discomfort to eyes or upper airways and no changes in the spirometric data could be registered; two individuals reported an odour of acetone at 50 mg/m^3, the odour was reported as “not uncomfortable”.
Workers in the microelectronics industry have reported severe eye irritation following exposure for a short time (30 minutes) to levels of about 3 mg/m^3 (8-hour TWA), exposures around 66 mg/m^3 were reported as being immediately uncomfortable (within 30 seconds) with minor eye irritation, and exposures above 200 mg/m^3 were found unbearable following a few seconds of exposure.

Several workers have experienced skin irritation and contact dermatitis on the hands after a few days of working with NMP; no signs of contact sensitisation have been reported.

133.3.3 Toxicity to reproduction

One case of intrauterine growth retardation followed by foetal demise at 31 weeks gestation has been reported in a laboratory worker exposed to NMP throughout the first trimester of pregnancy.

133.3.4 Mutagenic and genotoxic effects

No data have been found.

133.3.5 Carcinogenic effects

No data have been found.

133.4 Animal toxicity

133.4.1 Single dose toxicity

In the rat, the 4-hour LC_{50}-value has been reported as being greater than 5100 mg/m^3 or in the range of 3100-8800 mg/m^3. The reported oral and dermal LD_{50}-values ranged from 3600-7900 mg/kg and from 2500-10000 mg/kg, respectively, in the rat.

Skin irritation tests in rabbits indicated a low potential for skin irritation, whereas NMP is an eye irritant in this species; NMP did not show any skin sensitisation when tested in the guinea pig.

133.4.2 Repeated dose toxicity

Mortality occurred within 9 days in rats exposed to NMP (aerosol-vapour mixture, respirable aerosol with >95% of the droplets being below 10 µm in diameter) at 1000 mg/m^3 (6 hours a day, 5 days per week); marked pulmonary oedema and congestion, bone marrow effects (haemorrhage, hypoplasia, and necrosis in the haemopoietic cells), and prominent atrophy and necrosis in the lymphoid tissue in the thymus, spleen and lymph nodes were observed in dead animals.

No histopathological lesions have been observed in rats exposed by inhalation (6 hours a day, 5 days per week) to NMP at up to 500 mg/m^3 for 2 or 4 weeks; at 1750 mg/m^3 for 6 weeks; up to 1000 mg/m^3 for 13 weeks; or up to 400 mg/m^3 for 2 years; at levels above 4000 mg/m^3 (2 weeks) lesions were observed in the lungs and in the glandular stomach.
Following oral administration (dietary), no pathological lesions were observed in rats and mice when NMP was administered at dose levels up to 420 mg/kg b.w. per day (rats) or up to 500 mg/kg b.w. per day (mice) for 90 days; centrilobular hepatocellular hypertrophy has been observed in female rats (1344 mg/kg b.w. per day) and in both sexes of mice (1931 mg/kg b.w. per day) following dietary administration for 90 days.

Mild secretion from the nose has been observed in rats exposed to 1750 mg/m^3 and irritation of the respiratory tract has been reported following

exposure from about 1000 mg/m^3. One study has reported signs of lethargy and irregular respiration in rats after exposure to 100-1000 mg/m^3 for 3 to 4 hours. Following exposure to NMP at 620 mg/m^3 (6 hours a day, 7 days per week for 90 days), no indications of neurotoxic effects in the central nervous system of rats were observed.
Tremor, restlessness, piloerection, and defensive reactions have been reported in rats 2 weeks following oral administration (2066 mg/kg b.w per day by gavage).
In a 90-day dietary study, male rats showed an increase in mean foot splay values (from 433 mg/kg b.w. per day) and a higher incidence of low arousal and slight palpebral closure (at 1057 mg/kg b.w. per day).

Increased organ weights have been observed in rats (increased thyroid weight in males at 420 mg/kg b.w. per day (dietary) for 13 weeks; increased absolute and relative liver (females only) and kidney weights at 1057 (males) / 1344 (females) mg/kg b.w. per day for 90 days) and in mice (increased liver, kidney, thyroid, and pituitary weights in males from 200 mg/kg b.w. per day (dietary) for 13 weeks).

