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Number 26

NTP Technical Report
on Toxicity Studies of

Ethylene Glycol Ethers

2-Methoxyethanol, 2-Ethoxyethanol, 2-Butoxyethanol

(CAS Nos. 109-86-4, 110-80-5, 111-76-2)

Administered in Drinking Water
to F344/N Rats and B6C3F$_1$ Mice

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This NTP report on the toxicity studies of ethylene glycol ethers is based primarily on 2-week, 13-week, and stop-exposure studies conducted in 1988 at EG&G Mason Research Institute, Worcester, MA.

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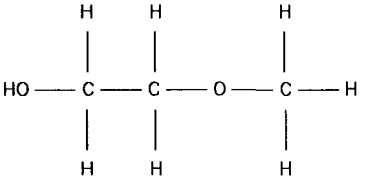
2-Methoxyethanol



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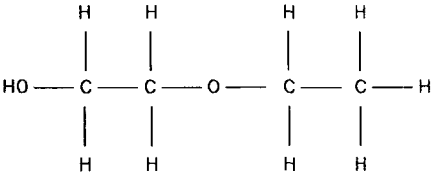
Molecular Weight 76.10
Synonyms Ethylene Glycol Monomethyl Ether, Methyl Cellosolve®

2-Ethoxyethanol



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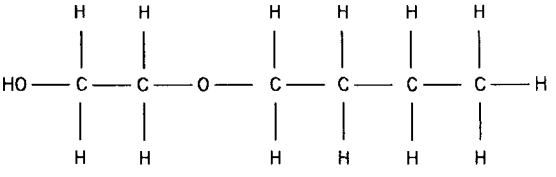
CAS Number 110-80-5
Molecular Weight 90.12
Synonyms Ethylene Glycol Monoethyl Ether, Cellosolve®

2-Butoxyethanol



Molecular Formula $C_6H_{14}O_2$
CAS Number 111-76-2
Molecular Weight 118.17
Synonyms Ethylene Glycol Monobutyl Ether, Butyl Cellosolve®

Glycol alkyl ethers represent a class of high-production-volume chemicals with widespread industrial applications as solvents and chemical intermediates. Comparative toxicity studies with three glycol ethers, 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol, were conducted in F344/N rats and B6C3F$_1$ mice in both 2-week and 13-week drinking water studies. Toxicologic endpoints evaluated in animals included histopathology, hematology, clinical chemistry, urinalysis, and reproductive system parameters. Genetic toxicity was also evaluated for each glycol ether in several *in vitro* and *in vivo* assays.

In the 2-week studies, groups of five male and five female rats and mice received 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol in the drinking water. Estimates of compound consumption based on water consumption by male and female rats ranged from 100 to 400 mg/kg for 2-methoxyethanol, 200 to 1600 mg/kg for 2-ethoxyethanol, and 70 to 300 mg/kg for 2-butoxyethanol. For mice, consumption values ranged from 200 to 1300 mg/kg for 2-methoxyethanol, 400 to 2800 mg/kg for 2-ethoxyethanol, and 90 to 1400 mg/kg for 2-butoxyethanol.

There were no chemical-related effects on survival for rats or mice in the 2-week studies. Decreased body weight gains were noted for both male and female rats treated with 2-methoxyethanol or 2-ethoxyethanol for 2 weeks, and there were dose-related decreases in water consumption for rats of each sex treated with the ethylene glycol ethers. Most of the changes in organ weights for rats and mice treated with the glycol ethers were sporadic (mice) or related to low final mean body weights (rats), except for thymic atrophy in male and female rats and testicular atrophy in males of both species receiving 2-methoxyethanol or 2-ethoxyethanol.

In the 13-week studies in rats, groups of 10 males and 10 females received 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol in the drinking water at concentrations ranging from 750 to 6000 ppm, 1250 to 20,000 ppm, or 750 to 6000 ppm, respectively. In the 13-week studies in mice, groups of 10 males and 10 females received 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol in the drinking water at concentrations ranging from 2000 to 10,000 ppm, 2500 to 40,000 ppm, or 750 to 6000 ppm, respectively. Estimates of compound consumption based on water consumption by male and female rats ranged from 70 to 800 mg/kg for 2-methoxyethanol, 100 to 2200 mg/kg for 2-ethoxyethanol, and 70 to 500 mg/kg for 2-butoxyethanol.

