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Toxicities of Ethylene Glycol and Ethylene Glycol Monoethyl Ether in Fischer 344/N Rats and B6C3F$_1$ Mice

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The toxicities of ethylene glycol (EG) and ethylene glycol monoethyl ether (EGEE) were studied in Fischer 344/N rats and B6C3F$_1$ mice. In a 13-week study, EG was administered in feed to groups of 10 rats and 10 mice of both sexes at dose levels of 0 (control), 0.32, 0.63, 1.25, 2.5 and 5.0%. Kidney/body weight ratios were elevated in the 2.5 and 5.0% dose groups of male and female rats relative to controls, while serum urea nitrogen and serum creatinine levels were elevated in the two highest dose groups of male rats. Toxic nephrosis and crystal deposits in renal tubules were observed in the 2.5 and 5.0% dose groups of male rats. Crystals were also observed in brains of male rats in the 5.0% dose group. Nephrosis was the only lesion observed in female rats (5.0% dose group). Mild, compound-related lesions were seen in kidneys (nephrosis) and livers (centrilobular degeneration) of male mice in the 2.5 and 5.0% dose groups. There were no adverse effects observed in female mice.

Groups of 50 rats and 50 mice of both sexes were administered EGEE by gavage in a 2-year study at dose levels of 0 (control), 0.5, 1.0 and 2.0 g/kg body weight. Testicular atrophy was observed in male rats that died early in this study and in the medium- and high-dose male mouse groups. Gross lesions noted at necropsy indicate that chronic treatment of rats with EGEE at dose levels of 0.5 or 1.0 g/kg body weight caused an apparent enlargement of the adrenal gland in male rats and interfered with the development of spontaneous lesions of the spleen (males and females), pituitary (males and females), testis (males), and subcutaneous tissue in the mammary gland region (females) that commonly occur in the aging Fischer 344/N rat. Histopathologic review of this study is in progress.

Introduction

Ethylene glycol (EG) and ethylene glycol monoethyl ether (2-ethoxyethanol, EGEE) were nominated to the National Toxicology Program for toxicity and carcinogenicity testing because of the large amounts of these chemicals which are produced each year and because of the potential for human exposure. EG is used in antifreeze and coolant mixtures for motor vehicles, in hydraulic fluids and heat exchangers, and as a solvent in the production of ethylene glycol esters, ether and resinous products (*1*). Production of EG in the United States in 1980 was estimated at 4.4 billion pounds (*2*). EGEE is used largely as a solvent in protective coatings (lacquers, varnishes, paints, and epoxy coatings) and as an intermediate in the production of ethylene glycol monoethyl ether acetate (*3*). It is also used as a solvent or vehicle for nitrocellulose, printing inks, metal and glass cleaners, hydraulic fluids, textile and leather dyeing solutions, varnish removers, lacquer thinners, and adhesives, as well as an anti-icing additive in aviation fuels (*3*). In 1980, 200 million pounds of EGEE were produced in the United States (*2*).

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Ethylene Glycol (EG)

Materials and Methods

The subchronic toxicity of EG in rats and mice was evaluated in a 13-week study conducted at Southern Research Institute (Birmingham, AL). EG was administered in the diet to groups of 10 male and 10 female Fischer 344/N rats and 10 male and 10 female B6C3F$_1$ mice. The oral route was selected because it was considered to be a potential route of human exposure. EG used in these studies was manufactured by the Ashland Chemical Company and was shown by gas chromatographic analyses to be greater than 99% pure. EG was also shown to be stable in rodent feed, with no measurable loss occurring after 2 weeks of storage at 25°C. Rats used in this study were obtained from the Frederick Cancer Research Center and mice were obtained from Harlan Industries. Rats were about 7 weeks of age at the start of the study and the mice were

about 9 weeks old at the start of the study. Five treatment groups plus an untreated control group were included for each sex and species. The dose levels of EG, mixed in NIH 07 feed, were 5.0, 2.5, 1.25, 0.63 and 0.32%. City tap water and the dosed feed mixtures or the control feed diet were available *ad libitum* throughout the study. The dose levels selected for this study were based on dosed feed studies of EG that had been reported in the scientific literature. Morris et al. (*4*) noted that 1% EG in feed had no effect on mortality of rats after 1 year of treatment, while survival was decreased at the 2% dose level. Blood (*5*) saw no effect on survival of male or female rats fed diets containing up to 4% EG for 3 months; however, there was 100% mortality at the 4% dose level by 6 months. We expected to reach no observable effect levels in rats or mice fed diets containing 5.0% to 0.32% EG for 13 weeks.

Results and Discussion

The only deaths observed in this study were in the highest dose group (5.0%) of male rats (Table 1). The four deaths occurred during weeks 11, 12 and 13 of the study. There were only nine male rats in the 0.63% dose group because of a sexing error which was not discovered until week 7 of the study. There were no deaths in any of the female rat groups. There was a greater than 10% depression of relative body weight gain for male rats in the 2.5 and 5.0% dose groups. Relative body weight data is a measure of the average weight gain of treated animals relative to the average weight gain of controls. There were no significant effects of EG on average body weight gain of female rats. There were no apparent differences in feed consumption between any of the male or female rat treatment groups and their respective control groups.

