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The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man

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THE TOXICOLOGY OF ETHYLENE GLYCOL MONOALKYL ETHERS AND ITS RELEVANCE TO MAN

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SUMMARY

1. Glycol ethers are a group of chemicals used widely as co-solvents and marketed under a variety of trade names. The current recommended exposure limits are mainly based on human case histories from the 1930's, but the results of recent animal studies have prompted a re-evaluation of their hazards. ECETOC set up a working group : to evaluate studies relevant to the toxicity to man of glycol ethers in general, and ethylene glycol monomethyl (EGME) and monoethyl (EGEE) ethers in particular, including evidence from human exposure; to advise on the significance for human exposure; to recommend studies which may be necessary to clarify the situation.

2. EGME and EGEE have been shown in experimental animals to cause haematological effects characterised by reductions in the numbers of circulating red and white blood cells, bone marrow and lymph node depression, testicular atrophy, foetotoxicity and teratogenicity. These effects have been observed in two or more animal species exposed by inhalation, dermal and oral routes. EGME appears to be two or three times more potent than EGEE. No-effect levels for inhalation and for oral exposure have yet to be defined for the effects of these compounds.

3. Ethylene glycol monoisopropyl (EGiPE) and ethylene glycol monobutyl (EGBE) ethers appear to have a different spectrum of activity. Their major effect on the blood is haemolysis. Effects on the testes are absent or less severe than with EGME and EGEE. There are no data on their teratogenic activity.

4. Case histories of human over-exposure have shown that EGME can cause haematological and also neurological effects similar to those seen in experimental animals. There are no comparable data for EGEE.

5. Consideration of the similarity in several animal species of the nature of the effects and of the exposure levels at which they occur, coupled with evidence that haematological effects of EGME are also seen in man, suggests that it would be unreasonable to disregard the evidence provided by the animal studies. It is prudent to assume, in the absence of evidence to the contrary, that in addition to the adverse effects on the haemapoietic system of man the effects on the testes and the developing embryo will also occur in exposed humans. No-effect-levels have not yet been found for either the testicular or teratogenic effects. The exposure levels at which these

effects have been found in animals suggest that the present recommended exposure control limits may not afford adequate safety margins for man.

6. Studies are being sponsored by the Chemical Manufacturers'Association to establish no-effect-levels for EGME and EGEE and to investigate the teratogenicity of EGBE.

7. Recommendations for improving our understanding of the situation have been made by the Working Group. The conclusions in this report should be reviewed when new data are available.

CONTENTS

A. INTRODUCTION

Glycol ethers are widely used chemicals. The monoalkyl ethers are usually produced by reacting ethylene oxide with the appropriate alcohol or by direct alkylation of a selected glycol with agents such as dialkyl sulfate. Ethylene glycol monoalkyl ethers are extensively used as co-solvents and are marketed under a variety of names. As they are miscible with water and a large number of organic solvents they are especially useful as co-solvents in many oil-water compositions. They are used as solvents for various resins, lacquers, paints, varnishes, dyes, inks, printing pastes, cleaning compositions, liquid soap and cosmetics. They are also used widely as chemical intermediates.

Glycol ethers are colourless liquids with mild ethereal odours and with boiling points, vapour pressures and evaporation rates dependent on the type of glycol and alkyl moiety. More detailed information about the production, properties and uses of glycol ethers is given in Appendix 1.

The current recommended exposure control limits (cf.Appendix 2) are mainly based on human studies such as those by Greenburg et al.(1938) which showed that overexposure of humans to ethylene glycol monomethyl ether was associated with disorders of the blood and central nervous system. These effects were later confirmed in animal studies. Attention has recently been focussed on glycol ethers as the result of studies demonstrating adverse effects on the reproductive processes of animals, namely testicular atrophy and foetal development. Since glycol ethers are widely used it was considered important to assess the available data. A Glycol Ether Working Group (WG; see list of members in H) was set up by ECETOC, with the following terms of reference :

"To evaluate studies relevant to the toxicity to man of glycol ethers in general and ethylene glycol monomethyl and monoethyl ethers in particular, including evidence from human exposure; to advise on the significance of such evidence for human exposure;to recommend studies which may be necessary to clarify the situation".

In this report the WG gives an assessment of the available data on ethylene glycol ethers. Some data on propylene glycol ethers have been included for comparison. The working group is aware of studies in progress but believes that the conclusions reached in this report are unlikely to be invalidated by the

results of these studies. Abbreviations of the names of the glycol ethers used in this report are given in Table 1.

