4 EFFECTS OF EXPOSURE

4.1 EFFECTS ON HUMANS

4.1.1 Case Studies and Miscellaneous Reports

The first known case study involving occupational exposure to EGME was reported in 1936 [Donley 1936]. A female worker was employed in a shirt factory where she "fused" collars by dipping them into a solvent mixture, followed by application of pressure to dry and stiffen them. The solvent mixture used in the collar fusing contained EGME (<3%), dimethyl phthalate (<3%), isopropyl alcohol (74%), and water (20%). The patient had worked at this job for six months without ventilation or respiratory protection when she was admitted to the hospital with symptoms of encephalopathy (i.e., headache, drowsiness, forgetfulness, and general apathy), signs of respiratory infection with coughing and sneezing, and blurred vision. Blood tests for erythrocytes, leukocytes, and hemoglobin were within normal ranges. Her diagnosis included psychosis, encephalopathy, acute rhinitis (inflammation of the nasal mucosa), bronchitis, and phlegmasia alba dolens (extreme edematous swelling of the leg) caused by occupational exposure to vapors from the solvent. An inquiry three months after the patient's discharge from the hospital revealed that she had fully recovered.

Parsons and Parsons [1938] reported case studies of two brothers, ages 22 and 20, who fused collars in the "dipping room" of a New York shirt factory. Rubber gloves were worn during this operation. The dipping fluid at the shirt factory contained two substances, EGME and "Solox" which contained ethyl alcohol (90%), methyl alcohol (4.4%), ethyl acetate (4.7%), and petroleum naphtha (0.9%). Both men were admitted to the hospital with symptoms of toxic encephalopathy (including personality change, dizziness, sleepiness, and apathy), nausea, weakness, burning eyes, and headache. An examination in the hospital revealed moderately severe anemia with leukopenia and lymphocytosis. A neurologic examination revealed general hypertonicity of all skeletal muscles, transitory right ankle clonus, moderate ataxia, and persistent dilation of pupils. The patients completely recovered one month after they were removed from exposure and treated for anemia. When followup blood tests were conducted about one year later the older brother, who was exposed to the dipping fluid for about one year, had an abnormal differential count (i.e., a relative lymphocytosis). The younger brother, who was exposed for only three months, had a normal differential count.

All ten workers in a small printing shop in Germany experienced discomfort when a printing press that used aniline-dye-based inks containing EGME was placed in operation [Groetschel and Schuermann 1959]. After one month of exposure, the workers experienced vomiting, intoxication, and deterioration of vision, hearing, and sense of taste. Attending physicians

also reported exhaustion, slowed reactions, irritability, vertigo, and disturbance of sleep patterns in the workers. Anemia and lymphopenia were found in the one individual whose blood was tested.

Zavon [1963] described case histories for five workers exposed to EGME in the printing department of a plant where plastic materials were made. EGME was used as a cleaning agent for the printing machines and the floor, and as a solvent in the printing ink, which also contained diethylene glycol monomethyl ether (DEGME). Few of the workers wore gloves while working and they were not required to wear clean work clothes or wash their hands before leaving work. Each worker saw a different private physician when symptoms developed and Zavon [1963] summarized the case reports. All five workers had worked in the printing department for the five months that EGME had been in use. The signs and symptoms reported were consistent with those reported previously in workers exposed to EGME, including drowsiness, personality change, memory loss, ataxic gait, tremors, slurred speech, hearing loss, loss of appetite, and apathy. All workers had low erythrocyte (RBC) counts and low hemoglobin (Hb) values. White blood cell (WBC) counts ranged from low to high. Two workers had abnormal differential counts and a third worker's differential count was slightly outside normal limits, but his bone marrow smear showed a hypocellular marrow with a decrease in the percentage of erythroid elements. Breathing zone samples taken under simulated conditions using EGME to clean the floor and equipment ranged from 61 to 3,960 ppm. Process changes and safe-handling requirements resulted in a reduction of EGME concentrations to below 40 ppm and the elimination of any reported health effects.

Nitter-Hauge [1970] reported the accidental poisoning of two men who each ingested about 0.1 liter of pure EGME that they believed was ethyl alcohol. They were admitted to the hospital with general weakness, disorientation, muscular restlessness, nausea, and vomiting. Clinical signs and symptoms appeared from 8 to 18 hr after ingestion and included cerebral confusion, pronounced hyperventilation, and profound metabolic acidosis (reduced alkali reserve in the blood and body fluids). Moderate renal failure developed in the older of the patients, along with a marked oxaluria (abnormally large amounts of oxalates in the urine). Both patients were treated intravenously with sodium bicarbonate and ethyl alcohol, and fully recovered over a period of approximately 4 weeks. The author [Nitter-Hauge 1970] concluded from this information that EGME hydrolyzed to methanol and ethylene glycol, which are metabolized to formic acid and oxalic acid, respectively.

Dermal absorption of EGME has caused a range of adverse health effects similar to those produced by the inhalation or ingestion of it [Ohi and Wegman 1978]. Two male workers were employed in an electroplating operation where they washed equipment by hand without protective gloves. EGME was substituted for acetone in the solvent bath. Air samples collected during the washing operation averaged 8 ppm; no estimate was made of the magnitude of skin absorption. The first worker, who was 48 years old, was hospitalized following 6 months exposure to EGME in the workplace. His symptoms included confusion, lethargy, sleepiness, impaired hearing, anorexia, weight loss, and personality change. On admission to the hospital, he had tremors of both upper extremities and reduced RBC and WBC counts. Bone marrow aspiration showed marrow depression consistent with a marrow toxin. His condition was diagnosed as metabolic encephalopathy and pancytopenia

(a reduction in the numbers of all formed elements of blood). Recovery was slow but uncomplicated, and within several weeks his blood count returned to normal. The second worker was a 45-year-old man who was admitted with similar symptoms following one month of using EGME on the job. His neurologic examination revealed poor concentration, orientation, reasoning, and memory. In addition, he had bone marrow depression. His symptoms disappeared within one week [Ohi and Wegman 1978].

Cohen [1984] described subacute hematopoietic effects in a male worker exposed to EGME in the microfilm production industry. The subject was a 32-year-old microfilm coating and mixing operator. His job entailed mixing chemicals and often standing directly over open 1,500-gallon kettles that contained 33% EGME. Methyl ethyl ketone (MEK) and propylene glycol monomethyl ether (PGME) were also present in small quantities. EGME was also used as a solvent in the manual cleaning of the kettles, usually done without gloves. Breathing zone samples revealed time-weighted average (TWA) concentrations of 18 to 58 ppm EGME (average, 35 ppm), 1 to 5 ppm MEK, and 4 to 13 ppm PGME. The worker had been employed for less than one year when signs and symptoms of EGME exposure appeared. His WBC and RBC counts, Hb, hematocrit (Hct), and platelets dropped to abnormally low levels. He also slept more, gained weight, had a decrease in appetite, felt fatigued, and was apathetic. The worker was removed from skin and inhalation exposure to EGME after 20 months on the job. Blood counts 1 and 3 months later revealed a return to normal limits. This case illustrates the development of reversible macrocytic anemia and subjective central nervous system (CNS) complaints (i.e., increased sleep needs, decreased appetite, fatigue, and apathy).

Bolt and Golka [1990] reported the occurrence of hypospadias at birth in two young boys whose mother had been occupationally exposed to EGMEA during her pregnancies. The woman had worked since 1974 in an industrial laboratory that produced lacquers and enameled wire. During her first pregnancy in 1980 to 1981, she cleaned the glassware 4 hrs a day using EGMEA as a solvent. Gloves were usually, but not always, worn. She cleaned the surfaces of laboratory desks by spreading EGMEA on a cloth and rubbing the desk surfaces with it. This was frequently done without the use of gloves. During her second pregnancy in 1983 to 1984, she cleaned the glassware for about an hour a day, generally under a laboratory hood. As before, EGMEA was used to clean the surfaces in the laboratory.

In 1981, the woman delivered a boy of normal birth weight with the following malformations: perineal hypospadia, micropenis, and pronounced bifid type of scrotum. The sex could not be determined without chromosomal analysis. Analysis of the chromosomes did reveal a normal male karyotype. Clinical examinations showed no further malformations. In 1984, the woman delivered a boy of normal birth weight with penile hypospadia and a bifid type of scrotum. Chromosomal analysis revealed a normal male karyotype.

Both children underwent surgery in the following years. The perineal and the penile hypospadias were corrected, chordee was removed in both children, and the undescended testes were removed to the scrotum. The older child was treated with chronic gonadotrophin, which led to normal-sized testes.

The authors stated that the risk of isolated hypospadia was between 1 in 300 and 1 in 1,800, while the risk for a boy whose brother has hypospadia was 1 in 24. They indicated that both the family history and medical examinations showed no overt risks other than the pronounced exposure of the mother to EGMEA during fetal development. The authors concluded that the hypospadias were actually caused by exposure to EGMEA.

There is only one case report that describes the effects of EGEE [Fucik 1969]. A 44-year-old woman who mistakenly ingested 40 ml of EGEE experienced adverse effects on the CNS, liver, and kidneys. After ingesting the EGEE, she suffered chest pains and vertigo, and lost consciousness. Upon hospitalization, signs and symptoms of EGEE exposure included restlessness, tachycardia, cyanosis, swelling of the lungs, tonoclonic spasms, and breath that smelled like acetone. Oxygen and chemical therapy were administered; 6 hr later the woman regained consciousness but was confused and markedly agitated. Her urine was positive for protein, acetone, and RBCs; liver enlargement and jaundice developed. After 44 days her condition improved. However, insomnia, fatigue, and paresthesia of the extremities persisted for one year.

4.1.2 Clinical and Industrial Hygiene Studies

4.1.2.1 Greenburg et al. [1938]

Greenburg et al. [1938] described a cross-sectional study of 19 workers exposed to EGME during the manufacture of fused shirt collars at the same factory studied by Parsons and Parsons [1938]. Greenburg et al. undertook this study following reports to the U.S. Department of Labor that two brothers employed in a collar fusing plant were hospitalized with aplastic anemia [Parsons and Parsons 1938]. The Greenburg et al. study included (1) a clinical examination with occupational history and (2) an environmental assessment of the loft area where EGME exposure took place during the collar fusing process. In the fused collar processing area, workers wearing rubber gloves rinsed shirts by hand in large open vats of the solvent containing EGME.

Air sampling was done after improvements were made to the exhaust and ventilation systems. EGME concentrations were 25 ppm with the windows open and 76 ppm with the windows partially closed. The authors stated that previous worker exposures were undoubtedly higher than the concentrations they measured. The occupational histories showed that the duration of exposure for the 19 workers ranged from 1 to 112 weeks prior to the examinations. Four of the 19 were exposed longer than 75 weeks; the other 15 workers were exposed fewer than 15 weeks. Sixteen of the 19 workers were employed in the collar fusing area when the medical examination was conducted.

Two clinical examinations of the exposed workers were conducted about 2 months apart. During the first exam, 11 exposed workers were examined. About 2 months later, 8 of the 11 original workers were reexamined and 8 additional workers were examined for the first time. Social, medical, and occupational histories were taken. Then the workers were given a complete medical examination with special attention to funduscopic tests, capillary

fragility tests, detailed hematologic studies, and neurologic findings. The results were normal for the funduscopic tests, capillary fragility tests, blood pressures, temperatures, pulse rates, and general physical status.

All 19 workers had abnormal hematology results, including low blood platelet counts. Nine of the workers' blood tests showed disturbed production of RBCs (erythropoiesis) including six subnormal RBC counts. The anemias were thought to be caused by a bone marrow toxin rather than hemolysis or peripheral toxicity. All 19 blood tests showed immature neutrophilic granulocytes indicative of bone marrow toxicity. Other results were normal, including Hct, bleeding time, coagulation time, erythrocyte sedimentation rate, and erythrocyte fragility.

Physical examinations of the workers revealed severe neurologic abnormalities. Two workers who were hospitalized for severe anemia and one who filed workman's compensation for multiple neuritis were not included in the physical findings. Four of the remaining 16 workers had symptoms of drowsiness or fatigue and the following neurologic abnormalities: clonus (rapid muscular contractions and relaxations) in one worker, mental retardation in two workers, exaggerated reflexes in four workers, and tremors of the hands in four workers. Four other workers did not complain of any symptoms; however, at examination two of these workers had abnormal reflexes, one had exaggerated knee and ankle reflexes, one had decreased knee jerks, and four had tremors of the hands. The remaining eight workers exhibited no abnormal symptoms.

4.1.2.2 Cook et al. [1982]

Cook et al. [1982] conducted a cross-sectional study of 65 male workers (40 with potential exposure to EGME during its manufacture and packaging) to determine if anemia, leukopenia, or infertility were present, and if these conditions were more prevalent among the exposed workers. The unrestricted, concurrent, nonexposed population consisted of 25 workers from plants where alkanolamines and salicylic acids were produced. In the EGME plant, the chemical was manufactured by a continuous enclosed process along with related products such as EGEE, polyols, polyoxy propylene glycols, brake fluids, butylene oxide, and polyglycols. In a separate packaging and distribution facility, EGME was loaded into drums, tank trucks, or rail cars; although drums were filled automatically, they were capped manually. Because of the potential for skin contact and absorption, continued use of rubber gloves was recommended during sampling and maintenance. There was also potential for exposure to ethylene, propylene, and butylene oxides, chlorobenzenes, and other ethylene glycol ethers. The control population had the potential for exposure to phenol, sodium phenate, potassium hydroxide, ammonia, propylene ethers of ethylene glycol, ethylene oxide, and propylene oxide. Industrial hygiene measurements taken in the production area indicated personal 8-hr TWA exposures of 0.4 ppm EGME or less. TWA air samples collected in the packaging and distribution facility for EGME indicated personal exposures of 5 to 9 ppm EGME and area concentrations of 4 to 20 ppm.