In the 13-week study in male and female mice (results not shown), there were no deaths in any of the control or EG treatment groups, and the relative weight gain data did not show any clear dose-related effect. There was no evidence of decreased feed consumption for any of the EG treatment groups and their respective control groups in the mouse study.

At the necropsy of animals which survived to the end of the study, organ weights were measured for the liver, right kidney, heart, brain, lungs and right testicle. The mean right kidney weights and kidney/body weight ratios for male and female rats treated with EG and the untreated control groups are presented in Table 2. Significant differences ($p < 0.01$) in the kidney/body weight ratio were observed for both male and female rats in the 2.5 and 5.0% dose groups relative to the controls.

The mean ratio of thymus to terminal body weight was significantly decreased ($p < 0.05$) in male rats in the 5.0% dose group relative to the controls (results not shown). There were no other dose-related organ to body weight differences in male or female rats. There were no differences in organ/body weight ratios in male or female mouse groups treated with EG relative to respective controls.

Serum and urine analyses were conducted on all rats and mice that survived to the end of the study. Blood

Table 1. Mortality and mean body weight changes of Fischer 344/N rats treated with ethylene glycol for 13 weeks.

Dose, % of feed	Males		Females	
	Mortality[a]	Relative weight change, %[b]	Mortality[a]	Relative weight change, %[b]
0 (control)	0/10	—	0/10	—
0.32	0/10	− 2	0/10	+11
0.63	0/9	− 6	0/10	+9
1.25	0/10	− 1	0/10	+6
2.5	0/10	− 13*	0/10	+6
5.0	4/10	− 17†	0/10	+2

[a]Number of deaths/number per group.
[b]Relative weight change = [weight change (dosed group)-weight change (control group)/weight change (control group)] × 100.
*$p < 0.05$, relative to controls.
†$p < 0.01$, relative to controls.

Table 2. Effect of ethylene glycol for 13 weeks on right kidney weight relative to terminal body weight in Fischer 344/N rats.

Dose, % of feed	Males		Females	
	Right kidney weight, g	Kidney/body wt. ratio ×1000	Right kidney weight, g	Kidney/body wt. ratio ×1000
0 (control)	1.06 ± 0.04	3.09 ± 0.07	0.63 ± 0.01	3.15 ± 0.05
0.32	1.07 ± 0.02	3.12 ± 0.06	0.63 ± 0.03	3.07 ± 0.13
0.63	1.08 ± 0.03	3.25 ± 0.06	0.67 ± 0.02	3.27 ± 0.07
1.25	1.09 ± 0.03	3.15 ± 0.06	0.65 ± 0.02	3.28 ± 0.05
2.5	1.47 ± 0.08	4.59 ± 0.20*	0.70 ± 0.02	3.47 ± 0.06*
5.0	1.62 ± 0.03	5.14 ± 0.22*	0.76 ± 0.02	3.80 ± 0.05*

*$p < 0.01$, relative to controls.

samples were obtained from the inferior vena cava of chloroform anesthetized rats and by cardiac puncture from similarly anesthetized mice. Serum chemistry profiles (calcium, chloride, inorganic phosphorous, sodium, potassium, urea nitrogen, creatinine, total bilirubin, total protein, albumin, pH, albumin/globulin ratio, and pCO_2) were determined with a CentrifiChem 500 centrifugal analyzer. The only alterations in serum chemistries in these studies which were statistically different from controls and which did not exceed normal limits were serum urea nitrogen and serum creatinine values (Table 3). The elevated serum urea nitrogen and creatinine levels in male rats treated with 2.5 or 5.0% EG in the feed are indicative of EG-induced renal toxicity in these dose groups. In female rats, serum urea nitrogen levels were elevated at all dose levels. However, because of the lack of a dose response in females and because these values did not exceed normal limits, we do not attribute any toxicological significance to these differences. Creatinine levels were decreased in three of the dose groups of female rats. The meaning of these findings is not clear since there were no significant differences in mean body weights between these groups of animals and the controls. Serum chemistries were unaffected in male or female mice treated with EG. There were no changes in urinary parameters (urobilinogen, bilirubin, glucose, pH, blood, ketones, protein, nitrite, specific gravity, and microscopic appearance) in any of the treatment groups of rats or mice.