For mice, consumption values ranged from 300 to 1800 mg/kg for 2-methoxyethanol, 600 to 11,000 mg/kg for 2-ethoxyethanol, and 100 to 1300 mg/kg for 2-butoxyethanol.

Chemical-related mortality occurred in male and female rats administered 4500 or 6000 ppm 2-methoxyethanol and in male and female rats administered 20,000 ppm 2-ethoxyethanol. No deaths occurred in rats administered 2-butoxyethanol or in mice administered 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol. Decreased body weight gains occurred in dosed rats and mice in all three studies; the greatest reductions in body weight gain were seen with 2-methoxyethanol.

In rats administered 2-methoxyethanol or 2-ethoxyethanol, treatment-related histopathologic changes were observed in the testes, thymus, and hematopoietic tissues (spleen, bone marrow, and liver). A dose-related degeneration of the germinal epithelium in the seminiferous tubules of the testes was more severe in 2-methoxyethanol-treated rats than in rats treated with 2-ethoxyethanol. In special stop-exposure studies in male rats in which administration of the glycol ethers was stopped after 60 days, marked degeneration of the seminiferous tubules was present in rats treated with 3000 ppm 2-methoxyethanol, and mild to moderate degeneration was observed in rats treated with 1500 ppm. Moderate to marked testicular degeneration was present in rats treated with 10,000 or 20,000 ppm 2-ethoxyethanol but not in rats treated with 5000 ppm. After 30 and 56 days of recovery from treatment with these chemicals, only partial recovery from testicular degeneration was observed. There was no testicular degeneration after 60 days of treatment with 1500 to 6000 ppm 2-butoxyethanol.

2-Methoxyethanol treatment for 13 weeks resulted in a progressive anemia associated with a cellular depletion of bone marrow and fibrosis of the splenic capsule. Anemia was also seen with 2-ethoxyethanol, but evidence of an adaptive response was indicated by increased hematopoiesis in the bone marrow, spleen, and liver. Toxicity with 2-butoxyethanol was limited to the liver and hematopoietic system. Cytoplasmic alteration and a minimal hepatocellular degeneration were present in the liver of male and female rats. A minimal anemia was present, and a hematopoietic response was evident in the bone marrow and spleen.

In mice, 2-methoxyethanol and 2-ethoxyethanol had similar effects on the testes, spleen, and adrenal gland (females only). A dose-related degeneration of the germinal epithelium

in seminiferous tubules of the testes was more severe with 2-methoxyethanol than with 2-ethoxyethanol. A dose-related increase in splenic hematopoiesis was also more prominent with 2-methoxyethanol. Both 2-methoxyethanol and 2-ethoxyethanol caused a prominent lipid vacuolization of the X-zone of the adrenal gland in female mice. There were no chemical-related lesions attributed to 2-butoxyethanol administration in mice.

All three of the glycol ethers were negative in *Salmonella typhimurium* mutation tests conducted with and without induced hamster and rat liver S9. In the mouse lymphoma L5178Y cell mutation assay, 2-ethoxyethanol was negative without S9 but was weakly positive in the presence of induced rat liver S9; 2-methoxyethanol and 2-butoxyethanol were not tested in this assay. At high concentrations, 2-ethoxyethanol induced sister chromatid exchanges (SCEs) in Chinese hamster ovary cells with and without S9. Chromosomal aberrations (Abs) were also induced by 2-ethoxyethanol, but only in the absence of S9 and without a delay in cell cycle. In contrast, 2-butoxyethanol induced cell cycle delay but did not induce SCEs or Abs with or without S9. 2-Ethoxyethanol was the only glycol ether tested for induction of sex-linked recessive lethal mutations in germ cells of *Drosophila melanogaster*; both feeding and injection trials were negative.