The clinical measures included Hb, WBC, RBC, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCHb), mean cell hemoglobin concentration (MCHC), hormone levels [i.e., luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone] and

sperm counts. Semen samples were available from six EGME-exposed workers and nine controls. Hematology results revealed no anemia or leukopenia in the EGME-exposed workers. No statistically significant differences were found in hematology test results, hormone levels, or sperm counts between the exposed workers and the controls. The investigators suggested that testicular size may have been reduced; however, the decrease in size approached but did not reach statistical significance ($P<0.05$) in either length ($P=0.19$) or width ($P=0.08$).

4.1.2.3 Markel and Moody [1982]

In a Health Hazard Evaluation [Markel and Moody 1982], NIOSH investigators evaluated exposure of workers to surfactant and emulsifier products used in a wet scrubbing system of a newspaper pressroom and reel room. The nonionic surfactant used in the air washer/demisting system was nonylphenoxy polyethylene oxyethanol and contained ethylene oxide and EGME. Although the surfactant was not used per se in the demisting system, it could have been present in pressroom air as a result of aerosolization of the surfactant solutions. Twenty workers were interviewed; half of them were asymptomatic. Five workers reported intermittent runny nose, nasal congestion and/or eye irritation at work. Of these five workers, three had histories of allergies or "sinusitis"; one of the five workers attributed his episodes of burning eyes to exposure to the mist produced by the de-mist system. The remaining five all complained of a "peculiar taste" in the mouth following exposure to the mist system. During splashing or siphoning by mouth of the surfactant solution, two workers noted an anesthetic effect on their lips. One worker experienced progressive ill health over a 10-month period. His symptoms consisted of headaches, fatigue, sores in the mouth, chronic eye irritation, shortness of breath, nausea, vomiting, trembling, and staggering. However, he stated that following termination of his work with the de-mist system, these symptoms abated. Environmental monitoring (five breathing zone and three general area measurements) indicated that the concentrations of EGME ranged from 0.3 to 0.5 ppm for six of the samples and were below the lower limit of detection (0.003 ppm) of the analytical method for the remaining two. The authors concluded that there was no evidence of EGME concentrations exceeding recommended levels and no evidence of ethylene oxide exposure.

4.1.2.4 Boiano [1983]

NIOSH conducted a health hazard evaluation in 1983 to evaluate worker exposure to two solvent cleaners, an image remover, and the paint remover used in a silk screening process [Boiano 1983]. The silk screener using the image remover was monitored for exposure to EGEEA and cyclohexanone, while the worker using the paint remover was monitored for a variety of organic solvents, one of which was EGEEA. Although the workers were primarily exposed by inhalation, they may have also been exposed by skin absorption because personal protective clothing was not always worn. The workers complained of headaches, lethargy, sinus problems, nausea, and heartburn. When they were away from work, their symptoms improved. The silk screener using image remover had TWA exposures to EGEEA ranging from 1.3 to 3.3 ppm, with short-term excursions to 3.8 ppm. The silk screener using paint remover had TWA exposures to EGEEA ranging from 0.5 to 3.9 ppm, with a short-term excursion to 4.0 ppm. Measured airborne exposures thus were

below occupational standards, but absorption through the skin may have contributed to the workers' overall exposure [Boiano 1983].

4.1.2.5 Gunter [1985]

In 1985, NIOSH conducted a health hazard evaluation of production areas at a plant that manufactured solid-state electronic circuits [Gunter 1985]. Soldering, degreasing, and circuit-board coating areas were evaluated. Workers in these areas had previously complained of narcosis, burning eyes, and dermatitis. Personal and area air samples were collected on charcoal tubes and analyzed for EGEE, EGEEA, Freon 113, toluene, MEK, xylene, petroleum distillates, methyl isobutyl ketone (MIBK), and methyl chloroform. Samples were also collected and analyzed for lead, zinc, and toluene diisocyanate (TDI). Six personal air samples taken for EGEE averaged 1.7 ppm; 14 personal air samples for EGEEA averaged 0.15 ppm. Samples taken for lead, zinc, and TDI were found to contain concentrations below even the lowest of the established limits (the NIOSH REL, the OSHA PEL, or the ACGIH TLV®).

4.1.2.6 Ratcliffe et al. [1986]

NIOSH conducted an evaluation for possible adverse effects on testicular function in male workers potentially exposed to EGEE during the preparation of ceramic shells used to cast metal parts [Ratcliffe et al. 1986]. The binder slurry included 50% EGEE and 50% ethanol. About 80 workers were employed in the investing departments at each of the sites where these ceramic shells were prepared. The potentially exposed male workers included those engaged in the preparation of binder slurry, hand dippers and grabbers who dipped molds into the slurry, shell processors who prepared and handled ceramic shells, supervisors, and process engineers. Although gloves were worn by some workers, no other chemical protective clothing or respirators were used. The comparison group consisted of men who worked elsewhere in the plant and who were not exposed to EGEE. Air samples, most of which were from the breathing zone, were collected. Because the potential for skin exposure existed, spot urine samples were taken and sent frozen to a laboratory to analyze for the presence of ethoxyacetic acid (EAA); blood samples were also drawn and analyzed. An evaluation of semen quality (pH, sperm concentration, and viability, motility, velocity, and morphology) was conducted. Brief examinations of the urogenital tract were also done. In addition, questionnaires were administered to determine personal habits and medical and work histories.

The NIOSH survey showed full-shift, breathing-zone exposures of EGEE ranging from nondetectable to 24 ppm. Collection of general area air samples at two sites revealed higher concentrations of EGEE (10 to 17 ppm) in the investment rooms, which contained open tanks of slurry, than in the mixing and storage rooms (5 to 7 ppm). However, analysis of quality control samples indicated that the measured airborne concentrations could be underestimated; recovery of analyte from field samples was below 100%, and as low as 60%. Analysis of blood samples collected at the end of the work shift from nine EGEE-exposed and four nonexposed workers revealed no detectable levels of EGEE in any of the samples. The concentrations of EAA in the urine of EGEE-exposed workers ranged from 16 to

163 mg/g creatinine for individual voids. No statistical testing was attempted because of the few data points.

A cross-sectional evaluation of semen quality was conducted among 37 men exposed to EGEE and a group of 38 men who were not exposed to EGEE. The average sperm count of the EGEE-exposed group was considerably lower than that of the nonexposed group (113 vs. 154 million per ejaculate, $P<0.05$). The mean sperm concentration of the unexposed group (60 million/ml) and that of the exposed group (48 million/ml) did not differ statistically from each other. It should be noted however, that the average sperm concentration for both groups was considerably lower ($P<0.001$) than the 70 million/ml these investigators had observed in similar studies of other working populations. No differences were observed with respect to other characteristics of semen quality or testicular size. It was concluded from this study that there was a possible effect of EGEE on semen quality.

4.1.2.7 Welch et al. [1988], Sparer et al. [1988], and Welch and Cullen [1988]

The effect of combined EGME and EGEE exposure on the reproductive potential of men who worked in a large shipbuilding facility was recently studied [Welch et al. 1988]. This site was selected for study because of a previous health hazard evaluation [Love and Donohue 1983] in which evidence of glycol ether exposure had been obtained. The shipyard employed 900 painters, 600 of whom were men. The painters were divided into four crews that included the shop men who mixed the paint formulations, interior and exterior painters, and the tank crew that painted interiors of ballast tanks and other confined spaces. The interior painting crew members were involved in a variety of jobs, using spray and brush painting; half-face cartridge respirators were available to these men, but their use was at the discretion of the individual painters. The tank crew applied paint primarily in spray form and wore supplied-air respirators. Cotton gloves were available for use. In the course of a year, many painters rotated from crew to crew. At the completion of a boat's construction, exterior painting was done; often all the painters were assigned to this job for a brief period of time. Prior to painting, exterior painters wearing air-supplied respirators sandblasted the boats. The entire study population for the semen, hematologic, and male reproductive studies consisted of 94 painters and 55 nonexposed controls, but only 73 of the 94 painters and 40 of the 55 nonexposed controls participated in the semen study. Urine was collected for the determination of EAA and methoxyacetic acid (MAA), the principal metabolites of EGEE and EGME (Section 4.2), respectively. At the clinic site, participants filled out questionnaires revealing personal habits and medical and work histories; a medical examination was performed at the same time.

Personal air samples were collected over six workshifts for three consecutive days and analyzed for EGME and EGEE [Sparer et al. 1988]. Because no tanks were being painted at the time of the study, sampling was performed only for the interior work. The industrial hygiene survey revealed that the painters were exposed to EGME at a TWA concentration of 0 to 5.6 ppm with a mean of 0.8 ppm and a median of 0.44 ppm, and to EGEE at 0 to 21.5 ppm with a mean of 2.6 ppm and a median of 1.2 ppm. Urine samples were obtained from each participant during the medical examination and when the participant brought his semen sample. Measurement of the urinary metabolites MAA and EAA [Smallwood et al. 1988]

confirmed that the painters had been exposed to EGME and EGEE; none of the controls' specimens had detectable MAA or EAA. Two other reproductive toxins, lead and epichlorohydrin, were present in the work environment. Epichlorohydrin was not detected in the air sampling. Exposure to lead was limited to sandblasting operations. Although there were significant air lead levels during blasting, the painters wore air-supplied respirators during this operation. A review of the shipyard biological monitoring data revealed that most blood lead levels were below 20 microgram (μg)%, with the highest single level being 40 μg% [Welch et al. 1988].

Serum samples were analyzed for testosterone, FSH, and LH. It was concluded that there was no pituitary or hypothalamic dysfunction in the exposed group relative to controls. Semen samples were analyzed for pH, sperm density, viability, count, motility, and morphology. The authors reported that although the semen of the exposed group had a significantly lower pH, no significant differences were found in measures of sperm motility, viability, and morphology (no statistics presented). They compared mean sperm density and count using analysis of variance. Sperm counts per ejaculate and per cubic centimeter (cc) of semen were lower (but not statistically significant) in the painters. The proportion of men with a sperm density ≤20 million/ml was higher in the exposed group than in the unexposed group (13.5% vs. 5%, P=0.12). The authors also compared the proportion of each group with oligospermia (defined as a count per ejaculate ≤100 million). Eight of the controls (20%) and 24 of the painters (32%) had oligospermia (P=0.2). The authors concluded that exposure to EGME and EGEE caused functional impairment by lowering sperm counts in this group of painters. In addition, when the authors controlled the analysis for the effects of smoking, they concluded that there was an increased odds ratio for a lower sperm count per ejaculate [Welch et al. 1988].

The effect of combined EGME and EGEE exposure on hematologic parameters was also assessed in these 94 painters and 55 controls [Welch and Cullen 1988]. Mean values for Hb, Hct, total and differential WBC count, and platelet count were assessed for painters and controls. Statistical analysis revealed no difference between exposed and nonexposed groups in mean Hb and Hct levels, and polymorphonuclear leukocyte and platelet counts. However, nine painters, and no controls were anemic. Similarly, five painters and no controls had mild to moderate granulocytopenia. A review of company medical records indicated that these abnormalities were acquired during employment. Analysis of blood lead levels appeared to eliminate lead as the cause of the abnormalities. Exposure to EGME and EGEE was suspected as being the cause of the hematologic disorders. However, because of the authors' inability to establish an exposure-effect relationship, they concluded that further investigation was necessary.

4.2 METABOLISM, UPTAKE, AND ELIMINATION

4.2.1 Studies in Animals

Studies have been conducted in animals to determine the metabolites of EGME and EGEE. Investigations by Tsai [1968] and Blair and Vallee [1966] demonstrated that EGME is a

possible substrate for alcohol dehydrogenase (ADH). Miller et al. [1982, 1983b] concluded that EGME was oxidized via ADH to methoxyacetaldehyde and via aldehyde dehydrogenase to MAA. In studies using radio-labeled EGME, MAA was identified as the major metabolite, and the urine as the major route of elimination [Miller et al. 1983b; Moss et al. 1985].

The metabolism of EGME to MAA has been evaluated as a bioactivation mechanism for EGME [Miller et al. 1982, 1983b; Foster et al. 1983; Brown et al. 1984]. Oral administration of MAA caused testicular changes; increases in embryo-fetal death; decreased fetal weights; increases in structural malformations; and urogenital abnormalities; and heart, tail, and limb defects [Miller et al. 1982; Foster et al. 1983; Brown et al. 1984; Ritter et al. 1985]. The preceding effects were similar to those caused by corresponding EGME doses.

Pretreatment of rats with pyrazole, an ADH inhibitor, inhibited the metabolism of EGME to MAA; however, pretreatment of rats with disulfiram, an aldehyde dehydrogenase inhibitor, had no significant effect on plasma or urinary metabolic profiles [Moss et al. 1985]. Administration of EGME by i.p. injection demonstrated extensive degeneration and necrosis of rat primary spermatocytes in the early and late pachytene stages of development. Pretreatment of rats with pyrazole appeared to protect against spermatocyte damage, while pretreatment with disulfiram had no effect on the degree of spermatocyte damage.

The role of EGME metabolism in the induction of paw malformations was also examined [Sleet et al. 1988]. Single oral exposures of mice to EGME or MAA produced comparable digit anomalies. The incidence of digit malformations was lower in i.v.-treated mice than in gavage-treated mice. When orally administered 1 hr before EGME, 4-methylpyrazole (4-MP), a potent ADH inhibitor, reduced the incidence of paw malformations in a dose-dependent manner. Oral administration of ethanol with and after EGME also caused reduced incidences of digit anomalies [Sleet et al. 1988]. These data are compatible with those of Romer et al. [1985], which demonstrated that ADH has a higher affinity for ethanol than for the glycol ethers [Sleet et al. 1988].