All animals in this study were subjected to a complete gross necropsy. Kidneys from the 2.5 and 5.0% dose groups of male rats had a granular, rough and/or pitted appearance. These were the only gross observations that appeared to be related to the administration of EG in any of the treatment groups. Tissues and organs were dissected from the carcass, fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues and organs were examined microscopically from high dose and control animals: gross lesions, tissue masses, abnormal lymph nodes, mandibular or mesenteric lymph nodes, salivary gland, thyroid, parathyroids, small intestine, colon, liver, gall bladder (mice only), prostate/testes or ovaries/uterus, lungs and mainstem bronchi, heart, esophagus, stomach, brain, thymus, trachea, pancreas, spleen, kidneys, adrenal glands, urinary bladder, pituitary gland, mammary gland, and sternebrae, femur or vertebrae including marrow. Any tissue or organ that was affected in the high dose groups was examined from lower dose animals until a no observable effect level was reached.

Chemically induced kidney lesions were observed in all male rats fed diets containing 2.5 or 5.0% EG (Table 4). Damaged kidneys contained deposits of transparent, doubly refractive crystals typical of calcium oxalate. Crystals were located mainly within tubular lumens in the renal cortex and were occasionally found in tubules in the medulla. Similarly appearing crystals were observed in the urinary bladder or urethral lumen of three of the highest dose (5.0%) male rats.

In male rats treated with EG, lesions of toxic nephrosis were superimposed on the background of minimal chronic nephropathy which is commonly seen in untreated male Fischer 344 rats. Severe toxic nephrosis

Table 3. Effect of ethylene glycol for 13 weeks on serum urea nitrogen and creatinine levels in Fischer 344/N rats.

	Urea nitrogen values, mg/dL		Creatinine values, mg/dL	
Dose, % of feed	Males	Females	Males	Females
0 (control)	17.0 ± 1.0	15.3 ± 0.7	0.73 ± 0.03	0.64 ± 0.06
0.32	22.3 ± 0.9	20.5 ± 0.7†	0.44 ± 0.03*	0.40 ± 0.05†
0.63	23.9 ± 0.6	18.8 ± 0.9†	0.81 ± 0.03	0.70 ± 0.05
1.25	20.8 ± 1.0	19.8 ± 0.5†	0.80 ± 0.02	0.67 ± 0.05
2.5	39.3 ± 2.8†	21.3 ± 0.7†	0.97 ± 0.07*	0.43 ± 0.03†
5.0	114.3 ± 14.0†	20.4 ± 0.5†	2.37 ± 0.29†	0.45 ± 0.04†

*$p < 0.05$, relative to controls.
†$p < 0.01$, relative to controls.

Table 4. Microscopic lesions in Fischer 344/N rats treated with ethylene glycol for 13 weeks.

	Mean severity (number of animals with lesion)[a]			
	Males			
Dose, % of feed	Nephrosis	Renal tubules, crystals	Brain crystals	Females, nephrosis
0 (control)	0.9 (9)	0 (0)	0 (0)	0 (0)
0.32	0.8 (8)	0 (0)	0 (0)	NE[b]
0.63	1.0 (9)	0 (0)	0 (0)	NE
1.25	0.9 (9)	0 (0)	0 (0)	0 (0)
2.5	3.0(10)	3.0(10)	0 (0)	0 (0)
5.0	4.0(10)	4.0(10)	2.0(10)	2.0(10)

[a]Severity of lesion: 1 = minimal; 2 = mild; 3 = moderate; 4 = severe.
[b]Not examined.

was diagnosed in the 5.0% dose group and moderate toxic nephrosis in the 2.5% dose group. Toxic nephrosis in the high dose groups involved distention and dilation of renal tubules, necrosis and regeneration of tubule epithelium, thickening of basement membranes, and fibrosis. The lumen of some tubules contained necrotic epithelial cells or aggregates of neutrophils. There was multifocal infiltration of lymphocytes, especially in areas of fibrosis.

Toxic lesions in the kidneys of female rats in the 5.0% dose group were multifocal and tended to be subcapsular. The cytoplasm of tubular lining cells was vacuolated, the nuclei were enlarged, and affected tubules were commonly surrounded by inflammatory cells. However, crystals such as those seen in the kidneys of treated male rats were not observed in the kidneys of female rats.

The brains of male rats in the 5.0% dose group contained clusters of transparent, doubly refractive crystals typical of calcium oxalate. There was no significant tissue response to the crystals.

Renal lesions diagnosed as mild toxic nephrosis were observed in about half the male mice in the 5.0% dose group and one mouse in the 2.5% dose group (Table 5). These lesions were characterized as tubular dilation, cytoplasmic vacuolization, and regenerative hyperplasia with piling up of nuclei. The lesions were focal and randomly distributed. There was no evidence of crystal formation in the affected tubules. Cytoplasmic vacuolization in epithelial cells of the convoluted tubules was seen in scattered foci in the treated and control groups of male mice. This lesion was slightly more severe in the highest dose group and may be compound-related. There were no adverse effects observed in female mice at any of the dose levels.

A degenerative change was present in the livers of all the male mice in the 5.0 and 2.5% dose groups. This change was characterized by the accumulation of an eosinophilic hyaline material that was not birefringent in the cytoplasm of hepatocytes adjacent to or close to central veins.