In summary, based on survival, decreased body weight gains, and histopathologic effects, the rank order of toxicity for the three glycol alkyl ethers was 2-methoxyethanol > 2-ethoxyethanol > 2-butoxyethanol; the toxic effects were more severe in rats than in mice. In the 13-week study of 2-methoxyethanol in rats, a no-observed-adverse-effect level (NOAEL) was not reached, since testicular degeneration in males and decreased thymus weights in males and females occurred at the lowest concentration administered (750 ppm). In the 13-week study of 2-ethoxyethanol in rats, the NOAEL for decreased thymus weights in males was 1250 ppm; for female rats treated with 2-ethoxyethanol for 13 weeks, the NOAEL for all histopathologic and hematologic effects was 5000 ppm. In rats treated with 2-butoxyethanol for 13 weeks, the NOAEL for liver degeneration was 1500 ppm in males and females.

For male mice treated with 2-methoxyethanol for 13 weeks, the NOAEL for testicular degeneration and increased hematopoiesis in the spleen was 2000 ppm. A NOAEL was not reached for female mice treated with 2-methoxyethanol, since adrenal gland hypertrophy and increased hematopoiesis in the spleen occurred at the lowest concentration administered (2000 ppm). For male mice treated with 2-ethoxyethanol for 13 weeks, the

NOAEL for testicular degeneration and increased hematopoiesis in the spleen was 20,000 ppm. For female mice in the 13-week study of 2-ethoxyethanol, the NOAEL for adrenal gland hypertrophy and increased hematopoiesis in the spleen was 5000 ppm. No clear chemical-related effects were seen in male or female mice administered 2-butoxyethanol for 13 weeks at concentrations as high as 6000 ppm.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of ethylene glycol ethers on December 2, 1992 are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies are appropriate and to ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

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SUMMARY OF PEER REVIEW COMMENTS

On December 2, 1992, the Technical Reports Review Subcommittee of the Board of Scientific Counselors for the National Toxicology Program met in Research Triangle Park, NC, to review the draft technical report on toxicity studies of ethylene glycol ethers.

Dr. Michael P. Dieter, NIEHS, introduced the short-term toxicity studies of ethylene glycol ethers by reviewing the rationale for study, experimental design, and results.

Dr. Bailey, a principal reviewer, said that the report was well written and organized. He asked that dosage information from the 2-week studies be clarified and that the types of caging used be specified. He also asked that additional information be given on exposure limits for the various glycol ethers.

Dr. Carlson, a second principal reviewer, also thought that the dosage information for the 2-week studies should be clarified, and he asked that thymus and testis weight information be added to the 2-week studies. He discussed the various no-observed-adverse-effect levels (NOAELs) given in the report and suggested changes to focus only on lesions thought to actually represent toxic changes.

Dr. Dieter responded by agreeing to adjust the way doses were expressed for the 2-week studies, adding that the cages were polycarbonate, and adding information concerning organ weight changes in the 2-week studies. He commented on the thoughts that went into the selection of the stated NOAELs and agreed to revisit the issue.

Dr. Ralph Gingell, Shell Oil Company, a member of the Chemical Manufacturers Association, Glycol Ethers Panel, commented that he felt the report was not clearly written and requested that it be split into three separate reports because of the different characters of the primary toxicities of the methoxy and ethoxy ethers versus the butoxy ether. He also disagreed with the information concerning potential uses and exposures as given in the report; he stated that only the butoxy ether is still used in consumer products or where there is a potential for human exposure. Dr. Gingell said that the information presented on the hematotoxicity of 2-butoxyethanol was consistent with other reports in the literature and that this toxicity is not an effect seen in humans. He also questioned the effects on testis weights and whether the effects reported for the liver of

rats administered 2-butoxyethanol might be an adaptive response to a large metabolic load on the liver.

Dr. Rodney Boatman, Eastman Kodak Company, also a member of the Chemical Manufacturers Association, Glycol Ethers Panel, questioned the speculation in the report that suggested that the variations in the mode or rate of metabolism of the glycol ethers might account for certain differences in the toxicities of the compounds; he also stated that none of the "minor metabolites" had been shown to be toxic.