Administration of EGEE by gastric intubation or inhalation resulted in two major urinary metabolites in rats, EAA and N-ethoxyacetyl glycine [Jonsson et al. 1982; Cheever et al. 1984]. In rats, the metabolism of EGEE proceeded chiefly through oxidation via ADH to EAA, with some subsequent conjugation of the acid metabolite with glycine [Jonsson et al. 1982; Cheever et al. 1984]. EAA was found in rat testes 2 hr after oral administration of EGEE. The data suggested that adverse testicular effects exerted by EGEE may be caused by its active metabolite EAA [Cheever et al. 1984].

Foster et al. [1987] examined the toxicity of MAA and EAA. Oral administration of equimolar doses of MAA or EAA in rats determined the initial target for testicular toxicity. Histologic examination of testes revealed testicular damage in all MAA-treatment groups, while EAA exerted testicular damage only at the highest dose. Pachytene spermatocytes were targets for reversible MAA and EAA toxicity. The addition of MAA or EAA to in vitro Sertoli cell and germ cell cultures caused depletion of pachytene spermatocytes within 24 hr [Foster et al. 1987].

An in vitro culture system utilizing rat embryos was used to assess potential adverse effects of MAA and EAA on fetal development [Rawlings et al. 1985]. At the highest dose used, both metabolites exerted adverse effects on fetal development in vitro. These effects included significant reductions in crown-rump length, head length, yolk sac diameter, and protein content of the embryo.

4.2.2 Studies in Humans

Recently EAA has been identified in the urine of workers exposed to EGEE vapor during physical exercise and at rest [Groeseneken et al. 1986a; Groeseneken et al. 1986c]. These findings are consistent with the previously described biotransformation studies in animals which identified EAA as the major metabolite of EGEE [Jonsson et al. 1982; Cheever et al. 1984]. The total amount of urinary EAA was related to the EGEE concentration in inspired air, uptake rate, pulmonary ventilation rate, oxygen consumption during exposure, and heart rate during and after exposure [Groeseneken et al. 1986c]. After the end of a 4-hr EGEE exposure period, maximal EAA excretion was achieved within 3 to 4 hr. EAA excretion then declined slowly with a biological half-life of 21 to 24 hr [Groeseneken et al. 1986c]. On the average, 23% of the absorbed EGEE was recovered as EAA within 42 hr. Respiratory frequency was also a contributing factor in urinary EAA concentration. About 64% of inhaled EGEE vapor was retained at rest, and retention increased as physical exercise was performed during exposure. The rate of EGEE uptake increased as exposure concentration or pulmonary ventilation rate, or both, increased. Individual uptake of EGEE appeared to depend on pulmonary ventilation or cardiac output, or both and not on anthropometric factors [Groeseneken et al. 1986b].

Groeseneken et al. [1988] compared urinary EAA excretion in man and rats after EGEE exposure (oral in rats and by inhalation in man). The human data were taken from Groeseneken et al. [1986c]. In rats the mean elimination half-life was determined to be 7.2 ± 1.5 hr; in man the half-life mean was 42 ± 4.7 hr. (This half-life of 42 hr differs from 23 hr reported in Groeseneken et al. [1986c].) The authors [Groeseneken et al. 1988] attributed the difference in half-lives to the averaging effect of pooling urine collections [Groeseneken et al. 1986c], especially during the first 12 hr.

EAA has also been identified in man as a metabolite of EGEEA [Groeseneken et al. 1987b]. EGEEA is believed to pass through the same metabolic pathway as EGEE after hydrolysis of the ester moiety. EAA excretion in workers exposed to EGEEA vapor was similar to EAA excretion in workers exposed to EGEE [Groeseneken et al. 1986c]. The maximal EAA excretion rate was achieved 3 to 4 hr after the end of the EGEEA exposure period; however, unlike EGEE exposure, a second peak EAA excretion appeared 3 hr later. On average, within 42 hr, 22.2% of absorbed EGEEA was metabolized and excreted as EAA [Groeseneken et al. 1987b]. In beagle dogs exposed to 50 ppm EGEEA for 5 hr, 80% of EGEEA was absorbed in 10 min and reached a plateau in 3 hr [Guest et al. 1984]. The pharmacokinetics of respiratory uptake were more complicated for EGEEA than for EGEE. Individual uptake of EGEEA was determined by pulmonary ventilation, cardiac output, height, and body fat. During exposure to EGEEA vapor, partial respiratory elimination of EGEE was observed.

This finding confirmed the hypothesis that EGEEA is first converted to EGEE by esterases [Groeseneken et al. 1987a].

MAA was detected in the urine of seven male volunteers exposed at rest to 5 ppm EGME [Groeseneken et al. 1989a]. MAA was present in the urine during and up to 120 hr after the beginning of exposure. The elimination half-life of MAA was estimated to be 77 hr. By extrapolation the total amount of MAA was estimated to be 85.5% of inhaled EGME.

No studies are available on the metabolism of EGMEA. However, based on the metabolism of EGEEA to EAA [Groeseneken et al. 1987a,b], EGMEA would be expected to act similarly and be metabolized to MAA.

A detailed description of the preceding studies may be found in Appendix B.

4.3 EFFECTS ON ANIMALS

Although kidney and liver damage, hematologic, CNS, reproductive, and teratogenic effects have been observed in experimental animals exposed to glycol ethers and their acetates, the type and severity of the response induced by each glycol ether are not identical. Therefore, each glycol ether and its corresponding acetate will be discussed separately following Section 4.3.1.

4.3.1 Acute Toxicity

Many experiments investigating the acute toxicity of glycol ethers to animals have been performed. These investigations led to the establishment of a lethal concentration or lethal dose for 50% of the exposed animals (LC_{50} or LD_{50}) in a variety of species by a variety of routes (inhalation, oral, dermal, injection). A summary of the available data by animal species is presented in Table 4-1.

4.3.1.1 Oral Administration

The toxicity of glycol ethers has been studied more extensively by oral administration than by any other route. Hematuria, narcosis, and digestive tract irritation were reported after oral administration of near-lethal or lethal concentrations of EGEE, EGEEA, EGME, or EGMEA in rats, mice, rabbits, and guinea pigs [Laug et al. 1939; Smyth et al. 1941]. However, the principal effect exerted by these glycol ethers in animals that did not die immediately was damage to the kidneys. Pathological examination revealed extreme tubular degeneration along with almost complete necrosis of the cortical tubules.

4.3.1.2 Inhalation Exposure

Waite et al. [1930] examined the effect on guinea pigs of a single inhalation exposure to EGEE vapor. The EGEE concentrations and periods of exposure ranged from those that produced death to those that caused no apparent effect after 24 hr of exposure. EGEE

Table 4-1.-Lethal doses or concentrations of glycol ethers

Species and sex	LD_{50}* oral (mg/kg)		LD_{50} oral (mg/kg)		LD_{50} i.p. (mg/kg)		LD_{50} i.v. (mg/kg)	LD_{50} dermal (mg/kg)		LC_{50} inhalation (ppm)	
	EGME	EGMEA	EGEE	EGEEA	EGME	EGEE	EGEE	EGME	EGEEA	EGME	EGEE
Rat:											
Male	2,460	3,930	3,000	3,900	----	----	----	----	----	----	----
	3,250	----	5,000	5,100	----	----	----	----	----	----	----
Female	3,400	----	2,300	2,900	----	----	----	----	----	----	----
	----	----	5,400	----	----	----	----	----	----	----	----
Not stated	----	----	3,204	----	----	----	2,691	----	----	----	----
Rabbit:											
Male	890	----	3,100	----	----	----	----	1,300	----	----	----
Not stated	----	----	----	----	----	----	840	----	10,500	----	----
Guinea pig:											
Male and Female	950	1,250	1,400	1,910	----	----	----	----	----	----	----
Not stated	----	----	2,584	----	----	----	----	----	----	----	----
Mouse:											
Female	----	----	----	----	2,150	1,709	----	----	----	1,480	1,820 (7 hr)
Not stated	----	----	3,991	----	----	----	3,600	3,600	----	----	----

*Abbreviations: i.p. = intraperitoneal; i.v. = intravenous; LD_{50} = median lethal dose; LC_{50} = median lethal concentration.

concentrations ranged from 500 to 6,000 ppm and were administered over a period of 1 to 24 hr. Guinea pigs exposed to 6,000 ppm for 24 hr exhibited inactivity, weakness, and dyspnea, and died by the end of the exposure; 3,000 ppm for 24 hr caused death within 24 hr following exposure; and exposure to 6,000 ppm for 10 hr, and 3,000 ppm or 1,000 ppm for 18 hr, caused death 1 to 8 days following exposure. Exposure to 6,000 ppm for 1 hr, 3,000 ppm for 4 hr, and 500 ppm for 14 hr caused no apparent harm. Gross pathological examinations of animals that died during and up to two days after exposure revealed congestion and edema of the lungs, distended and hemorrhagic stomachs, and congested kidneys.

Werner et al. [1943c] demonstrated an adverse effect of EGME and EGEE on the hematopoietic system. Groups of 14 or 16 white Swiss mice were subjected to single, 7-hr, inhalation exposures to EGME (930 to 6,800 ppm) and to EGEE (1,130 to 6,000 ppm). Although these vapors produced no typical narcotic action in mice, there was marked dyspnea. Histopathological examinations revealed slight damage to the lungs. The spleen consistently showed marked follicular phagocytosis, which indicated toxic action on the WBC [Werner et al. 1943c].

Groups of female rats developed increased osmotic fragility of erythrocytes when exposed by inhalation to EGEE, EGEEA, EGME, or EGMEA for 4-hr periods [Carpenter et al. 1956]. Of the four compounds, EGMEA (32 ppm) was the most toxic in terms of erythrocyte fragility, followed by EGEEA (62 ppm), and EGEE (125 ppm); EGME at 2,000 ppm only slightly affected erythrocyte fragility.

Ten male and ten female rats and two male and two female rabbits were exposed to 2,000 ppm EGEEA for 4 hr [Truhaut et al. 1979]. Only in rabbits was there a slight and transient hemoglobinuria or hematuria; no gross pathological lesions were noted in either species.

4.3.1.3 Dermal Exposure

A modified Draize "sleeve" technique was used to study the acute dermal toxicity of EGEEA in rabbits [Truhaut et al. 1979]. Death generally occurred 24 to 48 hr after the application of 10,500 mg EGEEA/kg. Although hemoglobinuria and/or hematuria were observed, there was little variation in Hb concentration and the number of RBCs (less than 15% to 20%) in blood; however, there was a considerable decrease in the number of WBCs (50% to 70%). In surviving animals, the WBC counts gradually returned to normal. Necropsy revealed bloody kidneys and blood in the bladder. When survivors were examined after the 2-week observation period, no gross lesions were noted.

4.3.1.4 Intraperitoneal, Intravenous, and Subcutaneous Administration

Karel et al. [1947] conducted the first toxicity study of the glycol ethers administered by intraperitoneal (i.p.) injection. Female albino Carsworth Farms mice (9 to 10 animals/dose) were injected intraperitoneally with varying doses of either EGEE or EGME and were observed for 7 days following injection. Gross and microscopic pathological studies were

conducted on animals that died during the first 7 days after injection or were sacrificed at the end of the 7-day observation period. The LD_{50} for EGME was 2,150 mg/kg and the LD_{50} for EGEE was 1,709 mg/kg. During the first 72 hr after injection of either EGME or EGEE, toxic reactions in the lymph nodes and spleen (lymphocyte degeneration followed by reticulum cell proliferation and phagocytosis of cellular debris) and mild renal glomerular and tubular degeneration were noted. During the fifth through seventh day, lymphoid regeneration occurred while renal tubular damage continued. Pulmonary congestion and atelectasis (collapse of the alveoli or a portion of the lung) were also observed in EGEE-treated mice.

Dogs and rabbits were given three 7.1-g injections (unspecified as to type or site) of EGME [Wiley et al. 1938]. At necropsy (2 to 3 days after last injection) histological examination of the dogs' organs revealed damage to the kidney, bladder, liver, and spleen. Multiple organs of the rabbits also demonstrated tissue damage—the lungs showed multiple hemorrhages, the spleen and liver were damaged, and the kidneys had various degrees of tubular degeneration.

Another group of investigators [Stenger et al. 1971] determined the acute LD_{50} for EGEE in the mouse, rat, and rabbit by intravenous (i.v.) administration of 3,600, 2,691, or 840 mg/kg, respectively. The following symptoms were observed: dyspnea, somnolence, ataxia, stomach distending to the side, and convulsions.

The acute toxic effects of EGME, EGMEA, EGEE, and EGEEA are summarized in Table 4-2.

4.3.1.5 Summary of Acute Toxicity

The acute toxicity of EGEE, EGEEA, EGME, and EGMEA has been investigated in a number of experiments with a variety of species and routes of exposure. Animals exhibited inactivity, weakness, and dyspnea. Necropsies revealed congested lungs, hemorrhagic stomachs, congested kidneys, and damage to the bladder, liver, and spleen [Waite et al. 1930; Wiley et al. 1938; Karel et al. 1946; Carpenter et al. 1956; Truhaut et al. 1979]. The principal toxic effect of these compounds was damage to the kidneys [Waite et al. 1930; Laug et al. 1939; Smyth et al. 1941; Gross 1943], which included extreme tubular necrosis and degeneration. Additional adverse effects included increased erythrocyte osmotic fragility and damaged spleens [Werner et al. 1943c; Carpenter et al. 1956; Truhaut et al. 1979].

4.3.2 Male Reproductive Effects

A number of experimental animal studies have demonstrated the adverse effects of glycol ethers on the male reproductive system. These effects include testicular atrophy, decrease in fertility, germ cell depletion, decrease in sperm motility, and an increase in the number of abnormal sperm cells. Although a brief summary of these studies follows, a detailed description of them may be found in Appendix B.