The objectives of these subchronic studies were to characterize dose-related toxic effects from repeated exposure to EG and to provide a basis for selection of dose levels for 2-year chronic studies. The 1.25% dose level appears to be a no-effect level for EG-induced renal toxicity (toxic nephrosis and crystals deposited in the kidney) in male rats treated for 13 weeks. This dose level corresponds to 0.6 to 1.0 g/kg/day. In a chronic study conducted by Union Carbide Corp. (*6*), renal tubular lesions were seen in the 1 g/kg dose group of male rats at a 6-month sacrifice, while calcium oxalate crystals with associated chronic nephritis were observed in male rats treated at this dose level for 12 months. In Blood's study (*4*), there was 100% mortality in a group of male rats fed diets containing 1% EG for 24 months; oxalate stones were observed in the kidneys of 11 of the 16 animals in that group. EG-induced renal toxicity in male rats is apparently progressive, and therefore, in order to insure good survival in a 2-year study, the maximum dose level for male rats should be somewhat less than 1%. Toxic lesions in the kidneys of female rats were observed microscopically only in the 5.0% dose group. Therefore, a maximum dose level of 2.5% appears to be suitable for a 2-year study in female rats. That dose level corresponds to 1.0 to 1.5 g/kg/day. Dose levels used in the Union Carbide study (*6*) included 0.2 g/kg/day for males and 1.0 g/kg/day for females. Since these dose levels appear to be adequate for an evaluation of the potential carcinogenicity of EG in rats, the NTP did not consider it necessary to perform a 2-year study of EG in rats.

In male mice, the no observable effect level for kidney and liver lesions produced by EG is 1.25%. However, the effects in the 2.5% dose group were considered to be minimal to mild and probably not life-threatening. There were no adverse effects seen in female mice at any of the dose levels (*7*). A 2-year study of EG administered in feed to groups of 60 B6C3F, male and female mice is in progress at Southern Research Institute. The dose levels for male mice are 0 (control), 0.625, 1.25 and 2.5%, while the dose levels for female mice are 0 (control), 1.25, 2.5 and 5.0%.

Ethylene Glycol Monoethyl Ether (EGEE)

Materials and Methods

EGEE used in the subchronic and chronic studies conducted at Gulf South Research Institute (New Iberia, LA) was manufactured by Union Carbide

Table 5. Microscopic lesions in male B6C3F$_1$ mice treated with ethylene glycol for 13 weeks.

Dose, % of feed	Mean severity (number of animals with lesion)[a]		
	Nephrosis	Kidney cytoplasmic vacuolization	Liver, centrilobular degeneration
0 (control)	0 (0)	0.8 (6)	0 (0)
0.32	NE[b]	NE	NE
0.63	NE	NE	NE
1.25	0 (0)	0.5 (3)	0 (0)
2.5	0.2 (1)	1.0 (8)	1.6(10)
5.0	1.1 (5)	2.0 (9)	2.4(10)

[a]Severity of lesion: 1 = minimal; 2 = mild; 3 = moderate; 4 = severe.
[b]Not examined.

Corporation. The purity of the bulk chemical was shown by vapor phase chromatography to be greater than 99%. In addition the chemical was shown to be stable in water. The route of administration originally selected for treating rats and mice with EGEE was dosed water. In a 14-day repeated dose study in which EGEE was administered in drinking water at dose levels ranging from 0.63% to 10%, there was a depression in weight gain in rats at all dose levels and a concomitant decrease in water consumption. Because of a concern that a palatability problem might obscure any toxicity due to the chemical treatment, the route of administration was changed from dosed water to gavage in a water vehicle.

Results and Discussion

Mortality data from the 14-day repeated dose study of EGEE administered by gavage to groups of five male or five female F344/N rats or $B6C3F_1$ mice are presented in Table 6. The treatment with EGEE was five times per week for 2 weeks. In each of these studies, there was 100% mortality at the 5.0 g/kg dose level and no mortality at the 1.25 g/kg dose level. These results are in line with LD_{50} values which were reported to vary from 3.0 to 5.5 g/kg body weight in rats (*7–10*) and from 4.0 to 4.8 g/kg body weight in mice (*7,10*).

A 2-year gavage study of EGEE dissolved in deionized water was conducted in Fischer 344/N rats and $B6C3F_1$ mice. Rats and mice of both sexes were administered EGEE at dose levels of 0 (control), 0.5, 1.0 or 2.0 g/kg body weight. Gavage volumes were adjusted to the weight of the animals to provide 5 mL/kg for rats and 10 mL/kg for mice. Rats were obtained from the Charles River Breeding Laboratories (Portage, MI) and were about 7 weeks of age when placed on the study, i.e., the age when they received their first dose. Mice were obtained from Charles River Breeding Laboratories (Stoneridge, NY) and were about 8 weeks of age when they received their first dose. For both species and sexes, animals were separated into various weight classes and randomly distributed into treatment and control groups. Rats and mice were housed five per cage. Treatment and control groups contained 50 animals per sex per species. EGEE solutions or deionized water were administered to the animals five times per week for 103 consecutive weeks. This was followed by a 1-week observation period. City tap water and NIH 07 diet were available *ad libitum* throughout the study. The environmentally controlled animal rooms had average temperatures between 72 and 76°F and the relative humidity varied between 40 and 70%. Filtered fresh air was provided at the rate of 12 room changes per hour. Fluorescent lighting was provided on a cycle of 12 hr on and 12 hr off. Animals were checked twice daily for mortality and signs of morbidity. Individual animal body weights were measured weekly for the first 13 weeks of the study and then monthly until the end of the study. Animals were also examined weekly for clinical signs.