Responding to Dr. Gingell's comments, Dr. Dieter said that the literature concerning the different sensitivities of rodent and human red blood cells to the hemolytic action of 2-butoxyethanol was adequately cited in the report and that the other comments would be considered.

Following these comments, Dr. Klaassen accepted the report on behalf of the peer review panel.

INTRODUCTION

Chemical and Physical Properties, Production, Use, and Exposure

Three of the simplest glycol alkyl ethers, 2-methoxyethanol (methyl Cellosolve® or ethylene glycol monomethyl ether), 2-ethoxyethanol (Cellosolve® or ethylene glycol monoethyl ether), and 2-butoxyethanol (butyl Cellosolve® or ethylene glycol monobutyl ether) are colorless organic liquids with a mild, non-residual odor, a sweetish odor, or a mild ether odor and with odor thresholds of 2.3, 2.7, and 0.10 ppm, respectively (Amoore and Hautala, 1983). They are miscible with water and many organic solvents. Chemical and physical properties for the three compounds are listed in Table 1.

The three glycol alkyl ethers are produced by reaction of ethylene oxide with their respective alcohols or by direct alkylation of ethylene oxide with agents like dimethyl, diethyl, or dibutyl sulfate (Rowe and Wolf, 1982). The products of these reactions are not pure glycol alkyl ethers. The glycol alkyl ethers must be separated from diethers and higher glycols.

2-Methoxyethanol is used as a jet fuel deicer (Meridian Research, Inc., 1987), as a plasticizer, and in the manufacture of printed circuit boards; it is also used in ink, photography, and dyeing applications. 2-Ethoxyethanol is used as a solvent and a chemical intermediate for the synthesis of ethylene glycol monoethyl ether acetate. 2-Butoxyethanol is used as a solvent, chemical intermediate, and component of herbicides and brake fluid. A complete review of the uses of these glycol alkyl ethers can be found in two National Institute for Occupational Safety and Health (NIOSH) criteria documents, one for 2-butoxyethanol (1990) and the other for 2-methoxyethanol and 2-ethoxyethanol (1991).

TABLE 1 Chemical and Physical Properties of the Ethylene Glycol Ethers[1]

Parameter	2-Methoxyethanol	2-Ethoxyethanol	2-Butoxyethanol
Specific gravity	0.962	0.926	0.898
Boiling point (°C)	124.2	135.0	170.8
Freezing point (°C)	–85	–100	–77
Vapor pressure (mm Hg at 25°C)	9.7	5.75	0.88
Refractive index	1.400	1.406	1.417
Flash point (°C), closed cup	39	43	62
Autoignition temperature (°C)	285	235	238
Flammability limits (vol. % in air)	1.8–14.0	1.70–15.6	1.10–12.7
Water solubility	miscible	miscible	miscible
Vapor density (air=1)	2.6	3.1	4.1
ppm in saturated air (25°C)	12,800	7600	1200

[1] Adapted from NIOSH (1990, 1991).

Because of the widespread applications of the glycol alkyl ethers and their large annual production volume, large numbers of U.S. workers are potentially exposed. For example, over the last 5 years, about 70 million pounds of 2-methoxyethanol, 110 million pounds of 2-ethoxyethanol, and 350 million pounds of 2-butoxyethanol were produced (SRI International, 1992). For detailed exposure data, refer to tables 3-3 to 3-5 in NIOSH criteria documents 90-118 (1990) and 91-119 (1991). The NIOSH-recommended exposure limits for 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol in air are 0.1 ppm (0.3 mg/m^3), 0.5 ppm (1.8 mg/m^3), and 5 ppm (24 mg/m^3), respectively, as time-limited averages for up to 10 hours per day during a 40-hour workweek (NIOSH, 1990, 1991). The threshold limit value-time weighted averages for skin exposure recommended by the American Conference of Governmental Industrial Hygienists for 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol are 5 ppm, 5 ppm, and 25 ppm, respectively (ACGIH, 1991-1992).