Table 4-2.—Acute toxicity of EGEE, EGEEA, EGME, and EGMEA

Compound	Species	Route of administration and dose	Observed effects	Reference
EGEE	Mouse	i.p.: LD_{50}* 1,709 mg/kg	Damage to lymph nodes and spleen, renal glomerular and tubular degeneration, pulmonary congestion	Karel et al. 1947
	Mouse	i.v.: LD_{50} 3,600 mg/kg	Dyspnea, somnolence, ataxia, distended stomach	Stenger et al. 1971
	Mouse	Oral: LD_{50} 3,991 mg/kg	Hematuria, renal tubular degeneration and cortical necrosis	Laug et al. 1939
	Mouse	Inhalation: 1,130–6,000 ppm for 7 hr	Dyspnea, damaged lung, toxic effect on white blood cells	Werner et al. 1943c
	Mouse (F)	Inhalation: LC_{50}* 1,820 ppm for 7 hr	Death	Werner et al. 1943a
	Rat	i.v.: LD_{50} 2,691 mg/kg	Dyspnea, somnolence, ataxia, distended stomach	Stenger et al. 1971
	Rat	Oral: LD_{50} 3,204 mg/kg	Hematuria, renal tubular degeneration and cortical necrosis	Laug et al. 1939
	Rat (M)	Oral: LD_{50} 3,000 mg/kg	Narcosis, digestive tract irritation, kidney damage	Smyth et al. 1941
	Rat (M)	Oral: LD_{50} 5,000 mg/kg	Narcosis, lung and kidney damage	Carpenter et al. 1956
	Rat (F)	Oral: LD_{50} 5,400 mg/kg	Narcosis, lung and kidney damage	Carpenter et al. 1956
	Rat (M)	Oral: LD_{50} 2,300 mg/kg	Death	Cheever et al. 1984
	Rat (F)	Inhalation: 125 ppm for 4 hr	Increase in osmotic fragility	Carpenter et al. 1956
	Rabbit	i.v.: LD_{50} 840 mg/kg	Dyspnea, somnolence, ataxia, distended stomach	Stenger et al. 1971
	Rabbit (M)	Oral: LD_{50} 3,100 mg/kg	Narcosis, lung and kidney damage	Carpenter et al. 1956
	Rabbit (M)	Dermal: LD_{50} 3,296 mg/kg	Death	Carpenter et al. 1956
	Guinea pig	Oral: LD_{50} 2,584 mg/kg	Hematuria, renal tubular degeneration and cortical necrosis	Laug et al. 1939

EGEE	Guinea pig (M,F)	Oral: LD_{50} 1,400 mg/kg	Narcosis, digestive tract irritation, kidney damage	Smyth et al. 1941
	Guinea pig (M,F)	Oral: LD_{50} 1,400 mg/kg	Narcosis, lung and kidney damage.	Carpenter et al. 1956
	Guinea pig	Inhalation: 0.05%, 0.3%, for 1–24 hr or 0.06%	No effect (0.05% for 14 hr; 0.3% for 4 hr; 0.6% for 1 hr); death (0.6% for 24 hr; 0.3% for 24 hr); inactivity, weakness, dyspnea (0.6% for 18–24 hr); congestion and edema of the lungs, hemorrhagic and distended stomachs, congested kidneys (0.6% for 18–24 hr; 0.3% for 18–24 hr)	Waite et al. 1930
EGEEA	Rat (M)	Oral: LD_{50} 5,100 mg/kg	Narcosis, digestive tract irritation, damaged kidneys	Smyth et al. 1941
	Rat (F)	Oral: LD_{50} 2,900 mg/kg	Hemoglobinuria, hematuria, renal lesions	Truhaut et al. 1979
	Rat (M)	Oral: LD_{50} 3,900 mg/kg	Hemoglobinuria, hematuria, renal lesions	Truhaut et al. 1979
	Rat (F)	Inhalation: 62 ppm for 4 hr	Increased osmotic fragility	Carpenter et al. 1956
	Rat (M,F)	Inhalation: 2,000 ppm for 4 hr	No effect	Truhaut et al. 1979
	Rabbit (M,F)	Inhalation: 2,000 ppm for 4 hr	Slight, transient hemoglobinuria and/or hematuria	Truhaut et al. 1979
	Rabbit (M,F)	Dermal: LD_{50} 10,500 mg/kg	Hemoglobinuria, hematuria, decreased white blood cell count, blood in kidneys and bladder	Truhaut et al. 1979
	Guinea pig (M,F)	Oral: LD_{50} 1,910 mg/kg	Narcosis, digestive tract irritation, damaged kidneys	Smyth et al. 1941
EGME	Mouse (F)	i.p.: LD_{50} 2,150 mg/kg	Damage to lymph nodes and spleen, renal glomerular and tubular degeneration	Karel et al. 1947
	Mouse	Inhalation: 930 to 6,800 ppm for 7 hr	Dyspnea, damage to lungs and white blood cells	Werner et al. 1943c

(Continued)

*Abbreviations: LC_{50} = median lethal concentration; LD_{50} = median lethal dose.

Table 4-2 (Continued).—Acute toxicity of EGEE, EGEEA, EGME, and EGMEA

Compound	Species	Route of administration and dose	Observed effects	Reference
EGME	Mouse (F)	Inhalation: LC_{50} to 1,480 ppm	Death	Werner et al. 1943c
	Rat (M)	Oral: LD_{50} 246 mg/kg	Digestive tract irritation, damaged kidneys	Smyth et al. 1941
	Rat (M)	Oral: LD_{50} 3,250 mg/kg	Narcosis, lung and kidney damage	Carpenter et al. 1956
	Rat (F)	Oral: LD_{50} 3,400 mg/kg	Narcosis, lung and kidney damage	Carpenter et al. 1956
	Rat (F)	Inhalation: 2,000 ppm for 7 hr	Slight increase in osmotic fragility	Carpenter et al. 1956
	Rabbit	Injection: 2,130 mg	Damage to tissues of kidney, bladder, liver, and spleen degeneration of testes	Wiley et al. 1938
	Rabbit (M)	Oral: LD_{50} 890 mg/kg	Narcosis, lung and kidney damage	Carpenter et al. 1956
	Rabbit (M)	Dermal: LD_{50} 1,289 mg/kg	Death	Carpenter et al. 1956
	Guinea pig (M,F)	Oral: LD_{50} 950 mg/kg	Digestive tract irritation, damaged kidneys	Smyth et al. 1941
	Guinea pig (M,F)	Oral: LD_{50} 950 mg/kg	Narcosis, lung and kidney damage	Carpenter et al. 1956
	Dog	Injection: 2,130 mg	Damage to tissues of kidney, bladder, liver, and spleen	Wiley et al. 1938
EGMEA	Rat (M)	Oral: LD_{50} 3,930 mg/kg	Narcosis, digestive tract irritation, damaged kidneys	Smyth et al. 1941
	Rat (F)	Inhalation: 32 ppm for 4 hr	Increased osmotic fragility	Carpenter et al. 1956
	Guinea pig (M,F)	Oral: LD_{50} 1,250 mg/kg	Narcosis, digestive tract irritation, damaged kidneys	Smyth et al. 1941

4.3.2.1 EGEE and EGEEA

EGEE administered by a variety of routes (subcutaneous, intravenous, oral, and inhalation) produced a marked toxic effect on the testes of many animal species. The testicular effects included edema [Morris et al. 1942; Stenger et al. 1971], an absence of testicular germ cells [Stenger et al. 1971; Nagano et al. 1979], and testicular atrophy [Morris et al. 1942; Nagano et al. 1979; Barbee et al. 1984; Terrill and Daly 1983a; Melnick 1984]. EGEEA has also caused testicular atrophy and depletion of spermatocytes in mice [Nagano et al. 1979]. Testicular degeneration in rats treated orally with EGEE or EGEEA was restricted to the later stages of primary spermatocyte development and secondary spermatocytes [Foster et al. 1983]. In detailed toxicologic studies, Creasy and Foster [1984] and Oudiz and Zenick [1986] concluded that primary spermatocytes in the pachytene stage of meiosis were the initial and major sites of morphologic damage from EGEE. Exposure of rats to EGEE has also resulted in reversible impairment of testicular function that was reflected in significantly decreased sperm counts ($P \leq 0.01$) and increased abnormal forms ($P \leq 0.05$) in the semen [Oudiz et al. 1984]. EGEE treatment has also resulted in lowered epididymal weights [Oudiz et al. 1984]. Oral treatment of rats with single doses of EAA affected diplotene, diakinetic, and secondary and early pachytene spermatocytes [Foster et al. 1987].

4.3.2.2 EGME and EGMEA

The testicular toxicity of EGME has been demonstrated in a number of species by a variety of routes. Adverse effects on the testes included the degeneration of germinal epithelium [Wiley et al. 1938; Miller et al. 1981; Foster et al. 1983; Chapin and Lamb 1984; Hobson et al. 1986], testicular atrophy [Nagano et al. 1979; Miller et al. 1981; Chapin and Lamb 1984; Hobson et al. 1986; Anderson et al. 1987; Exon et al. 1991; Smialowicz et al. 1991], and depletion of germ cells [Nagano et al. 1979; Miller et al. 1981; Foster et al. 1983; Hobson et al. 1986; Anderson et al. 1987]. In one inhalation study, microscopic testicular lesions were observed in rats only at the highest exposure level (300 ppm), but concentration-related testicular lesions were observed in rabbits at 30, 100, and 300 ppm [Miller et al. 1983a]. Miller et al. [1983a] concluded from this study that male rabbits were more sensitive than male rats to EGME vapor. A single 4-hr inhalation exposure to 625 ppm EGME damaged spermatids [Samuels et al. 1984].

Oral administration of 500 mg EGME/kg/day for 4 days caused maturation depletion of middle and late stage spermatids and maturation arrest of pachytene spermatocytes; partial recovery occurred four weeks after treatment and full recovery was achieved at 8 weeks [Foster et al. 1983]. A definite order of spermatocyte sensitivity to EGME has been demonstrated: dividing spermatocytes > early pachytene spermatocytes > late pachytene spermatocytes > midpachytene spermatocytes > leptotene/zygotene spermatocytes [Creasy and Foster 1984]. Anderson et al. [1987] concluded that testicular degeneration was restricted to later stages of primary spermatocyte development and secondary spermatocytes.

A partially reversible decrease in fertility was observed in male rats exposed to EGME by inhalation [Rao et al. 1983] or ingestion [Chapin et al. 1985a; Anderson et al. 1987]. Changes in fertility were correlated with changes in testicular histology and sperm morphology [Chapin et al. 1985b].

Nagano et al. [1979] demonstrated testicular toxicity of EGMEA administered orally to mice. Adverse effects included decreased testes weights and varying dose-related degrees of testicular seminiferous tubule atrophy.

The effects of EGEE, EGME, and their acetates on the male reproductive system are summarized in Tables 4-3 and 4-4.

4.3.3 Effects on the Female Reproductive System and the Developing Embryo

A number of experimental animal studies have investigated the effects of the glycol ethers on the female reproductive system and the developing embryo. Adverse maternal effects include prolonged gestation, reduced body weight, and reduced body weight gain. Adverse developmental effects include lethality, skeletal and visceral malformations, cardiovascular defects, and altered behavioral test responses.

4.3.3.1 EGEE and EGEEA

Treating pregnant females of various species with EGEE has caused adverse maternal and developmental effects. Effects on the dams included death [Schuler et al. 1984], reduced food consumption [Andrew et al. 1981; Hardin et al. 1981], reduced body weight and body weight gain [Andrew et al. 1981; Hardin et al. 1981], and prolonged gestation periods [Nelson et al. 1981]. Effects on the offspring included embryolethality [Stenger et al. 1971; Tinston 1983]; fetal skeletal, renal, cardiovascular, and ventral body wall defects [Stenger et al. 1971; Andrew et al. 1981; Hardin et al. 1981; Doe 1984a]; and reduced body weights [Andrew et al. 1981; Hardin et al. 1981; Hardin et al. 1982]. Altered behavioral test responses and altered neurochemical concentrations in the brain were also observed in the offspring of dams exposed by inhalation to EGEE [Nelson et al. 1982a].

Pregnant rabbits exposed by inhalation to EGEEA exhibited reduced body weight gain and food consumption and an increase in fetal resorptions [Doe 1984a; Tyl et al. 1988]. Embryolethality, visceral and skeletal abnormalities, and reduced fetal weights were observed in the offspring of dams treated with EGEEA [Doe 1984a; Nelson et al. 1984a; Hardin et al. 1984; Tyl et al. 1988].

4.3.3.2 EGME and EGMEA

Treating pregnant females of various animal species with EGME by various routes has caused adverse maternal and developmental effects. Effects on the dams included lethality, increased gestation period, decreased food consumption, and decreased body weight gain [Doe et al. 1983; Hanley et al. 1984a; Wickramaratne 1986]. Effects on the offspring included lethality, decreased fetal weights, decreased litter sizes, skeletal and visceral malformations, digit anomalies, and cardiovascular defects [Nagano et al. 1981; Doe et al. 1983; Hanley et al. 1984a; Horton et al. 1985; Toraason et al. 1985; Wickramaratne 1986; Greene et al. 1987; Hardin and Eisenmann 1987; Scott et al. 1989]. Although rabbits

Table 4-3.—Reproductive effects of EGEE and EGEEA

Compound	Species	Route of administration and dose	Observed effects	Reference
EGEE	Mouse (M)*	Oral: 500, 1,000, 2,000, or 4,000 mg/kg per day, 5 days/wk for 5 wk	Testicular atrophy (1,000 and 2,000 mg/kg per day); death (4,000 mg/kg per day)	Nagano et al. 1979
	Mouse (M)	Oral: 0.5, 1, or 2 g/kg per day for 2 yr	High mortality rate (2 gm/kg); testicular atrophy at 1 or 2 g/kg	Melnick 1984
	Rat (M)	s.c.: 93, 186, 372, or 744 mg/kg per day for 4 wk	Microscopic testicular changes (372 and 744 mg/kg per day)	Stenger et al. 1971
	Rat (M)	Oral: 1.45% in diet for 2 yr	Testicular enlargement, edema, and tubular atrophy	Morris et al. 1942
	Rat (M)	Oral: 46.5, 93, 186, 372 or 744 mg/kg per day for 13 wk	Microscopic testicular changes (186 and 744 mg/kg per day)	Stenger et al. 1971
	Rat (M)	Oral: 250, 500, or 1,000 mg/kg per day for 11 days	Decreased testes weights, spermatocyte depletion and degeneration (500, 1,000 mg/kg per day)	Foster et al. 1983
	Rat (M)	Oral: 250, 500, or 1,000 mg/kg per day for 11 days	Microscopic testicular lesions (500 and 1,000 mg/kg per day)	Creasy and Foster 1984
	Rat (M)	Oral: 0.5, 1, or 2 g/kg per day for 2 yr	High mortality rate (2 gm/kg); testicular atrophy at all doses	Melnick 1984

(Continued)

*Abbreviations: M = male; F = female.