Moribund animals and animals that survived to the end of the study were killed by exsanguination while under phenobarbital anesthesia and immediately subjected to a complete necropsy. Organs and tissues from all animals were preserved in 10% neutral-buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin. The same tissues listed in the ethylene glycol studies were examined microscopically from all animals in this study.

Kaplan-Meier survival curves for the 2-year studies of EGEE are shown in Figures 1–4. The most obvious effect is the marked reduction in survival of the high dose groups, i.e., the 2000 mg/kg or 2 g/kg dose groups. As a consequence of the high mortality rates, the high-dose groups for both sexes of both species were terminated at 17 to 18 weeks of the study. The control and other treatment groups, the 1.0 and 0.5 g/kg dose groups, were continued on the study until the scheduled terminal sacrifice. Statistical analysis (*11*) of the survival data showed that the survival of the medium-dose group of male rats (1000 mg/kg dose group) was significantly reduced ($p < 0.05$) in comparison to the survival of the control group (Fig. 1). Survival of the low dose group was not significantly different from the survival of the control group. At the end of the study there were 30 survivors in the control group, 38 survivors in the low-dose group, and 18 survivors in the medium-dose group. Survival of the medium-dose group of female rats (Fig. 2) was not significantly different from survival of the control group, while survival of the low-dose group was significantly increased ($p < 0.01$) in comparison to survival of the control group. At the end of this study there were 26 survivors in the control group, 46 survivors in the low-dose group, and 25 survivors in the medium-dose group.

For male or female mice there were no significant differences in survival between either the medium- or

Table 6. A 14-day study of ethylene glycol monoethyl ether administered by oral gavage to F344/N rats and $B6C3F_1$ mice: mortality data.

	Number of deaths/number per group			
Dose, g/kg body wt.	Male rats	Female rats	Male mice	Female mice
0 (control)	0/5	0/5	0/5	0/5
0.3	0/5	0/5	0/5	0/5
0.6	0/5	0/5	0/5	0/5
1.25	0/5	0/5	0/5	0/5
2.5	3/5	4/5	0/5	2/5
5.0	5/5	5/5	5/5	5/5