Absorption, Disposition, Metabolism, and Excretion

The metabolism of 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol has been investigated in rats, rabbits, guinea pigs, and dogs. In several studies, the three glycol alkyl ethers were shown to undergo oxidization catalyzed by alcohol dehydrogenase to intermediate aldehydes, which then underwent further oxidation catalyzed by aldehyde dehydrogenase to their respective acids (Carpenter *et al.*, 1956; Jonsson and Steen, 1978;

Jonsson *et al.*, 1982; Miller *et al.*, 1983a,b; Cheever *et al.*, 1984; Moss *et al.*, 1985). The acid metabolites were found in the urine; in the case of 2-ethoxyethanol, some of the ethoxyacetic acid was conjugated with glycine to form *N*-ethoxyacetyl glycine (Jonsson *et al.*, 1982; Cheever *et al.*, 1984). Subsequent investigations using radiolabeled 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol administered to rats in the drinking water revealed another metabolic product, ethylene glycol, in the urine. These studies also demonstrated that the fraction of the dose metabolized to ethylene glycol and carbon dioxide was inversely proportional to chain length (Medinsky *et al.*, 1990). This result was confirmed in an inhalation study of 2-butoxyethanol in rats (Sabourin *et al.*, 1992a). The elimination of 10% to 20% of the dose of each glycol alkyl ether as ethylene glycol in the urine suggested that dealkylation prior to oxidation to the alkoxyacetic acids could occur, which would represent an alternate pathway of metabolism and avoid formation of acid metabolites.

Additionally, metabolism studies of all three glycol alkyl ethers were conducted after human inhalation exposures, and the presence of the respective alkoxyacetic acids in the urine was confirmed (Groeseneken *et al.*, 1986a,b, 1987, 1988, 1989; Johanson *et al.*, 1986, 1988). These investigations also demonstrated that the half-life of 2-methoxyacetic acid in humans was greater than 70 hours (compared to about 12 hours in pregnant rats and 20 hours in pregnant macaque monkeys), and that dermal exposure to 2-butoxyethanol resulted in systemic uptake and the appearance of butoxyacetic acid in the urine.

Toxicity

ANIMAL TOXICITY

Glycol alkyl ethers exhibit a spectrum of toxicity, dependent upon dose, carbon chain length, route of exposure, and species investigated. Reviews of the toxicity of 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol were published by NIOSH in 1990 and 1991. Alkoxyacetic acids are the primary metabolites of the ethylene glycol mono-*n*-alkyl ethers and are considered to be the toxic agents (Ghanayem *et al.*, 1989). The target organs and systems that exhibited toxicity with these compounds included the kidney, liver, hematopoietic system, central nervous system, and reproductive system. Some glycol alkyl ethers are toxic to certain populations of rapidly dividing cells, such as embryonic stem cells (Nagano *et al.*, 1981), bone marrow stem cells (Hong *et al.*, 1988,

1989), tumor cells (Houchens *et al.*, 1984; Dieter *et al.*, 1990), renal tubule cells (Karel *et al.*, 1947; Dodd *et al.*, 1983), and spermatocytes (Creasy and Foster, 1984; Oudiz and Zenick, 1986; Anderson *et al.*, 1987). However, there is a remarkable specificity in the toxicity of the three glycol alkyl ethers that are the subjects of this report. For example, 2-methoxyethanol is a potent teratogen (Nagano *et al.*, 1981; Horton *et al.*, 1985; Greene *et al.*, 1987; Feuston *et al.*, 1990), but it exhibits little of the erythrocytic hemolysis produced by 2-butoxyethanol (Bartnik *et al.*, 1987; Ghanayem *et al.*, 1987a; Ghanayem, 1989); these toxic effects are seen with 2-ethoxyethanol only at higher doses. 2-Methoxyethanol and 2-ethoxyethanol are potent spermatotoxins (Creasy and Foster, 1984; Oudiz and Zenick, 1986; Anderson *et al.*, 1987) while 2-butoxyethanol is ineffective in this capacity. 2-Methoxyethanol was more than twice as effective as 2-ethoxyethanol in delaying tumor progression in a leukemia transplant model, and it was equally more potent in reducing the number of mouse, rat, or human leukemia cells in culture. 2-Butoxyethanol, on the other hand, was ineffective whether tested *in vivo* or *in vitro* in this system (Dieter *et al.*, 1990).