Table 4-3 (Continued).—Reproductive effects of EGEE and EGEEA

Compound	Species	Route of administration and dose	Observed effects	Reference
EGEE (cont'd)	Rat (M)	Oral: 936, 1,972, or 2,808 mg/kg per day for 5 days	Increased abnormal forms of sperms and decreased sperm count (936 mg/kg); azoospermia and oligozoospermia, decreased epididymal wts (1,972, 2,808 mg/kg per day)	Oudiz et al. 1984
	Rat (M)	Oral: 0 or 936 mg/kg per day 5 days/wk for 6 wk	Decreased sperm count and percent normal morphology at weeks 5 and 6; decreased sperm motility at week 6 (pachytene spermatocyte the most sensitive target)	Oudiz and Zenick 1986
	Rat (F,M)	Inhalation: 25, 100, or 400 ppm, 6 hr/day, 5 days/wk for 13 wk	No biologically significant effects.	Terrill and Daly 1983b; Barbee et al. 1984
	Rabbit (F,M)	Inhalation: 25, 100, or 400 ppm, 6 hr/day, 5 days/wk for 13 wk	Testes weight was decreased (400 ppm) and microscopic testicular changes (males)	Terrill and Daly 1983a; Barbee et al. 1984
	Dog (M)	Oral: 46.5, 93, or 186 mg/kg per day for 13 wk	Microscopic testicular changes (186 mg/kg per day)	Stenger et al. 1971
EGEEA	Mouse (M)	Oral: 500, 1,000, 2,000, or 4,000 mg/kg per day, 5 days/wk for 5 wk	Testicular atrophy, depletion of spermatocytes (1,000, 2,000, or 4,000 mg/kg per day)	Nagano et al. 1979
	Rat (M)	Oral: 726 mg/kg per day for 11 days	Testicular atrophy, spermatocyte depletion and degeneration	Foster et al. 1984

Table 4-4.—Reproductive effects of EGME and EGMEA

Compound	Species	Route of administration and dose	Observed effects	Reference
EGME	Mouse (M)*	Oral: 62.5, 125, 250, 500, 1,000, or 2,000 mg/kg per day, 5 days/wk for 5 wk	Testicular atrophy (250–2,000 mg/kg per day); no germ cells (1,000–2,000 mg/kg per day)	Nagano et al. 1979
	Mouse (M)	Oral: 500, 750, 1,000, or 1,500 mg/kg	Reduced testes weight at wk 2–5 (500–1,500 mg/kg); increased abnormal sperm morphology (500–1,500 mg/kg); degeneration of late spermatocytes and spermatids (1,000–1,500 mg/kg)	Anderson et al. 1987
	Mouse (F)	Inhalation: 10 or 50 ppm on g.d. 6–15; sacrifice on g.d. 18	Decreased maternal body weight gain	Hanley et al. 1984a
	Rat (M)	Oral: 50, 100, 250, or 500 mg/kg per day for 11 days	Decreased testicular weight at days 2, 4, 7, and 11 (500 mg/kg per day group); decreased testicular weight at days 7 and 11 (250 mg/kg per day group)	Foster et al. 1983
	Rat (M)	Oral: 50, 100, 250, or 500 mg/kg per day for 11 days	Degeneration of pachytene spermatocytes at 24 hr (100, 250, 500 mg/kg per day); no testicular effects (50 mg/kg for 11 days); degeneration in spermatid population (500 mg/kg per day for 4 days; 250 mg/kg per day for 7 days); absence of spermatid and late spermatocyte populations after 11 days of 250 and 500 mg/kg per day; partial depletion and degeneration of spermatids and spermatocytes with 100 mg/kg per day for 11 days	Foster et al. 1983
	Rat (M)	Oral: 500 mg/kg per day for 4 days, then sacrificed at 2-wk intervals	Maturation depletion of middle and late stage spermatids, maturation arrest of pachytene spermatocytes (2 wk); partial recovery (4 wk); full spermatogenesis in majority of tubules from all animals (8 wk)	Foster et al. 1983

(Continued)

*Abbreviations: M = male; F = female.

Table 4-4 (Continued).—Reproductive effects of EGME and EGMEA

Compound	Species	Route of administration and dose	Observed effects	Reference
EGME (cont'd)	Rat (M)	Oral: 500 mg/kg per day for 4 days; animals sacrificed at 0, 2, 4, and 8 wk post-exposure	Decreased testicular weights at 0, 2, and 4 wk; return to normal size in 8th wk; increased seminal vesicle weights at wk 8	Foster et al. 1983
	Rat (M)	Oral: 150 mg/kg per day for 5 days; animals sacrificed on g.d. 1, 2, 4, 7, and 10 after initial dosing	Spermatocyte degeneration at day 1; no significant increase in production of testis fluid and androgen-binding protein at day 2, 4, 7, and 10; reduced testes weight at day 2 and after	Chapin and Lamb 1984
	Rat (M)	Oral: 50, 100, or 200 mg/kg per day for 5 days then mated with 2 female rats/wk for 8 wk. After 8-wk interval, mated again for 5 days	Decreased pregnancies at wk 4 (200 mg/kg per day); reduced fertility at wk 5 (100 mg/kg per day); decreased number of live fetuses at wk 4-16 (200 mg/kg per day); fewer pups/litter at wk 5 (100 mg/kg per day); increased numbers of resorptions at wk 5 and 6, and at wk 3-16 increase in pre-implantation loss (200 mg/kg per day); increase in pre-implantation loss at wk 2 and 5 (100 mg/kg per day)	Chapin et al. 1985a
	Rat (M)	Oral: 50, 100, or 200 mg/kg per day for 5 days at weekly intervals, for 8 wk, efferent duct ligations, and following day animal was sacrificed	Decreased sperm/g cauda epididymis at wk 2 and remained low for 8 wk (100, 200 mg/kg per day); lower counts only at wk 5 (50 mg/kg per day); decreased sperm motility at wk 3-8 (200 mg/kg per day) and wk 4-8 (100 mg/kg per day); recovery began at wk 6. Increased abnormal sperm morphology at wk 3 (200 mg/kg per day) and wk 5 (100 mg/kg per day) and remained so	Chapin et al. 1985a
	Rat (M)	Oral: 50, 100 or 200 mg/kg per day for 5 days at weekly intervals, for 8 wk, efferent duct ligations, and animals sacrificed 16 hr later	Abnormal sperm morphology at wk 4 with recovery by wk 8 (50 mg/kg per day); abnormal sperm morphology at wk 1 with 50% recovery by wk 8 (100 mg/kg per day); severe testicular effects at wk 1, with 50% recovery by wk 7 (200 mg/kg per day) At wk 2 decreased numbers of sperm and increased numbers of immature germ cells (100, 200 mg/kg per day); transient mild increase in numbers of immature germ cells and decreased sperm density; elevated amount of protein in rete testis fluid at wk 2-5 (200 mg/kg per day) and wk 4-6 (100 mg/kg per day)	Chapin et al. 1985b

EGME (cont'd)	Rat (M)	Oral: 500, 750, 1,000, or 1,500 mg/kg	Reduced testes weight (500–1,500 mg/kg) at wks 3, 4, and 5; reduced sperm counts at wks 4, 5, 6, 7 (500–1,500 mg/kg); increased abnormal sperm morphology (500–1,500 mg/kg); 100% sterility (750–1,500 mg/kg); depletion of early pachytene spermatocytes (1,000–1,500 mg/kg)	Anderson et al. 1987
	Rat (M)	Oral: 2,000 or 6,000 ppm in drinking water for 10 days	Reduction in testes weights (6,000 ppm)	Exon et al. 1991
	Rat (M)	Oral: 50, 100, or 200 mg/kg per day for 10 days	Reduction in testes weights and elevated serum testosterone levels (200 mg/kg per day)	Smialowicz et al. 1991
	Rat (M)	Inhalation: 100, 300, or 1,000 ppm, 6 hr/day for 9 days	Microscopic testicular changes (1,000 ppm)	Miller et al. 1981
	Rat (M,F)	Inhalation: 30, 100, or 300 ppm, 6 hr/day, 5 days/wk for 13 wk	Reduced testicular weight and microscopic lesions (300 ppm) in males. Reduced body and thymus weights at 300 ppm in males and females	Miller et al. 1983a
	Rat (M)	Inhalation: 30, 100, or 300 ppm, 6 hr/day, 5 days/wk for 13 wk, then paired with unexposed females for breeding	Decreased male fertility (300 ppm), partially reversed when bred 13 and 19 wk after last exposure	Rao et al. 1983
	Rat (F)	Inhalation: 30, 100, or 300 ppm, 6 hr/day, 5 days/wk for 13 wk, then paired with unexposed males for breeding	No effect on female fertility	Rao et al. 1983
	Rat (M)	Inhalation: 100 or 300 ppm, 6 hr/day for 10 days	No effect at 100 ppm; testicular atrophy at 300 ppm	Doe et al. 1983

(Continued)

Table 4-4 (Continued).—Reproductive effects of EGME and EGMEA

Compound	Species	Route of administration and dose	Observed effects	Reference
EGME (cont'd)	Rat (M)	Inhalation: 150, 300, 625, 1,250, 2,500, or 5,000 ppm for 4 hr; sacrificed on day 14	Microscopic testicular changes and atrophy (1,250, 2,500, 5,000 ppm); damaged spermatids (625 ppm)	Samuels et al. 1984
	Rat (M)	Inhalation: 1,000 or 2,500 ppm for 4 hr; sacrificed on day 1, 2, 3, 4, 5, 8, 10 15, and 19 post exposure	Reduced testes weight at 48 hr (1,000, 2,500 ppm) and testicular atrophy on days 1-19	Samuels et al. 1984
	Rabbit (M)	Injection: (route and dose not specified)	Microscopic testicular changes	Wiley et al. 1938
	Rabbit (M,F)	Inhalation: 30, 100, or 300 ppm, 6 hr/day, 5 days/wk for 13 wk	Reduced testicular weight and microscopic lesions (300 ppm); dose-related increase in incidence and severity of testicular lesions (30, 100, 300 ppm); reduced thymus and body weights at 300 ppm (M,F)	Miller et al. 1983a
	Guinea pig (M)	Dermal: 1 g/kg per day, 5 days/wk for 13 wk	Decreased testicular weights, severe testicular atrophy, degeneration of seminiferous tubules with complete loss of spermatogenic cells	Hobson et al. 1986
EGMEA	Mouse (M)	Oral: 62.5, 125, 250, 500, 1,000, or 2,000 mg/kg per day, 5 days/wk for 5 wk	Testicular atrophy (500-2,000 mg/kg per day); no germ cells (2,000 mg/kg per day)	Nagano et al. 1979

demonstrated a greater sensitivity to EGME vapor than rats or mice, the results established no-observed-effect levels of 10 ppm EGME in these three species [Hanley et al. 1984a]. Oral EGME treatment of mice on different days of gestation caused exencephaly and digit anomalies [Horton et al. 1985]. The authors concluded that 100 mg/kg of EGME was the no-observed-effect level for digit malformations after a single oral dose of EGME on g.d. 11. Oral EGME treatment of nonhuman primates during gestation resulted in a missing digit on each forelimb of one dead embryo [Scott et al. 1989]. An altered response in avoidance conditioning and altered neurochemical levels in the brain were observed in the offspring of dams treated with EGME vapor [Nelson et al. 1984a]. Feuston et al. [1990] demonstrated that treatment of pregnant rats with a single dermal application of EGME caused statistically significant increases ($P<0.05$) in both the mean number of resorptions and the mean percentage of resorptions, as well as in visceral, external, and skeletal malformations. In this study, the authors established a NOAEL of 250 mg/kg on g.d. 12 for developmental effects. No studies have been reported using EGMEA; it would, however, be expected to have the same effects as EGME (see Section 4.2).

The effects of EGEE, EGEEA, and EGME on the female reproductive system and the embryo are summarized in Tables 4-5, 4-6, and 4-7.

4.3.4 Hematology

EGEE, EGEEA, EGME, and EGMEA exert adverse hematologic effects. These effects include increased osmotic fragility and decreased levels of Hb, Hct, platelets, RBCs, WBCs, and MCV. The following studies, which show these effects, are described in detail in Appendix B.

4.3.4.1 EGEE and EGEEA

Adverse hematological effects were observed in a number of species following administration of EGEE or EGEEA by oral, inhalation, and dermal routes. These effects included hemolysis [von Oettingen and Jirouch 1931] and increased osmotic fragility [Carpenter et al. 1956]. Other investigations demonstrated that EGEE and EGEEA caused decreased Hb concentrations, decreased numbers of RBCs, WBCs, and platelets, reduced Hct levels, and decreased MCVs [Werner et al. 1943a,b; Stenger et al. 1971; Nagano et al. 1979; Truhaut et al. 1979; Terrill and Daly 1983a; Barbee et al. 1984; Doe 1984a; Tyl et al. 1988]. These effects were shown to be reversible in only one study [Werner et al. 1943b]. In another study [Tyl et al. 1988], EGEEA caused an increase in WBC levels.