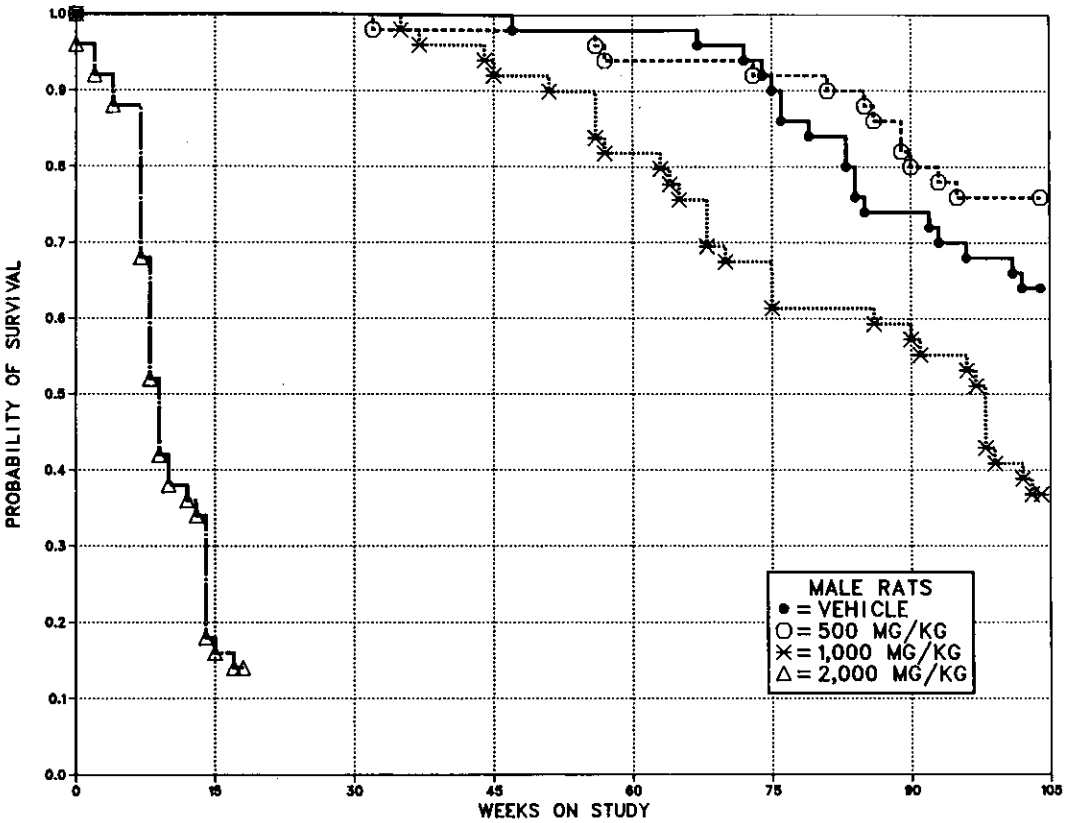

FIGURE 1. Kaplan-Meier survival curves for male rats administered ethylene glycol monoethyl ether by gavage.

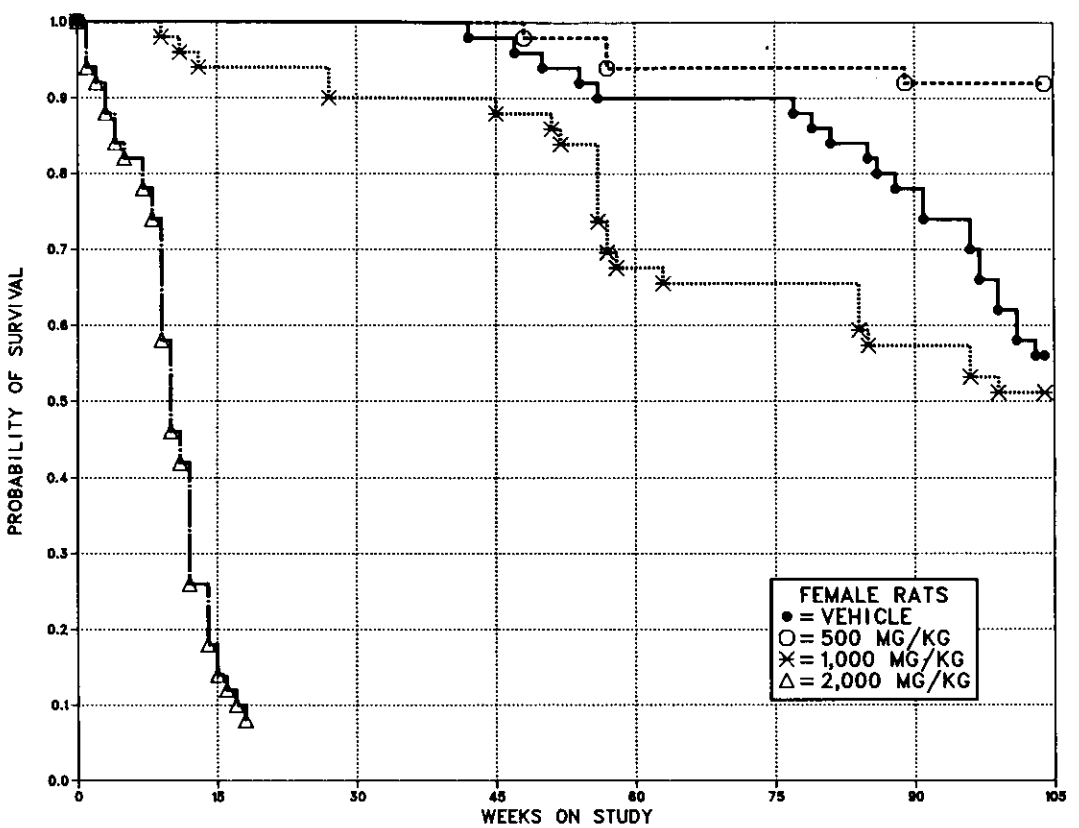

FIGURE 2. Kaplan-Meier survival curves for female rats administered ethylene glycol monoethyl ether by gavage.