HUMAN TOXICITY

As early as 1936 and 1938, case studies of adverse health effects related to exposure to 2-methoxyethanol in shirt factories were reported (Donley, 1936; Parsons and Parsons, 1938). Also, occupational exposures to 2-methoxyethanol dermally or by inhalation in printing (Groetschel and Schuermann, 1959; Zavon, 1963), electroplating (Ohi and Wegman, 1978), and microfilm production operations (Cohen, 1984) induced reversible toxicity that affected the hematopoietic and central nervous systems. An accidental poisoning resulted in reversible renal toxicity in two men who ingested pure 2-methoxyethanol (Nitter-Hauge, 1970). One case was reported in which a woman ingested 40 mL 2-ethoxyethanol, resulting in toxicity to the central nervous system, liver, and kidneys that persisted for up to 1 year (Fucik, 1969). In two other cases, women attempted suicide by ingesting 2-butoxyethanol, which resulted in reversible hematotoxicity (Rambourg-Schepens *et al.*, 1988; Gijsenbergh, 1989). In a study conducted in the 1950s, four men and three women were voluntarily exposed by inhalation to 2-butoxyethanol for as long as 8 hours at a concentration of 100 ppm or for two 4-hour exposure periods at concentrations as great as 200 ppm. In this study, inhalation exposure to 2-butoxyethanol resulted in various symptoms of toxicity, including nose and throat irritation, headaches, and vomiting; erythrocyte osmotic fragility was unchanged (Carpenter *et al.*, 1956). Butoxyacetic acid was excreted in the urine of the subjects.

Numerous epidemiological studies of adverse health effects related to exposure to glycol alkyl ethers have also been conducted. These studies have investigated exposure to 2-methoxyethanol in shirt factories (Greenburg *et al.*, 1938) as well as in manufacturing and packaging operations (Cook *et al.*, 1982), exposure to 2-ethoxyethanol in the preparation of ceramic molds (Ratcliffe *et al.*, 1986), and exposure to 2-methoxyethanol and 2-ethoxyethanol in shipbuilding facilities (Sparer *et al.*, 1988; Welch and Cullen, 1988; Welch *et al.*, 1988). Exposures to concentrations of at least 76 ppm 2-methoxyethanol for up to 112 weeks were reported in the 1938 study; these exposures resulted in bone marrow toxicity, anemia, and severe neurotoxicity. There was no evidence of 2-methoxyethanol toxicity in the 1982 study in which the highest air concentration of 2-methoxyethanol was 20 ppm. In the 1986 study, dermal and inhalation exposures of up to 24 ppm 2-ethoxyethanol resulted in spermatotoxicity and urine concentrations of ethoxyacetic acid ranging from 16 to 163 mg/g creatinine. In the 1988 studies, the combined exposure to 2-methoxyethanol and 2-ethoxyethanol by inhalation at concentrations up to 5 ppm 2-methoxyethanol and 22 ppm 2-ethoxyethanol was confirmed by identification of specific alkoxyacetic acid metabolites in the urine. The toxic responses in these studies included lowered sperm counts and suggested that anemia and granulocytopenia could have been related to exposure.

REPRODUCTIVE TOXICITY

2-Methoxyethanol and 2-ethoxyethanol are both potent male reproductive toxicants in mice, rats, guinea pigs, rabbits, and dogs. In these animals, exposure to 2-methoxyethanol or 2-ethoxyethanol by the subcutaneous, dermal, oral, or inhalation route resulted in testicular atrophy and decreased fertility caused by spermatotoxicity (Stenger *et al.*, 1971; Nagano *et al.*, 1979; Foster *et al.*, 1983; Miller *et al.*, 1983a; Chapin *et al.*, 1985; Hobson *et al.*, 1986; Oudiz and Zenick, 1986). The most sensitive cells were shown to be the primary spermatocytes in the pachytene stage of meiosis and secondary spermatocytes (Creasy and Foster, 1984; Oudiz and Zenick, 1986; Anderson *et al.*, 1987). In contrast, there is ample evidence from studies in mice and rats that administration of 2-butoxyethanol by the oral gavage or inhalation route has no effect on the male reproductive system (Nagano *et al.*, 1979; Doe, 1984a; Krasavage, 1986).