Reduced body weight gain has been observed following oral administration to rats (from 1800 mg/kg b.w. per day (dietary) for 4 weeks, from 517 mg/kg b.w. per day (gavage) for 4 weeks, from 433/565 mg/kg b.w. per day (dietary, males/females) for 90 days), to mice (from 200 mg/kg b.w. per day (dietary) for 13 weeks), and in dogs (apparently from 25 mg/kg b.w. per day (dietary) for 13 weeks), and in rats following inhalation of 400 mg/m^3 for 2 years.

A very common observation following exposure to NMP is a discoloration of the urine (dark yellow to orange to red-brown (depending on exposure level) from around 400 mg/m^3 following inhalation and from around 170 mg/kg b.w. per day following oral administration).

133.4.3 Toxicity to reproduction

Testicular effects (reduced weight and damage) were observed in rats following exposure to NMP by inhalation (3000 mg/m^3 for 90 days) or by oral administration (ca. 2000 mg/kg b.w. per day for 4 weeks).

No reproductive effects were noted in rats exposed by inhalation (480 mg/m^3 for up to 100 days; 2-generation study), but a slight decrease in foetal weight was detected among F1 offspring; following oral administration (500 mg/kg b.w. per day for 13 months; multi-generation study), reproduction was affected and parental effects (reduced body weight and feed consumption) were noted.

In teratology studies, no developmental effects were observed in rats or rabbits exposed by inhalation (rats: 350 mg/m^3; rabbits: 1000 mg/m^3; 6 hours/day on days 6-15 and 6-20 (rat), or 7-19 (rabbit)), following oral administration (rats: 330 mg/kg b.w./day on gestation days 6-15; rabbits: up to 175 mg/kg b.w./day on gestation days 6-18), or following dermal administration (rats: 500 mg/kg b.w./day on gestation days 6-15; rabbits: 1000 mg/kg b.w./day on gestation days 17-19). At higher dose levels (480-620 mg/m^3 (inhalation); 400-500 mg/kg b.w./day (oral administration); 750 mg/kg b.w./day (dermal administration)), foetotoxic effects (a lower foetal body weight) have been observed in rats in the absence of maternal toxicity.

Generally, no malformations have been observed at dose levels, which did not induce maternal toxicity. A neurobehavioral teratology study has shown an impairment of higher cognitive functions related to solving difficult tasks in rats exposed at 620 mg/m^3 on gestation days 7-20, a dose level that did not induce maternal toxicity.

133.4.4 Mutagenic and genotoxic effects

NMP has been tested in several short-term tests with negative result in every of these *in vitro* and *in vivo* tests, both with and without metabolic activation, except for a positive result for aneuploidy in yeast when tested under special circumstances.

133.4.5 Carcinogenic effects

NMP was not carcinogenic in rats when tested for carcinogenicity by inhalation (up to 400 mg/m^3) for 2 years. A two-year carcinogenicity study (feeding) has been performed in rats; however, the results of the study have apparently not been published.

134 Evaluation

Air saturated with NMP at 20°C has an NMP concentration of around 1400 to 1600 mg/m^3; thus, higher airborne exposure levels consist of a mixture of vapour and aerosol. When comparing the results of the inhalation studies, some discrepancies are observed; these may be due to a different vapour/aerosol ratio as well as particle size distribution between the reported studies.

NMP is readily absorbed following inhalation, oral administration and dermal application, and distributed widely to organs and tissues; in humans, a close correlation between inhalation exposures and the plasma concentration of NMP has been observed. NMP is rapidly metabolised and excreted with the major route of excretion being the urine (85-88%). Four metabolites have been identified in the urine with 5-hydroxy-N-methyl-2-pyrrolidone being the major one.

Volunteers did not report any discomfort to eyes or upper airways following exposure to NMP at concentrations up to 50 mg/m^3 for 8 hours on four different days, whereas workers (in the microelectronics industry) have reported severe eye irritation following exposure for a short time (30 minutes) to levels of about 3 mg/m^3 (8-hour TWA). This discrepancy may be due to NMP work processes performed at temperatures above the boiling point of NMP (202°C) in the microelectronics industry (in relation to operations with ovens at temperatures up to 240°C) and thus, the 8-hour TWA concentration may have contained periods of high peak exposures or the warm NMP vapour may have condensed to an aerosol, which is irritating to the eyes. Individuals have reported an odour of acetone following exposure to NMP around 50 mg/m^3, the odour was reported as “not uncomfortable”. Workers have experienced skin irritation and acute irritant contact dermatitis of the hands has been reported.