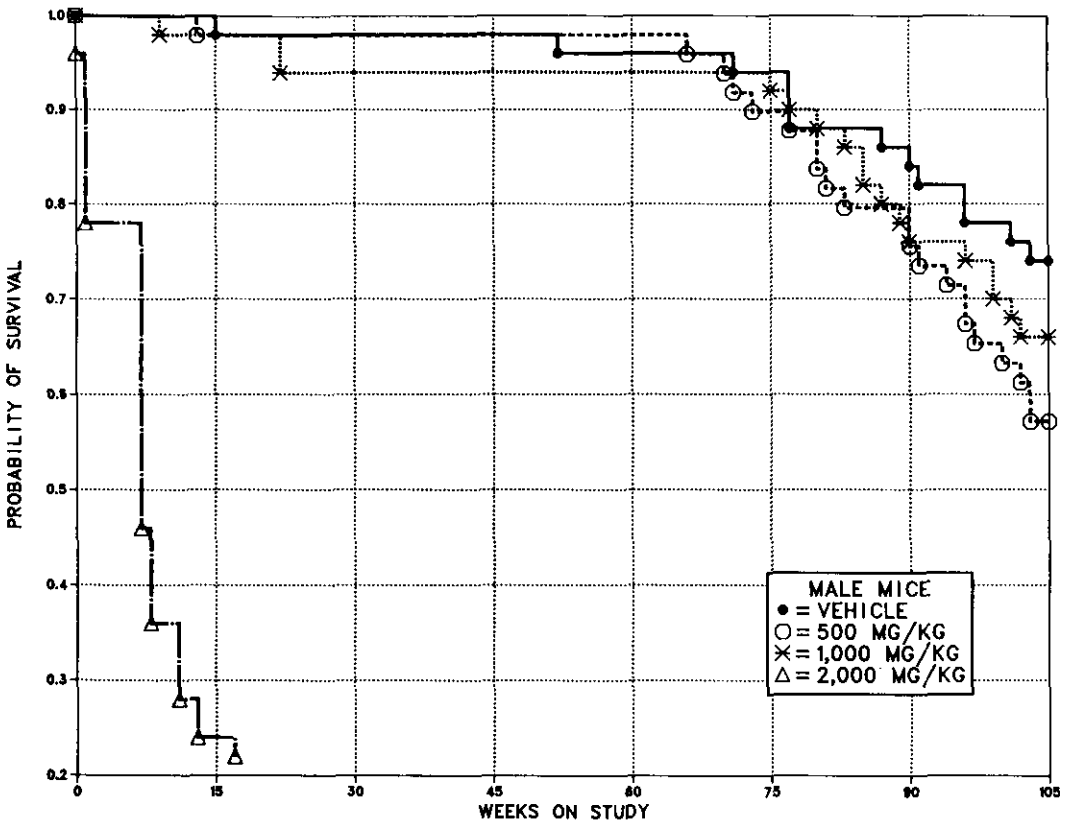

FIGURE 3. Kaplan-Meier survival curves for male mice administered ethylene glycol monoethyl ether by gavage.

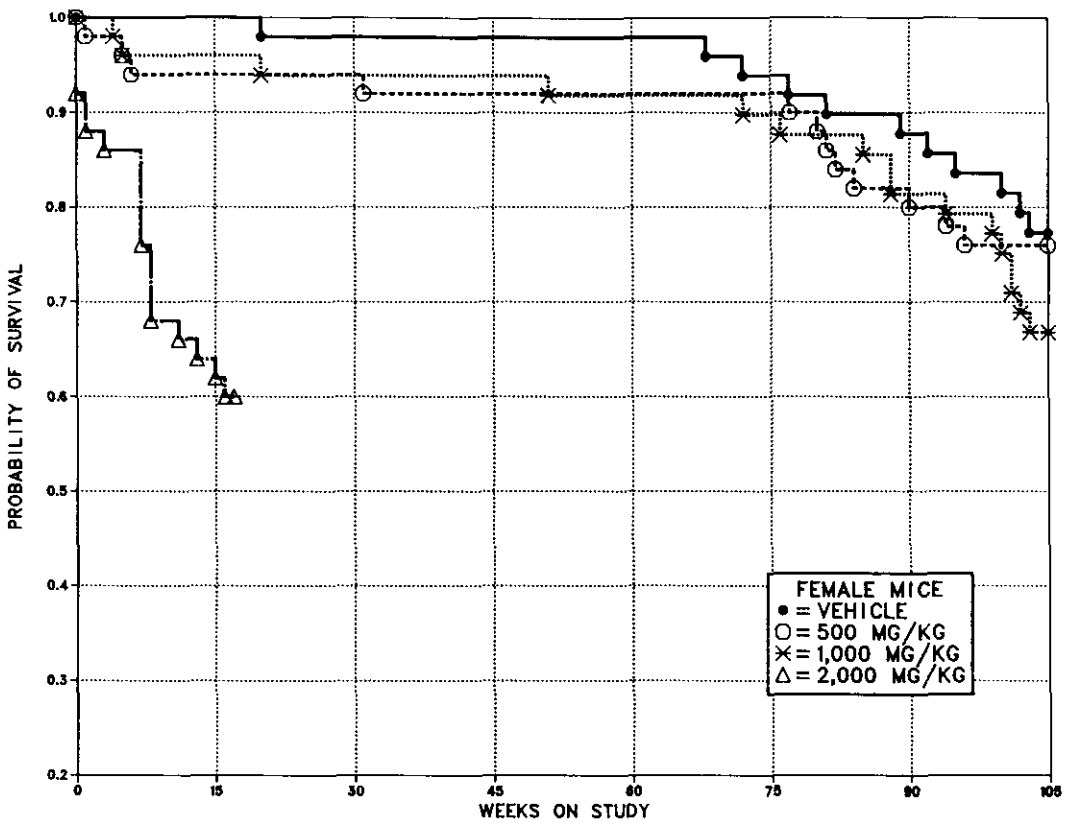

FIGURE 4. Kaplan-Meier survival curves for female mice administered ethylene glycol monoethyl ether by gavage.