Table 1

Names		Abbreviations	CAS-No.
ethylene glycol-methyl ether	$HO-CH_2CH_2-O-CH_3$ 2-methoxyethanol	EGME	109-86-4
ethylene glycol-ethyl ether	$HO-CH_2CH_2-OCH_2CH_3$ 2-ethoxyethanol	EGEE	110-80-5
ethylene glycol-isopropyl ether	$HO-CH_2CH_2-O-CH(CH_3)_2$ 2-isopropoxyethanol	EGiPE	109-59-1
ethylene glycol-n-propylether	$HO-CH_2CH_2-O-CH_2CH_2CH_3$ n-propoxyethanol	EGnPE	2807-30-9
ethylene glycol-butyl ether	$HO-CH_2CH_2-O-CH_2CH_2CH_2CH_3$ 2-butoxyethanol	EGBE	111-76-2
ethylene glycol-phenyl ether	$HO-CH_2CH_2-O-C_6H_5$ 2-phenoxyethanol	EGPhE	122-99-6
2-Propylene glycol-methyl ether	$CH_3-CH(OH)CH_2-O-CH_3$ 1-methoxypropanol-2	PGME	107-98-2
Ethylene glycol	$HO-CH_2CH_2-OH$	EG	107-21-1
Ethylene glycol-acetate	$HO-CH_2CH_2OOC-CH_3$	EGAc	542-59-6
Ethylene glycol methyl-ether acetate	$CH_3COOCH_2CH_2-O-CH_3$	EGMEAc	110-49-6
Ethylene glycol ethyl-ether acetate	$CH_3COO-CH_2CH_2-O-C_2H_5$	EGEEAc	111-15-9

B. TOXICOLOGY OF GLYCOL ETHERS

1. Haematological and Testicular Effects.

The adverse effects of EGME on the testes and bone marrow of experimental animals have been known for many years. Since both tissues contain rapidly dividing cells it was considered appropriate to review the effects of glycol ethers on both systems in parallel.

1.1. Historical Data

1.1.1. Human data

a) Haematological effects. The first report of haematological changes ascribed to EGME intoxication appears to have been that made by Donley (1936) in which a 45 year old caucasian woman, in addition to exhibiting neurological symptoms, was found to have anaemia (red blood cell count $3.5 \times 10^6 mm^{-3}$, and a haemoglobin value of 85%).* The white blood cells were unaffected. Whether EGME caused these changes is uncertain; the woman had been exposed to a solvent containing 3% EGME and 74% isopropyl alcohol.

Parsons & Parsons (1938) described two cases of exposure to EGME in which the predominant symptom was fatigue. Both subjects had reduced erythrocyte and leucocyte counts and were diagnosed as having aplastic anaemia. In the same year, Greenburg et al.(1938) reported a variety of haematological abnormalities in workers employed in a collar-fusing plant in which EGME was used as a 33% solution in denatured alcohol. In 19 subjects there was a general immaturity of neutrophils with some abnormal forms, and in the majority of cases a lower number of platelets. Blood haemoglobin concentrations were high in relation to the number of erythrocytes which, together with a slightly higher mean corpuscular volume, suggested a macrocytic type of anaemia. Since the reticulocyte counts were normal and serum bilirubin concentrations generally low, the abnormal blood picture was ascribed to an effect on the bone marrow rather than on the blood itself. The solvent mixture used in this factory contained EGME, ethyl alcohol and small amounts of ethyl acetate and methyl alcohol. Since ethyl alcohol has never been associated with such effects and the exposure levels to ethyl acetate

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* In this report the UK system for numbers is used, ie. ten thousand is 10,000 and one point two is 1.2

and methyl alcohol were so low,it seems unlikely that these substances could be responsible for the observed effects. All the subjects were in their late teens or early twenties and the duration of exposures quoted ranged from 1 to 12 months.Atmospheric concentrations at the time of intoxication were not measured although later investigations suggested that they were in the order of EGME 76 ppm, ethyl alcohol 215 ppm, methyl alcohol 16 ppm, ethyl acetate 5 ppm and petroleum naphtha 1 ppm.