NMP is of low acute toxicity in the rat with the 4-hour LC_{50}-values reported being greater than 5100 mg/m^3 or in the range of 3100-8800 mg/m^3. The available studies do not suggest that NMP is a skin irritant or sensitiser, whereas NMP has shown eye irritancy in rabbits.

In one study of rats, mortality occurred within 9 days following inhalation exposure to NMP (aerosol-vapour mixture) at 1000 mg/m^3; marked pulmonary oedema and congestion, bone marrow effects, and prominent atrophy and necrosis in the lymphoid tissue in the thymus, spleen and lymph nodes were observed in dead animals. In all the other studies on rats exposed repeatedly to NMP by inhalation (most studies: 6 hours a day, 5 days per week), histopathological lesions (including testicular damage) were observed only at very high exposure levels (above 3000 mg/m^3). Other signs of exposure (mild secretion from the nose and lethargy and irregular respiration for 3 to 4 hours from start of exposure) have been noted at lower exposure levels (predominantly as an aerosol). Exposure of rats to NMP at 620 mg/m^3 (6 hours a day, 7 days per week for 90 days) did not cause neurotoxic effects

in the central nervous system. In a 2-year inhalation study, the highest dose level (400 mg/m^3, 6 hours a day, 5 days per week to the vapour predominantly) did not cause any adverse effects and no clinical signs of exposure were reported; thus 400 mg/m^3 is considered as being a NOAEL in the rat for NMP as a vapour with respect to clinical effects as well as to chronic toxicity.
A very common observation is a discoloration of the urine (inhalation from around 400 mg/m^3); however, this effect, although not been further investigated, is not considered as being an adverse effect as no renal lesions have been detected except for a chronic progressive nephropathy observed in a 2-year study on rats, which is a very common naturally occurring lesion in the kidneys of ageing rats.

No reproductive effects were noted in a two-generation study on rats exposed by inhalation to 480 mg/m^3; in a multigeneration study on rats, oral administration (500 mg/kg b.w. per day for 13 months) affected reproduction and parental effects were noted.
Several teratology studies have investigated the developmental toxicity of NMP in rats (most studies) and in rabbits; generally, no malformations were observed at dose levels, which did not induce maternal toxicity. Foetotoxic effects in form of a lower foetal body weight have been observed in some studies on rats at dose levels (480-620 mg/m^3 (inhalation); 400-500 mg/kg b.w./day (oral administration); 750 mg/kg b.w./day (dermal administration)) that did not induce maternal toxicity. A neurobehavioral teratology study has shown an impairment of higher cognitive functions related to solving difficult tasks in rats exposed at 620 mg/m^3 on gestation days 7-20, a dose level that did not induce maternal toxicity. A number of studies have reported foetotoxic effects only at dose levels, which also affected the maternal animals (predominantly evidenced as a reduced body weight and/or body weight gain); however, if a correction for the gravid uterine weight is done, no difference in body weight and/or body weight gain is observed between exposed animals and the controls.
Based on the available data, it is considered that developmental effects (lower foetal body weight, neurobehavioral effects) may occur at dose levels, which do not affect the maternal animals.

The mutagenicity and genotoxicity tests available indicate that NMP is not a mutagenic or genotoxic substance. No carcinogenic effects were observed in rats exposed by inhalation (up to 400 mg/m^3, 6 hours a day, 5 days per week for 2 years).

134.1.1 Conclusion

Based on the available data, the critical effect in humans following exposure to airborne NMP is considered to be the irritative effects on the eyes and the respiratory tract; the critical effect in humans following dermal contact is considered to be skin irritation. Data obtained from studies on experimental animals do not indicate that other effects, including neurotoxic effects, than the irritative ones should be expected to occur following exposure to the levels of NMP eliciting these irritative effects.