Table 7. Mean body weights of F344/N rats and B6C3F$_1$ mice treated with ethylene glycol monoethyl ether for 2 years.

Dose, g/kg body wt.	Mean body weight at 104 weeks, g (% of control)			
	Male rats	Female rats	Male mice	Female mice
0 (control)	421	315	44	40
0.5	339 (81)	239 (76)	40 (91)	38 (95)
1.0	304 (72)	225 (71)	41 (93)	39 (98)

low-dose groups and the respective control groups (Figs. 3 and 4). At the end of the study of male mice there were 36 survivors in the control group, 28 survivors in the low-dose group, and 33 survivors in the medium-dose group. At the end of the study of female mice there were 36 survivors in the control group, 38 survivors in the low-dose group, and 32 survivors in the medium-dose group.

Dose-related depressions in mean body weight were apparent in both the male and female rat studies (Table 7). The differences in mean body weight between the control and treatment groups became apparent at about 15 weeks, and continued throughout the course of the study. The mean body weights of the male or female mouse groups treated with EGEE did not appear to differ markedly from the mean body weights of the respective control groups (Table 7).

Two types of gross lesions were commonly seen in the high-dose rats and mice that were terminated at 17 to 18 weeks of the study due to high early mortality (Table 8). First, the testes of the high-dose male rats and mice were generally decreased in size. Stenger et al. (*10*) and Nagano et al. (*12*) reported that EGEE causes testicular atrophy in male rats and mice. Microscopic examination of the testes of the high-dose male rats and mice confirmed the diagnosis of testicular atrophy in the present study. Second, stomach ulcers were observed in many of the high-dose male and female rats and the high-dose male mice. These lesions were probably a major contributing cause of death in the high-dose groups. There were no consistent gross lesions seen in the high-dose female mice.

Changes in the frequencies of gross lesions in the low-and medium-dose groups of rats and mice relative to the respective control groups in the 2-year study of EGEE are summarized in Table 9. There was an increased incidence of enlarged adrenal glands in male rats treated with EGEE in comparison to control male rats. A comparable change in treated female rats or in treated male or female mice was not observed at necropsy. Also, there were decreased incidences of enlarged spleens in the male and female rat groups treated with EGEE in comparison to the incidences in the control male and female rat groups. Pituitary changes, including enlargement, presence of a mass, and/or discoloration, which are relatively common in the aging Fischer 344/N rat were decreased in incidence in both male and female rat groups treated with EGEE relative to their respective control groups. Subcutaneous tissue masses in the mammary gland region were decreased in incidence in female rats treated with EGEE in comparison to control female rats. In the male Fischer 344/N rat, the common testicular changes which include enlarged testis with or without evidence of a mass were decreased in incidence in rats treated with EGEE compared to controls. Additionally, testis size was generally reduced in male mice treated with EGEE relative to control male mice. The histopathologic review of this study is in progress.

Table 8. Gross lesions in high dose (2.0 g/kg) Fischer 344/N rats or B6C3F$_1$ mice treated with ethylene glycol monoethyl ether.

Species/sex	Lesion
Rats, male	Testis, reduced in size Stomach, ulceration
Rats, female	Stomach, ulceration
Mice, male	Testis, reduced in size Stomach, ulceration
Mice, female	None

The following conclusions were made from the 2-year study of EGEE. Repeated administration of EGEE at the 2.0 g/kg dose level was lethal to rats and mice. Early mortality in the high-dose groups of rats and mice appeared to be due to stomach ulcers. EGEE caused testicular atrophy in male rats and mice. This effect was

Table 9. Gross lesions in Fischer 344/N rats or B6C3F$_1$ mice treated with ethylene glycol monoethyl ether for 2 years.

Lesion	Male rats	Female rats	Male mice	Female mice
Adrenal: enlarged	↑	—	—	—
Spleen: enlarged	↓	↓	—	—
Pituitary: enlarged, mass and/or discoloration	↓	↓	—	—
Subcutaneous tissue: mass in mammary gland region	—	↓	—	—
Testis: enlarged and/or mass	↓		—	
reduced in size	—		↑	

apparent in high-dose male rats which died early in the 2-year study and in the medium- and high-dose male mice. Gross observations indicate that chronic treatment with EGEE at dose levels of 0.5 or 1.0 g/kg body weight caused an apparent enlargement of the adrenal gland in male rats and interferes with the development of spontaneous gross lesions of the spleen, pituitary, and testis that commonly occur in the aging male Fischer 344 rat. Chronic treatment with EGEE also caused a decrease in the incidences of enlarged spleens and pituitaries and of subcutaneous masses in the mammary gland region in the aging female Fischer 344 rat.

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