Zavon (1963) presented case histories of 5 workers exposed to EGME. All had worked in a printing department where the floor and the printing machines were cleaned with EGME. Estimations of atmospheric EGME in simulated conditions gave concentrations ranging from 61 to 3960 ppm. Erythrocyte counts and haemoglobin levels were generally below normal and a bone marrow smear on one subject showed hypoplasia with a reduced number of erythroid elements. All patients experienced weakness and lethargy which may have originated from either their anaemia or the central nervous depressant effects of the solvent.

Ohi & Wegman (1978) described two cases of poisoning believed to have resulted from skin contamination with EGME. Both showed signs of anaemia. There was no increase in the numbers of reticulocytes in either case and a study of bone marrow biopsies revealed hypoplasia. In one patient slight haemoglobinuria was also observed,suggesting a haemolytic process.

b)Testicular Effects.There have been no reports of an association between exposure to glycol ethers and adverse effects on human testes. One of the subjects investigated by Zavon (1962) complained of impotence but this may have been a manifestation of general debility resulting from anaemia and CNS effects.

1.1.2. Experimental data

a)Haematological effects.Haematological changes similar to those described in humans have been found in cats exposed to 1.1 to 1.3 mg/l (350 ppm) of EGME vapour several hours per day for 6 days(Flury & Wirth, 1933) and in rats exposed to atmospheres containing between 300 and 400 ppm of either EGME, EGEE, EGnPE, EGiPE or EGBE, 7 hours per day, 5 days per week for 5 weeks (Werner et al.,1943a). The exposure conditions were not sufficient to produce any overt signs of toxicity.

A summary of the histological and haematological findings is given in Table 2 :

Table 2

	decreased RBC(1)	Hb(2)	increased retics(3)	increased juvenile granulocytes	bone marrow hypoplasia	haemo-siderosis spleen	decreased extra medullary haema-poiesis
EGME	-	-	+	+	+	-	-
EGEE	-	-	+	+	+	+	-
EGiPE	+	+	+	+	+	-	+
EGnPE	+	+	+	+	-	-	+
EGBE	+	+	+	+	-	-	+
Control	-	-	-	-	-	-	-

(1) Red Blood Cell Count
(2) Haemoglobin Concentration in Blood
(3) Reticulocyte Count

+ present
- absent

A reduction in erythrocytes and haemoglobin concentration and a compensatory elevation in reticulocytes, occurred with the n-propyl, isopropyl and butyl ethers after one week of treatment,followed by a gradual return to normal. These changes which suggested a haemolytic process were absent in animals exposed to equimolecular concentrations of the methyl and ethyl ethers. This evidence of a haemolytic effect following exposure to EGiPE, EGnPE and EGBE was supported by the observation of haemoglobinuria in mice exposed to these compounds (Werner et al., 1943-a). In terms of in vitro haemolytic activity, the order of potency was EGBE, EGiPE, EGnPE, EGEE, EGME, which parallels their order of efficacy in reducing the surface tension of water. Although there was no appreciable change in the leukocyte count with any of the solvents tested, the percentage of juvenile granulocytes increased. Examination of bone marrow from the middle of the femoral shaft showed replacement of fat by active marrow in some animals from all treatment groups.

Werner et al.,(1943-b) exposed groups of two dogs to atmospheres containing EGME (750 ppm), EGEE (840 ppm) or EGBE (415 ppm), 7 hrs/day, 5 days/week for 12 weeks. All three materials caused a decrease in the haemoglobin concentration, erythrocyte count and haematocrit value which were maximal after 4 to 6 weeks. On cessation of treatment there was a gradual recovery. Examination of blood smears showed marked microcytosis and hypochromia in the animals treated with EGME and EGEE and a similar but less pronounced effect in animals treated with EGBE. A reduction in total white cells noted in the EGEE group at the end of the first week was attributed to a decrease in the number of lymphocytes.An increase in the number of juvenile granulocytes similar to that found previously in rats was noted for both EGME and EGEE between 1 and 8 weeks. Although all three materials were shown to produce anaemia in the dog there was no evidence, even for EGBE, that this was the result of a haemolytic process.

It would therefore appear that there is some similarity between the effects of EGME in dogs and man in that the erythroid elements are affected as well as the white cells.In rodents it is the white cell population that is predominantly affected.