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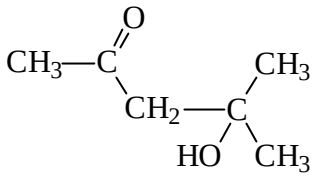
Appendix 18: 1-Hydroxy-4-methyl-2-pentanone (HMP)

136 General description

136.1 Identity

Molecular formula: $C_6H_{12}O_2$

Structural formula:



Molecular weight: 116

CAS-no.: 123-42-2

Synonyms: Diacetone alcohol
4-Hydroxy-2-keto-4-methylpentane
2-Methyl-2-pentanol-4-one
Acetonyldimethylcarbinol
Diketone alcohol
2-Hydroxy-2-methyl-4-pentanone
4-Hydroxy-4-methylpentan-2-one
4-Hydroxymethyl isobutyl ketone

136.2 Physico / chemical properties

Description: 4-Hydroxy-4-methyl-2-pentanone (HMP) is a colourless liquid with a sweetish odour.

Melting point: - 44 °C

Boiling point: 169 °C

Density: 0.93 g/ml at 25°C

Vapour pressure: 0.97 mmHg (129 Pa) at 20°C

Concentration of saturated vapours: 6165 mg/m^3 (1276.3 ppm) (calculated)
1550 ppm at 20°C and 760 mmHg (Patty)

Conversion factor: 1 ppm = 4.83 mg/m^3 at 20°C and 760 mmHg
1 mg/m^3 = 0.21 ppm

Solubility: Completely miscible in water at 20°C. Also readily soluble in e.g., alcohol and ether.

Partition coefficient: Log $P_{o/w}$ = -0.098 (estimated)

References: A&H (1989), DPIMR (1996), HSDB (2001).

137 Toxicokinetics

137.1 Absorption, distribution, metabolism and elimination

No data were available on the uptake of HMP. However toxicological data indicate that the substance is absorbed both after inhalation and after oral intake.

HMP was identified in serum as the major metabolite following a single intraperitoneal administration of 450 mg/kg of methyl isobutyl ketone (MiBK) to guinea pigs. MiBK and HMP had serum half-lives of 78 minutes and 66 minutes, and clearance times of 6 hours and 16 hours, respectively. The authors stated that a hydroxylation product like HMP would commonly be eliminated in urine as *O*-sulfate or *O*-glucuronide or may enter the intermediary metabolism to be eliminated as CO_2 or to be incorporated into tissues. (DiVincenzo et al. 1976).

The metabolic fate of 4-hydroxymethyl-4-methyl-2-pentanone (HMP) was investigated in Charles River CD-1 mice by measuring blood and brain concentrations at 15, 30, 60 and 90 minutes following intraperitoneal injection. Administration of 290 mg HMP/kg b.w. resulted in levels from approximately 430, 300, 160 and 50 µg/ml blood and 430, 300, 150 and 60 µg/g brain at 15, 30, 60 and 90 minutes after dosing. No metabolites were detected. (Granvil et al. 1994).

137.2 Mode of action

No information was found.

138 Human toxicity

138.1 Single and repeated dose toxicity

12 subjects of both sexes were exposed by inhalation to 100 ppm HMP (equivalent to 483 mg/m^3) for 15 minutes. The majority of them found the odour and taste objectionable and complained about eye, nose and throat irritation although they indicated that they could work an 8 hour-day in 100 ppm. (Silverman et al. 1946).

Pulmonary discomfort and eye, nose and throat irritation at 400 ppm (equivalent to 1932 mg/m^3) was reported (unpublished data by Shell - quoted from Patty's 1982).

HMP is reported to be defatting to the skin following repeated exposure (Patty's 1982).

A man who had painted a floor over three days with a paint containing HMP and ethanol as solvents was diagnosed to have glomerulonephritis about 40 days after the exposure. The man recovered within a year after immunosuppressive therapy. (von Scheele et al. 1976 – quoted from A&H 1989 and DPIMR 1996).

138.2 Toxicity to reproduction

No information was found.

138.3 Mutagenic and genotoxic effects

No information was found.

138.4 Carcinogenic effects

No information was found.