4.3.4.2 EGME and EGMEA

The effect of EGME and EGMEA treatment on the blood and the hematopoietic system has been investigated in a variety of species by a variety of routes. Adverse effects of EGME and EGMEA include decreased Hb, Hct, RBC, and WBC levels, and altered MCVs [Werner et al. 1943a,b; Miller et al. 1981, 1983a; Nagano et al. 1979; Grant et al. 1985; Hobson et al. 1986]. Carpenter et al. [1956] showed that EGME (2,000 ppm) and EGMEA (32 ppm)

Table 4-5.—Developmental effects of EGEE

Species	Route of administration and dose	Observed effects	Reference
Mouse (F)*	s.c.: 46.5 or 93 mg/kg per day on g.d. 1-18	No embryotoxic or teratogenic effects	Stenger et al. 1971
Mouse (F)	Oral: 3.6 g/kg per day on g.d. 7-14	Maternal death (10%); embryonic death (100%)	Schuler et al. 1984
Rat (F)	s.c.: 23, 46.5, 93 mg/kg per day on g.d. 1-21	Fetal skeletal defects (93 mg/kg per day)	Stenger et al. 1971
Rat (F)	Oral: 11.5, 23, 46.5, 93, 186 or 372 mg/kg per day on g.d. 1-21	Complete resorption of all litters (372 mg/kg per day); embryonic death increased (46.5-186 mg/kg per day); fetal skeletal defects and lower body weight (93-186 mg/kg per day)	Stenger et al. 1971
Rat (F)	Inhalation: before pregnancy, 150 or 650 ppm 7 hr/day, 5 days/wk for 3 wk; then 200 or 765 ppm, 7 hr/day on g.d. 1-19	No effect on fertility; slight maternal toxicity (765 ppm); embryonic death (100% at 765 ppm); fetal cardiovascular and skeletal defects and reduction in growth (200 ppm)	Andrew et al. 1981 Hardin et al. 1981
Rat (F)	Inhalation: 900 ppm, 7 hr/day on g.d. 14-20;	Extended gestation duration by 48 hr	Nelson et al. 1981
Rat (F)	Inhalation: 100 ppm, 7 hr/day on g.d. 7-13 or 14-20	Prolonged gestation (0.7 day); altered behavioral test results; altered neurochemical concentrations in brain	Nelson et al. 1982a

Rat (F)	Inhalation: 200 ppm, 7 hr/day on g.d. 7–13	Increased dopamine levels in cerebrum; increased norepinephrine levels in cerebrum and cerebellum	Nelson et al. 1982b
Rat (F)	Inhalation: 10, 50, or 250 ppm, 6 hr/day on g.d. 6–15	Fetotoxic, reduced ossification, skeletal variants (250 ppm)	Doe 1984a
Rat (F)	Dermal: 1.0 or 2.0 ml/day on g.d. 7–16	Reduced maternal weight gain (2.0 ml/day); embryonic death (100% at 2.0 ml/day and 76% at 1.0 ml/day); fetal cardiovascular defects and skeletal variations and reduced fetal body weight (1.0 ml/day)	Hardin et al. 1982
Rat (F)	Dermal: 1.0 ml/day on g.d. 7–16	Increase of visceral malformations	Hardin et al. 1984
Rabbit (F)	s.c.: 23 mg/kg per day on g.d. 7–16	No embryotoxic or teratogenic effects	Stenger et al. 1971
Rabbit (F)	Inhalation: 160 or 615 ppm, 7 hr/day on g.d. 1–18	Embryonic death (100% at 615 ppm and 22% at 160 ppm); fetal renal, cardiovascular, and ventral body wall defects and skeletal variations (160 ppm); reduced maternal food consumption (160, 615 ppm); maternal death (615 ppm)	Andrew et al. 1981 Hardin et al. 1981
Rabbit (F)	Inhalation: 10, 50, or 175 ppm, 6 hr/day on g.d. 6–18	Skeletal variations in fetus (175 ppm)	Doe 1984a
Rabbit (F)	Inhalation: 50, 150, or 400 ppm on g.d. 6–18	Decreased number of live fetuses, gravid uterus weights and litter weights, increased post implantation loss, early and late fetal deaths (400 ppm)	Tinston 1983

*F = female.

Table 4-6.—Developmental effects of EGEEA

Species	Route of administration and dose	Observed effects	Reference
Rat (F)*	Inhalation: 130, 390, or 690 ppm, 7 hr/day on g.d. 7-15	Embryonic death (100% at 690 ppm and 56% at 390 ppm); reduced fetal weights and increased visceral malformations (130 and 390 ppm)	Nelson et al. 1984b
Rat (F)	Inhalation: 50, 100, 200, or 300 ppm, 6 hr/day on g.d. 6-15	Reduced weight gain and food consumption, elevated liver weight (100-300 ppm); embryo/fetotoxicity (100-300 ppm), external (300 ppm), visceral, and skeletal malformations (200-300 ppm)	Tyl et al. 1988
Rat (F)	Dermal: 1.4 ml/day on g.d. 7-16	Reduced maternal body weight; embryonic death (100%); reduced fetal body weights and visceral malformations and skeletal variations	Hardin et al. 1984
Rabbit (F)	Inhalation: 25, 100, or 400 ppm, 6 hr/day on g.d. 6-18	Reduced maternal body weight gain and food consumption (400 ppm); increased resorptions (400 ppm) and reduced fetal body wt (100 ppm); major vertebral column malformations (400 ppm)	Doe 1984a
Rabbit (F)	Inhalation: 50, 100, 200, or 300 ppm, 6 hr/day on g.d. 6-18	Decreased weight gain, reduced gravid uterine wt; elevated absolute liver wt (100-300 ppm); embryotoxicity (200-300 ppm); fetotoxicity (100-300 ppm); external, visceral, and skeletal malformations (200-300 ppm)	Tyl et al. 1988

*F = female.

Table 4-7.—Developmental effects of EGME

Species	Route of administration and dose	Observed effects	Reference
Mouse (F)*	Oral: 31.25, 62.5, 125, 250, 500 or 1,000 mg/kg per day on g.d. 7-14	Embryonic death (250-1,000 mg/kg per day); 100% dead (1,000 mg/kg per day), 1 alive (500 mg/kg per day); reduced fetal weights (125-250 mg/kg per day); gross anomalies and skeletal malformations (250 mg/kg per day); increased skeletal malformations (62.5-125 mg/kg per day); retarded fetal ossification (31.25-1,000 mg/kg per day); bifurcated or split cervical vertebrae	Nagano et al. 1981
Mouse (F)	Oral: 1,400 mg/kg per day on g.d. 7-14	100% embryonic death	Schuler et al. 1984
Mouse (F)	Oral: 250 mg/kg on g.d. 7-14 Oral: 250 mg/kg per day on g.d. 7-9, 8-10, or 9-11; Oral: 250 mg/kg on g.d. 7-8, 9-10, or 10-11; Oral: 250 or 500 mg/kg per day on g.d. 10, 11, 12, or 13	Exencephaly and paw lesions; reduced fetal weights, increased embryolethality in all dosage groups except single 500 mg/kg on g.d. 12 or 13; increased exencephalic fetuses (250 mg/kg on g.d. 7-9, or 8-10); increased digit malformations (250 mg/kg on g.d. 8-10, or 9-11, or 10 and 11); increased paw malformations (500 mg/kg on g.d. 9, 10, 11, or 12); forepaw anomalies (500 mg/kg on g.d. 9, 10, or 11); hindpaw syndactyly (500 mg/kg on g.d. 12)	Horton et al. 1985
	Oral: 100, 175, 250, 300, 350 400, or 450 mg/kg on g.d. 11	Increased digit anomalies (250-450 mg/kg); present at 175 mg/kg, but not statistically significant; NOAEL = 100 mg/kg	Horton et al. 1985
Mouse (F)	Oral: 250 mg/kg per day on g.d. 7-14; sacrificed on g.d 18	Gross malformations (exencephaly and paw lesions)	Horton et al. 1985
Mouse (F)	Oral: 25, 50, or 100 mg/kg per day on g.d. 7-13	100% resorption (100 mg/kg per day); increased cardiovascular defects (50 mg/kg per day); increased numbers of fetuses with aberrant QRS complexes (25-50 mg/kg per day)	Toraason et al. 1985

(Continued)

*F = female.

Table 4-7 (Continued).—Developmental effects of EGME

Species	Route of administration and dose	Observed effects	Reference
Mouse (F)	Oral: 100, 250, or 350 mg/kg on g.d. 11, then sacrificed 2, 6, 24, or 48 hr later and embryos removed	No maternal toxicity; forelimb bud cytotoxicity as early as 2 hr post EGME treatment, with maximum effect at 6 hr (350 mg/kg)	Greene et al. 1987
Mouse (F)	Oral: 100, 175, 250, 300, 350 400, 450, or 500 mg/kg on g.d. 11 and embryos removed 6 or 24 hr later	Paw malformations induced in dose-dependent manner (all dose levels except 100 mg/kg)	Greene et al. 1987
Mouse (F)	Oral: 304 mg/kg on g.d. 11, sacrificed on g.d. 18	No maternal toxicity; paw malformations	Hardin and Eisenmann 1987
Mouse (F)	Inhalation: 0, 10, or 50 ppm on g.d. 6–15; sacrificed on g.d. 18	Slight fetotoxicity (50 ppm): minor skeletal variations	Hanley et al. 1984a
Rat (F)	Inhalation: 100 or 300 ppm, 6 hr/day on g.d. 6–17, then litters delivered	Reduced maternal body weight gain and 100% embryonic death (300 ppm), prolonged gestation and reduced number of pups and live pups (100 ppm)	Doe et al. 1983
Rat (F)	Inhalation: 3, 10, or 50 ppm on g.d. 6–15	Minor skeletal variations (50 ppm)	Hanley et al. 1984a
Rat (F)	Inhalation: 25 ppm, 7hr/day on g.d. 7–13 or 14–20	Significant differences in avoidance conditioning of offspring from mothers exposed on g.d. 7–13; neurobehavioral deviations in offspring	Nelson et al. 1984a
Rat (F)	Dermal: 3%, 10%, 30%, or 100% solutions at 10 ml/kg, 6 hr/day on g.d. 6–17	100% maternal deaths (100% soln); 100% fetal death (30%); reduced litter sizes (10%)	Wickramaratne 1986

Rat (F)	Dermal: 250, 500, 1,000 or 2,000 mg EGME/kg on g.d. 12	Reduced maternal body weight gain day after EGME application (all EGME exposures and times except for 250 mg on g.d. 12)	Feuston et al. 1990
	2,000 mg EGME/kg on g.d. 10, 11, 12, 13, or 14	Increase in mean number of resorptions, mean percentage resorptions (2,000 mg/kg on g.d. 10); decrease in fetal body weights (1,000 or 2,000 mg/kg on g.d. 10 and 12). Increases in external,visceral, or skeletal malformations (500, 1,000, or 2,000 mg/kg on g.d. 12)	
Rabbit (F)	Inhalation: 3, 10, or 50 ppm on g.d. 6–18	Reduced maternal body weight gain, increased absolute liver weight, increased resorption rate, reduced mean fetal body weights (50 ppm); increased incidence of skeletal and visceral malformations (50 ppm); 10 ppm = no effect level	Hanley et al. 1984a
Monkey (F)	Oral: 12, 24, or 36 mg/kg on g.d. 20–45	Embryonic death — 3 of 13 or 23% at 12 mg/kg, 3 of 10 or 30% at 24 mg/kg, 8 of 8 or 100% at 36 mg/kg; 1 embryo (36 mg/kg group) was missing one digit on each forelimb	Scott et al. 1989

caused increased osmotic fragility of RBCs. Histopathology of EGME- and EGMEA-exposed animals revealed reduced bone marrow cellularity, lymphoid atrophy of the thymus and gut-associated lymphoid organs, decreased hepatocyte size, and reduced thymus weights [Miller et al. 1981, 1983a; House et al. 1985]. In addition, serum total protein, albumin, and globulin levels were reduced [Miller et al. 1981, 1983a], while serum creatinine kinase and lactate dehydrogenase activity were increased [Hobson et al. 1986].

The hematologic effects of EGEE, EGME, and their acetates are summarized in Tables 4-8 and 4-9.

4.3.5 Immunology of EGME and MAA

Houchens et al. [1984] examined the effect of EGME and EGEE on cell-mediated immunity using an allograft rejection assay. In this model, mice that are allogenic in relation to the leukemic cell tumor used survive when challenged with the tumor unless they have been immunosuppressed; the tumor will grow in syngeneic mice unless chemical treatment has a direct cytotoxic effect on the tumor cells. Day zero was the tumor implantation day. Allogeneic $B6C3F_1$ mice were given 600, 1,200, or 2,400 mg EGEE/kg or 300, 600, or 1,200 mg EGME/kg orally on days -12 to -0, or 100 mg/kg cyclophosphamide (Cy) i.p. on day -1. Sham-treated controls were given oral doses of water on days -12 to -0 and -5 to -1, respectively. The mice were then challenged with 100 (10^2), 3,000 (3×10^3), 300,000 (3×10^5), or 3,000,000 (3×10^6) L1210 cells i.p. on day zero. Syngeneic $CD2F_1$ mice were challenged with 100,000 (10^5) L1210 cells on day zero and were treated on days 1 to 5 and 8 to 12 with the same doses of EGME and EGEE used for the $B6C3F_1$ mice. Water-treated syngeneic mice died with a median survival time of 8 days. In the syngeneic mice there was no direct antitumor activity of EGME or EGEE against the L1210 tumor at the doses tested because there was no effect on the median survival time. Neither EGME nor EGEE were toxic to the syngeneic mice, as determined by weight loss or early death. The authors [Houchens et al. 1984] suggested that higher doses might be tolerated and have some direct cytotoxic effect on the tumor.