Carpenter et al.(1956) demonstrated an increase in osmotic fragility of erythrocytes in rats, mice and rabbits and to a lesser extent in guinea pigs, rhesus monkeys and humans exposed to EGBE. Repeated inhalation exposure in rodents and dogs resulted in : haemoglobinuria; reduced erythrocyte counts; reduced haemoglobin concentration; increased reticulocytes; and haemolytic anaemia. The haemolytic effects of a number of other glycol ethers were investigated following a four-hour inhalation exposure in rats. The lowest concentrations causing increased erythrocyte fragility were EGME 2000 ppm, EGnPE 62 ppm, EGiPE 62 ppm, and EGBE 62 ppm. A concentration of EGPhE approaching saturation did not increase fragility. Potency was similar to that established by Werner et al.(1943-a). A further experiment was described in which 3 humans and 3 rats were simultaneously exposed for 8 hours in one day to an atmospheric concentration of 195 ppm. The osmotic fragility of the rat erythrocytes increased throughout the exposure, whereas the human erythrocytes were unaffected.

Repeated oral administration of PGME at doses from 1 to 6ml/kgbw/day* in mice, rats and dogs for 2, 13 and 14 weeks respectively, showed no clear or consistent effects on the haemapoietic system (Stenger et al.,1972).

A dose-related increase in Heinz bodies found in mice given 1 to 6ml/kgbw/day of PGME was considered "biologically irrelevant" because of the high doses used. In view of the lack of detail in this report it is impossible to judge the significance of these findings. There was no indication of Heinz-body formation in the rat or dog, or of haemolytic anaemia in any of the three species. Irrespective of the above findings, PGME clearly does not possess the same type of activity as EGME.

b) Testicular effects. The first report of degenerative changes in the seminiferous epithelium was made by Wiley et al.(1938). Two rabbits administered EGME showed degenerative changes and desquamation of the germinal epithelium, with giant cell formation. Degenerative lesions of the testes were also noted in 3 of 4 rabbits administered EG or EGAc. The precise doses and route of administration employed are not clear from the report, although it was stated that the animals were "injected". There were no reported effects on the blood or lymphoid organs .

A 90-day feeding study carried out in 1960 (Tyler,1981) in which rats were fed diets containing 0.01, 0.05, 0.25 and 1.25% EGBE caused testicular atrophy at the two highest levels. There was no mention of any haematological abnormalities.

Stenger et al.(1971) described testicular oedema in rats given subcutaneous doses of EGEE of approx. 200 and 400mg/kgbw/day for 4 weeks. Similar lesions were also reported in the testes of dogs given oral doses of approximately 50 to 200 µl/kgbw/day for 13 weeks. A reduction in haemoglobin concentration and haematocrit value was also noted.

Stenger et al.(1972) administered PGME to rats in doses up to 4 ml/kgbw per day (about 4000 mg/kgbw/day) for four weeks subcutaneously or 13 weeks orally. No testicular atrophy was reported. Three dogs treated with 2 or 3 ml/kgbw PGME, 5 days per week for 14 weeks were described as

* kgbw : kilogram body weight.

having an "abundance of spermiophages in the apertures of the epididymis". The meaning of these histological changes are not clear. They were not reported in the other species examined and cannot be equated with the gross testicular damage produced by EGME.

1.2. Recent Information

Nagano et al.(1979) reported the results of a series of experiments to investigate the testicular effects of a number of glycol ethers and glycol ether acetates when administered by gavage 5 times per week for 5 weeks to groups of 5 adult male mice. EG, EGEE and EGEEAc were given at doses of 500, 1000, 2000 and 4000 mg/kgbw; EGME or EGMEAc at 62.5, 125, 250, 500, 1000 and 2000 mg/kgbw; and EGBE or EGPhE at 500, 1000 and 2000 mg/kgbw. Dose-related decreases in testicular weight were found at a dose of 250 µg/kgbw for EGME, 500 mg/kgbw for EGMEAc and 1000 mg/kgbw for EGEE and its acetate. The effects were most pronounced with EGME and least with EGEEAc. Histologically, the testes showed atrophic changes with a loss of germinal epithelium. Although there were no reported effects on the interstitial cells there was a reduction in weight of accessory glands. One animal in each of the groups administered 1000 mg/kgbw EGBE and EGPhE was reported to have "atrophied seminiferous tubules". The significance of these lesions is, however, questionable in view of their low incidence and their occurrence in untreated animals.