139 Animal toxicity

139.1 Single dose toxicity

139.1.1 Inhalation

RTECS has reported a lowest lethal concentration (LC_{Lo}) of 1000 ppm (equivalent to 4830 mg/m^3) in rats exposed for 4 hours (NTIS –quoted from RTECS 2001).

A saturated vapour concentration (approximately equivalent to 6165 mg/m^3) was tolerated by rats for 8 hours without mortalities (Smyth Jr. and Carpenter 1948).

A concentration of 2100 ppm (equivalent to 10143 mg/m^3) in rats, mice, rabbits and cats first caused excitability, then somnolence. In rabbits, injury of the kidney was reported. No further details are given in the reference. (Von Oettingen 1943).

139.1.2 Oral intake

LD_{50}-values of 4000 mg/kg b.w. (Smyth Jr. and Carpenter 1948) and 2520 mg/kg b.w. (RTECS 2001) in rats, and an LD_{50}-value of 3950 mg/kg b.w. in mice (Union Carbide 1956 – quoted from A&H 1989, IUCLID 2000 and RTECS 2001) have been reported for HMP.

A single gavage dose of 2 ml HMP/kg b.w. (equivalent to 1.86 g/kg b.w.) was administered to 20 rats. Four rats were used as controls. The treatment resulted in decreased haemoglobin and RBC count (approximately 75% of values before treatment), both returning to normal 6 days later. Liver effects including increase in numbers of histiocytes, cloudy swelling, vacuolisation and granulation of the liver cell cytoplasm were also recorded. The effects peaked at 24 hours and were reversible from one week after treatment. No damage was seen at day 35. (Keith 1932).

Four rabbits administered a single gavage dose of 2.4, 4 or 5 ml HMP/kg b.w. (equivalent to 2.2, 3.7 or 4.7 g/kg b.w.) had marked respiratory depression and narcosis. The two rabbits at the top dose died, while the two other animals recovered within 24 and 48 hours, respectively. (Walton et al. 1928).

Keith (1932) reported the maximum tolerated single dose of HMP in rabbits to be of 3 ml/kg b.w. (equivalent 2.79 g/kg b.w.).

139.1.3 Dermal contact

An LD50-value of 14.5 ml/kg b.w. (equivalent to 13750 mg/kg) was reported in rabbits (Smyth Jr. and Carpenter 1948).

139.1.4 Irritation

Exposure to 2100 ppm (equivalent to 10143 mg/m^3) HMP was irritating to mucous membranes in rats, mice, rabbits and cats (Von Oettingen 1943).

In an eye irritation test in rabbits, 0.005 ml or 0.02 ml HMP (equivalent to 4.65 mg or 18.6 mg) was applied to the centre of the cornea and examined 24 hours later. The irritation was evaluated to be severe and graded 5 on a 10 grade scale. (Carpenter & Smyth Jr. 1946).

In another eye irritation test using three rabbits performed according to OECD/EU guideline, HMP was reported to be mildly irritating with mean scores of 1.6, 2.0, 0.7 and 1.3 for chemosis and redness of the conjunctiva, iritis and corneal damage, respectively (IUCLID 2000).

Open application of 500 mg of undiluted HMP to rabbit skin produced mild irritation (unpublished data from Shell and Union Carbide 1959 – quoted from IUCLID 2000, RTECS 2001 and Patty's 1982).

RTECS has reported skin irritation in rabbits following a 24-hour open application of 10 mg HMP (RTECS 2001).

139.1.5 Sensitisation

No data were found.

139.2 Repeated dose toxicity

139.2.1 Inhalation

Groups of 12 female and 12 male Wistar rats were exposed to 0, 50, 225 or 1000 ppm HMP 6 hours/day, 5 days/week for 6 weeks (equivalent to 242, 1087 or 4830 mg/m^3). There was no mortality. In the high dose group, slight lethargy during and after exposure, reduction in female body weight gains at week 6 and increased liver and kidney weights were reported. Males of the high dose group had histological changes in the proximal kidney tubules (no details on the type of changes is reported). Animals of the mid dose group had increased liver weights while no effects were reported in the low dose group. (Unpublished study by Shell, 1979 - quoted from IUCLID 2000).

139.2.2 Oral intake

Oral administration to 4 rabbits of 2 ml HMP/day (equivalent to 1.86 g) for 12 days produced narcosis, kidney damage and death of three rabbits (Flury et al 1938 – quoted from Patty's 1982).