The results for the allogeneic mice were more complex. All allogeneic mice receiving either water or Cy and challenged with 3×10^6 tumor cells died with ascites. However, no more than one animal per group died when the mice were treated with EGME or EGEE and challenged with 3×10^6 tumor cells. Houchens et al. [1984] suggested that the compounds may in some way stimulate the immune system and provide a prophylactic action. Blood smears of allogeneic mice were made for differential counts the last day of dosing, the day of death when possible, and on survivors at day 43 after tumor implantation. In those mice not surviving until the day of sacrifice, differential counts showed evidence of monocytosis, which is indicative of monocytic leukemia. All surviving allogeneic mice were sacrificed and necropsied on day 43. Cholecystitis was present in 7% of the mice that had received EGEE and in 58% of the mice that had received EGME. The authors did not refer to the control group.

Exposure of laboratory animals to glycol ethers has been associated with thymus atrophy and leukopenia [Nagano et al. 1979; Truhaut et al. 1979; Miller et al. 1981, 1983a; Grant et al. 1985]. Because these effects could involve depletion of immunoresponsive cells,

Table 4-8.—Hematologic effects of EGEE and EGEEA

Compound	Species	Route of administration and dose	Observed effects	Reference
EGEE	Dog and beef blood	in vitro (1 cc)	Hemolysis	von Oettingen and Jirouch 1931
	Mouse	Oral: 0, 500, 1,000, or 2,000 mg/kg per day, 5 days/wk for 5 wk	Reduced WBC counts (2,000 ppm)	Nagano et al. 1979
	Rat	Inhalation: 370 ppm 7 hr/day, 5 days/wk for 5 wk	Increase in hemosiderin; fat replacement in bone marrow; decrease in myeloid cells	Werner et al. 1943a
	Rat	Inhalation: 125 ppm for 4 hr	Increased erythrocyte osmotic fragility	Carpenter et al. 1956
	Rat (M,F)*	Inhalation: 0, 25, 100 or 400 ppm, 6 hr/day, 5 days/wk for 13 wk	Decreased WBC in females (400 ppm)	Terrill and Daly 1983b; Barbee et al. 1984
	Rat (F)	Inhalation: 0, 10, 50 or 250 ppm on g.d. 6-15	Decreased Hb, Hct, and MCV (250 ppm)	Doe 1984a
	Rabbit	Oral: 186, 372, or 744 mg/kg per day, 7 hr/day for 13 wk	Decreased Hb and Hct; increased hemosiderin; hematopoietic foci in spleens	Stenger et al. 1971
	Rabbit (M,F)	Inhalation: 0, 25, 100, or 400 ppm, 6 hr/day, 5 days/wk for 13 wk	Decreased Hb, Hct, and RBC in males and females (400 ppm)	Terrill and Daly 1983a; Barbee et al. 1984
	Rabbit (F)	Inhalation: 0, 10, 50, or 175 ppm on g.d. 6-18	No effects	Doe 1984a
	Dog	Oral: 186 mg/kg per day, 7 hr/day for 13 wk	Decreased Hb and Hct; increased hemosiderin; hematopoietic foci in spleens	Stenger et al. 1971
	Dog	Inhalation: 840 ppm, 7 hr/day, 5 day/wk for 12 wk	Increased circulating immature granulocytes; increased hemosiderin	Werner et al. 1943b

(Continued)

*Abbreviations: M=male; F=female.

Table 4-8 (Continued).—Hematologic effects of EGEE and EGEEA

Compound	Species	Route of administration and dose	Observed effects	Reference
EGEEA	Dog and beef blood	in vitro (1 cc)	Hemolysis	von Oettingen and Jirouch 1931
	Mouse	Oral: 0, 500, 1,000, 2,000, or 4,000 mg/kg per day, 5 days/wk, for 5 wk	Reduced white blood cell counts (2,000 mg/kg per day); reduced packed erythrocyte volume (4,000 mg/kg per day)	Nagano et al. 1979
	Rat	Inhalation: 62 ppm for 4 hr	Increased erythrocyte osmotic fragility	Carpenter et al. 1956
	Rat	Inhalation: 2,000 ppm for 4 hr	No effect	Truhaut et al. 1979
	Rat (F)	Inhalation: 0, 50, 100, 200 or 300 ppm for 6 hr/day on g.d. 6-15	Increased WBC (200 and 300 ppm); reduced RBC, Hb, Hct, erythrocyte size (100, 200, and 300 ppm); reduced platelet counts (200 and 300 ppm)	Tyl et al. 1988
	Rabbit	Inhalation: 2,000 ppm for 4 hr	No effect	Truhaut et al. 1979
	Rabbit (F)	Inhalation: 0, 25, 100, or 400 ppm, 6 hr/day on g.d. 6-18	Reduced Hb; slight reduction in Hct, RBC, and MCV (400 ppm)	Doe 1984a
	Rabbit	Dermal: 10.5 g/kg	Decreased white blood cell count	Truhaut et al. 1979

Table 4-9.—Hematologic effects of EGME and EGMEA

Compound	Species	Route of administration and dose	Observed effects	Reference
EGME	Mouse (M)*	Oral: 500, 1,000, or 2,000 mg/kg, 5 times/wk for 5 wk	Decreased WBC counts (500 mg); decreased RBCs and Hb (1,000 mg)	Nagano et al. 1979
	Mouse (F)	Oral: 250, 500, or 1,000 μg/g, 10 times during 2 wk	Reduced thymus wts (500 and 1,000 μg/g)	House et al. 1985
	Mouse (M)	Oral: 500, 1,000, or 2,000 mg/kg, 5 times/wk for 5 wk	Decreased WBC and RBC counts (1,000 mg/kg); decreased Hb (2,000 mg/kg)	Nagano et al. 1979
	Mouse (M, F)	Inhalation: 100, 300, or 1,000 ppm 6 hr/day for 9 days	Decreased WBC counts, packed cell volume, and RBC counts (1,000 ppm); similar but less severe effects at 300 ppm	Miller et al. 1981
	Rat (M)	Oral: 100 or 500 mg/kg per day for 4 days; animals sacrificed on day 1, 4, 8, and 22 after last treatment	Day 1: (500 mg/kg per day) hemorrhagic bone marrow and sinus endothelial damage, return to normal on day 4; splenic medullary hemopoiesis abolished, partial recovery by day 4, return to normal on day 22; mild anemia; reduced Hct and Hb (day 4) and reduced RBC counts (day 8); reduced WBC counts and no return to normal; (100 mg/kg per day) reduced WBC on day 1	Grant et al. 1985
	Rat	Inhalation: 310 ppm, 7 hr/day, 5 days/wk for 5 wk	Increased levels of hemosiderin and immature granulocytes	Werner et al. 1943a
	Rat	Inhalation: 32 ppm for 4 hr	Increased osmotic fragility (hemolysis)	Carpenter et al. 1956
	Rat (F)	Inhalation: 2,000 ppm for 4 hr	Increased osmotic fragility	Carpenter et al. 1956
	Rat (M, F)	Inhalation: 100, 300, or 1,000 ppm 6 hr/day for 9 days	Decreased WBC counts, packed cell volume, and RBC counts (1,000 ppm); similar but less severe effects at 300 ppm; decreased Hb (300 ppm in F); reduced total serum protein, albumin, and globin (1,000 ppm in M)	Miller et al. 1981

(Continued)

*Abbreviations: M = male; F = female.

Table 4-9 (Continued).—Hematologic effects of EGME and EGMEA

Compound	Species	Route of administration and dose	Observed effects	Reference
EGME	Rat	Inhalation: 30, 100, or 300 ppm 6 hr/day, 5 days/wk for 13 wk	After 4 and 12 wks, decreased WBC counts, platelet counts, and HB, reduced total protein, albumin, and globin; thymic atrophy (300 ppm)	Miller et al. 1983a
	Rabbit	Inhalation: 30, 100, or 300 ppm 6 hr/day, 5 days/wk for 13 wk	After 4 and 12 wks, decreased WBC counts, platelet counts, Hb, and RBC counts; thymic atrophy (300 ppm)	Miller et al. 1983a
	Guinea pig(M)	Dermal: 1 g/kg per day, 5 days/wk for 13 wk	Decreased RBC counts, increased MCV, lymphopenia, neutrophilia, and increased serum creatinine kinase and lactate dehydrogenase activity	Hobson et al. 1986
	Dog	Inhalation: 750 ppm, 7 hr/day, 5 days/wk for 12 wk	Microcytic anemia; decreased Hb and Hct (at 4–6 wks); increased osmotic fragility (at 11–12 wks)	Werner et al. 1943b
EGMEA	Rat	Inhalation: 62 ppm for 4 hr	Increased osmotic fragility (hemolysis)	Carpenter et al. 1956

House et al. [1985] studied possible alterations in immune function and host resistance of mice following exposure to EGME or its metabolite MAA. Specific pathogen-free female $B6C3F_1$ mice were treated orally 10 times over a 2-week period with EGME or MAA to yield total doses of 0, 250, 500, or 1,000 mg/kg. A statistically significant reduction in thymus weights was seen in the 500 mg/kg groups of both compounds ($P<0.01$). However, no reduction was found in bone marrow cellularity or leukocyte counts. No significant alterations in immunopathology, humoral immunity, cell-mediated immunity, macrophage function, and host resistance to *Listeria monocytogenes* challenge were found in mice exposed to EGME or MAA. The authors concluded that care must be taken in interpreting thymus atrophy as evidence of functional immunotoxicity because EGME and MAA produced thymic atrophy without a concomitant decrease in immune function or host resistance [House et al. 1985].

Exon et al. [1991] investigated the effects of EGME on the immune function of male and female Sprague-Dawley rats. The animals (six per group) were exposed to EGME (2,000 or 6,000 ppm for males and 1,600 or 4,800 ppm for females) in deionized drinking water for 21 days; the unexposed group received only deionized drinking water. All rats were injected s.c. at the base of the tail vein with 1 mg of aqueous keyhole limpet hemocyanin (KLH) 7 days after beginning treatment with EGME; a second injection of KLH was administered on day 13 to initiate the production of immunoglobulin G (IgG) antibody. To determine the effect on delayed type hypersensitivity (DTH), the right footpad of each animal was injected with 100 µl of heat-aggregated KLH on day 20; the left footpad was injected with 100 µl of sterile saline.

On day 21, all animals were sacrificed by CO_2 asphyxiation. Serum was then collected by cardiac puncture, and the thymus, spleen, liver, right kidney, and right testis were removed. The mean dose of glycol ethers actually consumed was calculated on the basis of mean body weight and water consumed during the entire study. The actual exposure concentrations of EGME for male rats were determined to be 161 mg/kg per day (2,000 ppm) and 486 mg/kg per day (6,000 ppm). The actual exposure concentrations of EGME for female rats were determined to be 200 mg/kg per day (1,600 ppm) and 531 mg/kg per day (4,800 ppm).

The authors [Exon et al. 1991] reported the following results. The body and testis weights of male rats exposed to 6,000 ppm EGME were significantly reduced ($P\leq0.05$). Male and female rats exposed to either concentration of EGME had a dose-dependent reduction in thymus weights ($P\leq0.05$). Spleen weights were reduced ($P\leq0.05$) in female rats treated with 4,800 ppm EGME. Liver weights expressed as a percentage of body weight were significantly increased ($P\leq0.05$) in male rats treated with 2,000 ppm EGME; however, this effect was not apparent when the actual weights of the livers were compared. EGME exposure did not affect kidney weights in either sex.

Natural killer cytotoxic responses were enhanced ($P\leq0.05$) in male and female rats at either concentration of EGME, but specific IgG production to KLH was suppressed ($P\leq0.05$) in a dose-dependent manner in both sexes. Gamma interferon (γIF) production was decreased ($P\leq0.05$) in all EGME-treated male rats and in female rats exposed to 4,800 ppm EGME. Spleen cell numbers were reduced ($P\leq0.05$) in female rats exposed to both doses of EGME

and in male rats exposed to 6,000 ppm EGME. Interleukin-2 (IL2) production by spleen cells was decreased ($P \leq 0.05$) in female rats treated with 4,800 ppm EGME. No significant effects were observed on DTH reactions of either sex. EGME appears to exert immunomodulatory effects.

Smialowicz et al. [1991] studied the effects of EGME on the immune function of another strain of rat, the Fischer 344 (F344) rat. Adult male or female rats (six per group) were exposed by oral gavage to 25, 50, 100, or 200 mg/kg per day in a volume of 0.25 ml/100 g EGME in water for 2 or 10 consecutive days, depending on the experiments performed. Control rats were given 0.25 ml of water/100 g of body weight by oral gavage. Additional rats were treated by oral gavage with MAA (25 to 200 mg/kg per day) for 2 or 10 days. To generate an antibody response, the F344 rats were immunized in vivo on treatment day 9 or 4 hr before two treatments (separated by 24 hr) with either the sheep erythrocyte (SRBC) antigen or the trinitrophenyl-lipopolysaccharide (TNP-LPS) antigen. Forty-eight hours after the last treatment of EGME or MAA, the animals were sacrificed by asphyxiation with CO_2. Blood samples were obtained from the abdominal aorta, and the spleen, thymus, and mesenteric lymph nodes were removed.

Smialowicz et al. [1991] reported the following observations for male rats only (unless otherwise specified). EGME (50, 100, or 200 mg/kg per day for 10 days) caused a statistically significant ($P \leq 0.05$) dose-dependent decrease in thymus weights with no change in body or spleen weights. In rats immunized on day 9 of EGME treatment, the antibody response to SRBC antigen was enhanced ($P \leq 0.05$) at 50 mg EGME/kg per day; in contrast, the antibody response to TNP-LPS antigen was inhibited ($P \leq 0.05$) in a dose-dependent manner at 50, 100, or 200 mg EGME/kg per day.