The most striking haematological changes were also found with EGME, EGEE and their respective acetates. These effects consisted of a significant decrease in leukocyte count with a less marked reduction in erythrocytes, haematocrit value and the haemoglobin concentrations. A reduction in erythrocytes only was found in the groups given 500 or 1000 mg/kgbw EGBE. The acetates appear to be equipotent on a molar basis.

Table 3 shows the lowest dose level at which the testicular and haematological effects of the compounds studied were seen.

TABLE 3

Lowest dose levels (mg/kgbw) for testicular and haematological effects in mice (Nagano et al., 1979)

COMPOUNDS	TESTICULAR ATROPHY	REDUCED NUMBER OF RED BLOOD CELLS	REDUCED NUMBER OF WHITE BLOOD CELLS	REDUCED HAEMO-GLOBIN LEVEL	REDUCED HAEMA-TOCRIT
EGME	250	1000	500	1000	no effect
EGEE	1000	no effect	2000	no effect	no effect
EGMEAc	500	no effect	1000	2000	no effect
EGEEAc	1000	no effect	2000	no effect	4000
EGBE	1000*	500	no effect	no effect	no effect
EGPhE	1000*	no effect	no effect	no effect	no effect
EG	no effect	no effect	no effect	no effect	no effect

* equivocal data

Although erythrocyte count and haemoglobin concentration were reduced, there were no effects such as haemoglobinuria, splenomegaly or haemosiderosis indicating a haemolytic process. Reticulocyte counts were not made. These findings support the view that bone marrow suppression is most pronounced with the methyl and ethyl ethers.

A series of short-term inhalation and skin-application studies on EGBE carried out by Union Carbide (summarised by Tyler,1981) corroborated some of the previously-reported haematological changes caused by EGBE. Fischer 344 rats were exposed to 20, 86 and 245 ppm of EGBE vapour, six hours per day for 5 days, and for a further 4 days following 2 days without exposure. Haemoglobinuria was found after 2 days of treatment in the female rats exposed to 245 ppm. This effect was transitory and was not found in any other treatment group. Haematological changes were found in both male and female rats exposed to 245 ppm, although these were not specified. A decreased haemoglobin concentration and an increased mean corpuscular volume were seen in both sexes at 86 ppm EGBE.

The percutaneous toxicity of EGBE in rabbits was investigated by the application of 1 ml of 5, 25, 50 or 100% aqueous solutions under occluded conditions, the exposure schedule being as in the inhalation studies. Haemoglobinuria was found in many rabbits treated with the undiluted and 50% solutions but not with the lower concentrations. A reduction in leucocyte count was reported in the male rabbits exposed to the undiluted material although the author expressed some reservations on its relevance to treatment. Reductions in erythrocyte count, haemoglobin concentration and mean corpuscular haemoglobin were observed in female rabbits receiving the undiluted material.

The results of a 90-day inhalation study in rats reported in the same paper showed significant decreases in erythrocyte count, haemoglobin concentration and mean corpuscular haemoglobin in animals exposed to 77 ppm by inhalation for 6 hrs/day, 5 days/wk for 13 weeks. No changes were seen at the lower levels, ie. 5 or 25 ppm, and no histological changes occurred in the testes or bone marrow at any levels.

Results of a short-term study of the inhalation toxicity of EGME and PGME (Miller et al.,1981) substantiated the previous reports of haematological and testicular effects of EGME. In these experiments male and female Fischer 344 rats and B6C7F1 mice were exposed to 0, 100, 300 and 1000 ppm of EGME or 0, 300, 1000 or 3000 ppm of PGME for 6 hours/day for 9 days during an 11 day period. At the 1000 ppm level of EGME there was a reduction in red and white blood cell counts and haemoglobin concentration, a depletion of both myeloid and erythroid cells from bone marrow, and lymphoid atrophy. There was also a statistically-significant reduction in leucocyte count in the 100 ppm group which , although small in relation to historical control values, seems likely to be related to treatment in view of the marked effects seen at the higher dose levels.There was a loss of germinal epithelium, and giant cells were formed in the testes of all animals exposed to 1000 ppm EGME, although sperm was still present in the epididymides. Similar but less marked effects were reported at the 300 ppm level.In contrast there were no gross or histopathological changes that could be ascribed to PGME even at the 3000 ppm exposure level.

Studies (Dow Chemical Company,1982) in which rats and rabbits were exposed to 0, 30, 100 or 300 ppm of EGME for 6 hours/day, 5 days per week for 13 weeks have similarly shown leukocytopenia, severe bilateral