In a range finding test over 30 days, groups of 5 males and 5 female rats were administered 0, 10, 40 and 130 mg HMP/kg b.w. in the drinking water. There was no effect on survival, growth (body weight gain) or water consumption at any dose. Histopathological effects on the liver, the kidney, the spleen and/or the testes were reported at 40 mg/kg. No details on the organ or organs involved or the type of histopathological effects found, and the no report of findings at the high dose were given in the reference. The low dose was reported not to cause any effects. (Smyth Jr. & Carpenter 1948).

139.2.3 Dermal contact

No data were found.

139.3 Toxicity to reproduction

No information was found.

139.4 Mutagenic and genotoxic effects

139.4.1 *In vitro* studies

HMP was tested in Ames test using the plate incorporation assay in *Salmonella typhimurium* strains TA 1535, TA1537, TA1538, TA98 and TA100. A reverse mutation assay with *Escherichia coli* WP_2 and WP_2 *uvr A*, was also performed. Both assays were conducted with and without metabolic activation with 10% S9 mix. The concentrations tested were up to 4000 µg/plate. Reproducibility was secured by triplicating the assay. No reverse gene mutation was induced in either bacteria. (Brooks et al. 1988).

In a yeast mitotic recombination assay, HMP suspensions at concentrations of 0.01, 0.1, 0.5, 1.0 or 5.0 mg/ml was added to a *Saccharomyces cerevisiae* JD1 suspension containing 10×10^6 cells/ml with or without S9 mix. No mitotic gene conversion was recorded. (Brooks et al. 1988).

A chromosome aberration assay in rat liver RL_4-cells (epithelial- type cell line) was conducted with HMP concentrations of 750, 1500 2000, 3000 and 4000 µg/ml. No S9 mix was used as these cells are metabolically competent. Small increases in chromatid damage, breaks and acentric fragments were seen at 2000 and 4000 µg/ml but no clastogenic effects were observed at 3000 µg/ml. There was a slight, but not dose-related genotoxic effect of HMP. (Brooks et al. 1988).

HMP has been reported to reduce the number of mutants caused by [6]-gingerol (Nkamura and Yamamoto 1983 – quoted from A&H 1989).

139.4.2 *In vivo* studies

No studies were found.

139.5 Carcinogenic effects

No information was found.

140 Regulations

140.1 Ambient air

Denmark (C-value): 0.1 mg/m^3 (MST 2002)

140.2 Drinking water

-

140.3 Soil

-

140.4 Occupational Exposure Limits

Denmark: 50 ppm (240 mg/m^3) (At 2002)

US-ACGIH: 50 ppm (240 mg/m^3) (ACGIH 1991)

Germany: 50 ppm (240 mg/m^3) (MAK 1998)

140.5 EU-Classification

HMP is classified as an irritant (Xi; R36 – irritating to eyes) (MM 2002).

140.6 IARC

-

140.7 US-EPA

-

141 Summary

141.1 Description

4-Hydroxy-4-methyl-2-pentanone (HMP) is a colourless liquid organic solvent with a sweetish odour. It is soluble in water and organic solvents, and moderately volatile.

141.2 Toxicokinetics

It appears from toxicological data, that HMP is absorbed both by inhalation and by oral intake. Intraperitoneal administration of HMP to mice resulted in similar levels in the blood and the brain. The substance is expected to be eliminated in urine as conjugates, to enter the intermediary metabolism, or to be incorporated in the tissues.

141.3 Human toxicity

HMP was found to be irritating to eyes, nose and throat following exposure to concentrations from 483 mg/m^3 for 15 minutes. At this level, the odour was also objectionable.
A case study has reported glomerulonephritis following exposure over three days to paint containing HMP and ethanol.
Repeated exposure to high concentrations HMP has been reported to be defatting to the skin; however, no details are available.

No information was found on toxicity to reproduction, mutagenic and genotoxic effects, or carcinogenic effects of HMP.