When rats were immunized with either SRBC or TNP-LPS antigens and then treated with EGME 4 and 28 hr later (2 doses of EGME), the antibody response to both antigens was inhibited ($P < 0.01$). At 400 mg/kg per day, EGME inhibited the antibody response to SRBC; and at 100, 200, or 400 mg/kg per day inhibited the antibody response to TNP-LPS. The authors [Smialowicz et al. 1991] then compared the effect of 10 daily doses of EGME (25, 50, 100, or 200 mg/kg per day) on the antibody response to TNP-LPS in male and female rats immunized on day 9 of EGME treatment. The antibody response of both sexes was inhibited ($P < 0.05$), but male rats were more sensitive than female rats to the immunosuppressive effects of EGME. At concentrations of 50, 100, and 200 mg/kg per day, EGME inhibited the antibody response in the male rats. Although 50 mg EGME/kg per day had no effect on the antibody response in female rats, 100 and 200 mg EGME/kg per day inhibited the response in a dose-dependent manner.

No alterations were observed in natural killer cell activity, mixed lymphocyte reaction, or cytotoxic T lymphocyte responses. Lymphoproliferative responses to concanavalin A and phytohemagglutinin were reduced at 50 to 200 mg EGME/kg per day, and the mitogen responses of pokeweed and *Salmonella typhimurium* were reduced at 200 mg EGME/kg per day ($P < 0.05$). Interleukin-2 production was reduced ($P < 0.05$) in rats exposed to 50, 100, or 200 mg EGME/kg per day. Expulsion of adult *Trichinella spiralis* worms was reduced in rats treated with 200 mg EGME/kg per day and infected with *T. spiralis* larvae.

The authors then demonstrated that MAA (the metabolite of EGME) plays a role in EGME-induced immunosuppression. MAA administered by gavage (50, 100, or 200 mg EGME/kg per day) to male rats suppressed ($P<0.01$) the antibody response to TNP-LPS in animals immunized on day 9 of MAA treatment. Concomitant exposure of rats to EGME (100 or 200 mg/kg per day) and the alcohol dehydrogenase inhibitor 4-methylpyrazole (4-MP) blocked EGME-induced suppression of the TNP-LPS antibody response observed in rats treated with EGME only.

In this study [Smialowicz et al. 1991], the authors also examined the effect of EGME on male reproductive parameters. The results are presented in Section 4.3.2.2 and Table 4-4.

4.3.6 Carcinogenicity

The National Toxicology Program (NTP) of the U.S. Department of Health and Human Services tested EGEE for carcinogenicity in male and female rats and mice at 500, 1,000, and 2,000 mg/kg/day administered by gavage [Melnick 1982]. Because mortality was high in the 2,000 mg/kg/day groups, survivors were sacrificed after 16 weeks; males had testicular lesions. The final report of this study has not been published. Currently, prechronic carcinogenicity studies are in progress for EGEE and EGME [NTP 1988].

4.3.7 Mutagenicity

A limited number of studies of the potential mutagenicity of EGEE and EGME have been performed. Most of these were in vitro tests with microorganisms or mammalian cell cultures. EGME did not appear to be mutagenic, and EGEE was positive in one test system. No data are available concerning the mutagenicity of EGEEA and EGMEA.

4.3.7.1 EGEE

EGEE was not mutagenic in the Ames test using *S. typhimurium* TA1538, with or without metabolic activation [Kawalek and Andrews 1980], or *E. coli* scl-4-73 [Szybalski 1958]. EGEE was not mutagenic (up to 23 mg/plate) [Ong 1980] when tested in *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 with and without Aroclor-induced rat liver S9 supernatant. NTP reported that EGEE was not mutagenic at concentrations up to 10 mg/plate in the same four *Salmonella* strains with and without microsomal fractions prepared from Aroclor-induced rat and hamster livers [Melnick 1982].

EGEE was also tested in an NTP study at concentrations up to 9 mg/ml and was found to induce sister chromatid exchange in Chinese hamster ovary (CHO) cells in both the presence and absence of rat S9 mix. The response was weaker in the presence of rat S9 mix than in its absence. EGEE induced chromosomal aberrations in CHO cells in the absence of rat S9 mix, but failed to do so in its presence. EGEE was not mutagenic in the Drosophila, sex-linked, recessive lethal test [McGregor 1984].

4.3.7.2 EGME

Abbondandolo et al. [1980] assayed five organic solvents, including EGME, for their ability to induce forward mutations in the fission yeast, *Schizosaccharomyces pombe*, both with and without metabolic activation. An S10 post-mitochondrial fraction from phenobarbital-induced mouse liver was used for metabolic activation. EGME gave negative results in all forward mutation experiments.

EGME was not mutagenic (up to 200 mg/plate) to *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 with and without S9 mix [Ong 1980].

McGregor et al. [1983] tested EGME in various in vitro systems (i.e., bacterial and human embryonal intestinal fibroblasts) for mutagenic potential. In the bacterial mutation tests incubations were conducted both in the presence and absence of an adult male rat liver's post-mitochondrial supernatant fluid and NADPH-generating system (S9 mix). There was no evidence of mutagenicity in the Ames plate incorporation assay at levels up to 3 mg EGME/plate. In another experiment, alcohol metabolism was mediated by yeast B NAD+-dependent alcohol dehydrogenase, and no mutagenic effect was observed. Human embryonic intestinal fibroblasts in the presence of ^{3}H-thymidine were incubated with EGME both in the presence and absence of S9 mix. There was no indication of increased unscheduled DNA synthesis (UDS) in cells exposed to concentrations up to 10 mg EGME/ml. EGME had no effect on bone marrow cytogenetics and did not induce point mutations in the L5178Y mouse lymphoma TK^{+}/-cell assay in the presence of rat S9 mix [McGregor 1984]. In the sex-linked, recessive lethal test with *Drosophila*, results were inconsistent and reinvestigation was suggested. EGME was positive in both the mouse sperm abnormality test and the male rat dominant lethal test.

Mutagenic effects of EGEE and EGME are summarized in Table 4-10.

4.3.8 In Vitro Toxicity

The effects of EGME and MAA on lactate production and protein synthesis by cultured Sertoli cells were studied by Beattie et al. [1984], who suggested that alterations in Sertoli cell function induced by EGME or MAA could critically affect spermatocyte viability and maintenance of spermatogenesis. Sertoli cells were isolated from Sprague-Dawley CD rats and incubated with ^{3}H-labeled leucine. EGME or MAA was then added at 0-, 3-, or 10-mM concentrations, and spectrophotometric lactate determinations were made after 0, 1, 3, 6, 9, and 12 hr of incubation. EGME had no effect on lactate concentrations or rates of accumulation at any time point compared to controls. However, lactate concentration and rate of accumulation were both significantly decreased ($P<0.01$) by both 3 and 10 mM MAA at 6, 9, and 12 hr of incubation. No significant differences were seen between experimental and control plates in protein synthesis as measured by the incorporation of ^{3}H-labeled leucine into acid insoluble material at the end of 12 hr of incubation.

Table 4-10.—Mutagenic effects

Type of test	Compound	Test species and exposure	Results*	References
Bacterial, mutation	EGEE	S. typhimurium TA1538, with and without S9 mix	-	Kawalek and Andrews 1980
		S. typhimurium TA1535, TA1537, TA98, TA100, with and without rat S9 mix, a hamster S9 mix	-	Ong 1980; Melnick 1982
		E. coli scl-4-73	-	Szybalski 1958
	EGME	S. typhimurium TA1535, TA1537, TA98, and TA100, with and without S9 mix	-	Ong 1980
		S. typhimurium TA1535, TA1537, TA1538, TA98, TA100, with and without rat S9 mix and with alcohol dehydrogenase	-	McGregor et al. 1983
Yeast, mutation	EGME	Schizosaccharomyces pombe, with and without mouse S9 mix	-	Abbondandalo et al. 1980
Mammalian, in vitro, unscheduled DNA synthesis	EGME	Human embryonic intestinal fibroblast cells, with and without rat S9 mix	-	McGregor et al. 1983
Mammalian, in vitro, chromosomal aberrations	EGEE	CHO cells with S9 mix CHO cells without S9 mix	- +	McGregor 1984
Drosophila, sex-linked recessive lethal	EGEE EGME	3-day-old males 3-day-old males	- ?	McGregor et al. 1983 McGregor et al. 1983

(Continued)

*Abbreviations: - = no significant response; + = significant response; ? = unclear, further testing recommended.

Table 4-10 (Continued).—Mutagenic effects

Type of test	Compound	Test species and exposure	Results	References
Mammalian, in vitro, point mutations	EGME	L5178Y mouse lymphoma TK+/- cells with rat S9 mix	-	McGregor 1984
Rat bone marrow cytogenetics	EGME	Rats (M,F) exposed to 25 or 500 ppm 7 hr/day, for 1 or 5 days	-	McGregor et al. 1983
Mouse sperm abnormality	EGME	Mice exposed to 25 or 500 ppm, 7 hr/day for 5 days	+ (at 500 ppm)	McGregor et al. 1983
Male rat dominant lethal	EGME	Male rats exposed to 30, 100, or 300 ppm, 6 hr/day, 5 days/wk, for 13 wk	Male sterility at 300 ppm reversible	McGregor et al. 1983
		Male rats exposed to 30, 100, or 300 ppm, 7 hr/day, for 5 days, followed by 10 successive weekly matings	Male sterility at week 5 (500 ppm), reversible	McGregor et al. 1983

4.3.9 Cytotoxicity

The in vitro cytotoxicities of EGME, EGEE, and their corresponding alkoxyacetic acids (MAA and EAA) were studied using CHO cells [Jackh et al. 1985]. CHO cells were seeded into culture flasks, and after 4 to 5 hr test material was added to the medium. After 16 hr the medium was renewed and the cells were allowed to grow in colonies for 6 to 7 days prior to counting. Cloning efficiency was used as an indication of cytotoxicity. Concentrations that allowed approximately 50% of the seeded cells to form colonies (EC_{50}) were calculated. The EC_{50} for EGEE was 0.22 mmol/ml or 21.5 mg/ml and for EGME the EC_{50} was 0.49 mmol/ml or 37.5 mg/ml. EAA and MAA were more cytotoxic (EC_{50} = 0.04 to 0.05 mmol/ml or 4.6 mg/ml for both) than their parent compounds. The authors concluded that gross cytotoxicity to dividing cells is not the predominant mechanism for the reproductive, developmental, and myelotoxic effects of these glycol ethers [Jackh et al. 1985].

Chinese hamster V79 cells display a specific form of cell-to-cell communication called metabolic cooperation, which is characterized by the exchange of molecules between cells through permeable junctions formed at sites of cell contact [Hooper and Subak-Sharpe 1981]. Blockage of metabolic cooperation has been proposed as a mechanism of action of some teratogens [Trosko et al. 1982]. The effects of EGME and EGEE on cell-to-cell communication in Chinese hamster V79 cells were demonstrated in two separate studies [Welsch and Stedman 1984; Loch-Caruso et al. 1984]. In both studies, EGME and EGEE were able to block metabolic cooperation in vitro. The potencies were inversely related to the length of the aliphatic chain; in general, cytotoxicity increased with increasing aliphatic chain length. Loch-Caruso et al. [1984] concluded that because EGME was effective in blocking metabolic cooperation over a broad noncytotoxic range, blockage of intercellular communication may be its teratogenic mechanism. However, EGEE was more cytotoxic and interrupted cell communication over a narrower range of concentrations. The authors therefore concluded that interrupted intercellular communication may be mixed with cytotoxicity in the embryo and the dam, and thus is less specific as a mechanism of teratogenesis for EGEE.

Gray et al. [1985] also investigated the response of primary mixed cultures of Sertoli and germ cells prepared from testes of immature rats that had been exposed to EGEE and its alkoxyacetic acid metabolite, EAA. EGEE had no effect when added to the culture medium at concentrations up to 50 mM (4.505 mg) for up to 72 hr. In contrast, the following changes were induced when 2 to 10 mM of MAA, the major in vivo metabolite of EGME, was added for 24 to 72 hr. After 24 hr incubation of cultures with 5 mM MAA, pachytene spermatocytes were reduced in number and many of those remaining showed degenerative changes consisting of rounding up, increased cytoplasmic eosinophilia, and nuclear pyknosis. The number of pachytene spermatocytes was further reduced after 48 hr of incubation, and after 72 hr the cultures consisted of earlier spermatocytes, spermatogonia, and Sertoli cells with only occasional degenerate pachytene spermatocytes. At 10 mM MAA, pachytene spermatocytes were lost more rapidly, and cell debris in the Sertoli cells was observed more frequently. While no effect was observed at 1 mM MAA, 2 mM MAA caused a slightly increased frequency of pachytene spermatocyte degeneration. EAA, the major metabolite of EGEE in vivo, produced similar changes but was less potent than MAA. Although

cultures treated with 5 mM EAA showed some loss of pachytene spermatocytes, even after 72 hr frequent foci of these cells were still present and many appeared morphologically normal.

In view of the differences in the toxicity of MAA and EAA in cell culture, they were administered orally to rats at equimolar doses of 6.6 mM (592 mg MAA/kg and 684 mg EAA/kg, respectively) to characterize their relative testicular toxicity in vivo. Only MAA reduced testis weight. The effects of MAA were found mainly on the pachytene spermatocyte population; maturation depletion of the early round spermatid population was also evident, while leptotene and zygotene spermatocytes appeared unaffected. EAA had less severe effects, with only focal depletion of early pachytene spermatocytes and early round spermatids, while mid- and late-pachytene spermatocytes appeared normal. The authors concluded that the close correspondence between the testicular toxicity of MAA and EAA in vitro and in vivo suggests a similar mode of action in both cases.