141.4 Animal toxicity

141.4.1 Single dose toxicity

An LC_{LO} of 4830 mg/m^3 over 4 hours was reported in rats. A saturated vapour concentration was tolerated by rats for 8 hours. No deaths were reported following acute inhalation exposure of rats, mice, rabbits or cats up to concentrations of 10143 mg/m^3, which is higher than the saturated vapour concentration. However, no details were available in any of these reports.

Oral LD_{50}-values were reported to be 2520 or 4000 mg/kg b.w. in rats and 3950 mg/kg b.w. in mice. In rats, a sub-lethal dose of 1860 mg/kg b.w. was reported to cause decrease in haemoglobin and RBC count, and liver cell vacuolisation and granulation. In rabbits, an oral dose of 4.7 g/kg b.w. was lethal, while 2.2 and 3.7 mg/kg b.w. caused respiratory depression and narcosis.

A dermal LD_{50}-value in rabbits of 13750 mg/kg b.w. was reported.

HMP was reported to be irritating to the mucous membranes in rats, mice, rabbits and cats at concentrations of 10143 mg/m^3. Eye irritation reports are

conflicting as HMP was reported to cause severe irritation in an elder range-finding test but only mild irritation in another test, conducted according to OECD/EU guideline.

HMP has been reported to be a mild irritant to rabbits skin in one reference and to be skin irritating in another.

No data were available on the sensitising potential of the substance.

141.4.2 Repeated dose toxicity

Inhalation exposure of rats from 242 to 4830 mg/m^3 HMP (HMP) for 6 weeks resulted in slight lethargy, increased liver and kidney weights, and unspecified histological changes in the proximal renal tubules of male rats at the high dose, while only liver weights increase was reported at the mid dose and no effects were seen at the low dose.

Rats exposed to 40 mg HMP/kg b.w. in the drinking water for 30 days were reported to have histopathological changes in either the liver, the kidney, the spleen or the testes, while 10 mg/kg did not induce any changes.

141.4.3 Toxicity to reproduction

No information was found.

141.4.4 Mutagenic and genotoxic effects

HMP was negative in reverse mutation assays in *Salmonella typhimurium* and *Escherichia coli* strains with and without metabolic activation and did not induce mitotic gene conversion in a yeast recombination assay.
A slight increase in chromatid damage, breaks and fragments was seen in a chromosome aberration test in rat liver cells *in vitro*, but no dose-response relationship was established; HMP was concluded by the authors to be slightly clastogenic.
No *in vivo* data were found.

141.4.5 Carcinogenic effects

No information was found.

142 Evaluation

The toxicology database available on HMP is limited, with a number of elder studies being summarily described.

HMP is the major metabolite of methylisobutylketone. It appears to be absorbed following inhalation and oral exposure. No data were available on the metabolism and excretion of HMP.

Data in animals on HMP indicated a low acute toxicity by all three routes of exposure.

In humans, the substance was irritating to eyes, nose and throat at 483 mg/m^3. In animals, high concentrations were reported to be irritating to mucous membranes. Though elder data in rabbits indicated severe eye irritation from exposure to HMP, the substance is evaluated to be mildly irritating to the rabbit eyes on the basis of a guideline study. Skin irritation data in rabbits indicated that HMP is a mild to moderate skin irritant. The substance is reported to be defatting to the skin.
No data were available on the sensitising potential of the substance.

A human case of glomerulonephritis caused by mixed exposure to a paint containing HMP and ethanol as solvents has been reported. However, a causal relationship is not possible on the basis of one case of mixed exposure. Prolonged exposure of rats to 4830 mg/m^3 is reported to be toxic to the kidney in males, producing changes in the proximal tubules. As the effect is restricted to the male rats, it is considered that this could be due to the accumulation of $a_{2\mu}$-globulin. This effect is considered not to be relevant to humans.

HMP was negative in bacteria tests and in a yeast mutagenicity assay. A slightly clastogenic but not dose-related effect was seen in a chromosome aberration assay *in vitro*. No *in vivo* data on mutagenicity or genotoxicity were found. Overall, the available evidence indicated that the substance is not genotoxic.

No information on toxicity to reproduction or on carcinogenicity was found.

On the basis of the available data, HMP is evaluated to be irritating to the eyes and to the mucous membranes. Other end-points could not be evaluated because of insufficient data.

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