2-Methoxyethanol and, to a lesser extent, 2-ethoxyethanol are potent teratogens. Studies have been conducted in mice, rats, rabbits, and monkeys using dermal, oral, and inhalation routes of administration (Hardin *et al.*, 1981, 1982; Nagano *et al.*, 1981;

Doe, 1984b; Hanley *et al.*, 1984; Horton *et al.*, 1985; Greene *et al.*, 1987; Scott *et al.*, 1989; Feuston *et al.*, 1990). For 2-methoxyethanol, a no-observed-effect level of 10 ppm was established for pregnant mice, rats, and rabbits (Hanley *et al.*, 1984). Additionally, a no-observed-effect level of 100 mg/kg was established for pregnant mice when 2-methoxyethanol was administered in a single dose on Day 11 of gestation (Horton *et al.*, 1985). Adverse effects on maternal animals included prolonged gestation and reductions in body weights and weight gains. The toxicity of 2-butoxyethanol on the reproductive system of female F344 rats and CD-1 mice was limited to fetal mortality and decreased body weights and weight gains in the dams; these effects were noted only after administration of doses that caused death to 20% of the dams (LD_{20}). There was no teratogenicity in the offspring of dams that received doses of 2-butoxyethanol below the maternal LD_{20} (Schuler *et al.*, 1984; Tyl *et al.*, 1984).

CARCINOGENICITY

There have been no adequate carcinogenicity studies conducted with any of the glycol alkyl ethers.

IMMUNOTOXICITY

The results of a cell-mediated immunity assay in mice suggested that 2-methoxyethanol and 2-ethoxyethanol might stimulate the immune system. Allogenic mice given L1210 leukemia cells and dosed with up to 100 mg/kg 2-methoxyethanol or 2400 mg/kg 2-ethoxyethanol 12 days before transplant survived, while those without chemical treatment developed leukemia and died (Houchens *et al.*, 1984). However, a second study in mice used 2-methoxyethanol at doses of up to 1000 mg/kg, and while thymic atrophy occurred, no changes in bone marrow cellularity, leukocyte counts, or immune function were observed (House *et al.*, 1985). Two studies of the potential effects of 2-methoxyethanol on immune function conducted in Sprague-Dawley and F344 rats yielded conflicting data. Exon *et al.* (1991) reported that natural killer cell cytotoxic responses were enhanced in male and female Sprague-Dawley rats administered doses of 1600 to 6000 ppm 2-methoxyethanol in drinking water for 21 days; however, delayed type hypersensitivity was suppressed as was gamma interferon production and interleukin-2 production by spleen cells. The authors suggested that 2-methoxyethanol exerted immunomodulatory effects in the rats. However, Smialowicz *et al.* (1991) reported variable responses in F344 rats given 50 to 200 mg/kg 2-methoxyethanol per day in the plaque-

forming cell response to sheep red blood cell and trinitrophenyl-lipopolysaccharide antigens (depending on dose and schedule of 2-methoxyethanol administration), no alterations in natural killer cell activity, mixed lymphocyte, cytotoxic T-cell, or lymphoproliferative responses, and a reduction in interleukin-2 production by spleen cells.

GENETIC TOXICITY

None of the three glycol ethers, 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol, was mutagenic in *Salmonella typhimurium*, with or without S9 activation (McGregor *et al.*, 1983; Shimizu *et al.*, 1985; Zeiger *et al.*, 1985, 1992). Additional genotoxicity data are available for the monomethyl and monoethyl ethers; most of the results were negative, but a few positive responses were reported for each chemical in tests for induction of chromosome damage in mammalian cells.

2-Methoxyethanol, tested as a vapor, did not induce mutations in the *Drosophila* sex-linked recessive lethal assay (McGregor *et al.*, 1983). In addition, it did not cause gene mutations in the yeast *Schizosaccharomyces pombe*, assayed either in a host-mediated assay (Barale *et al.*, 1979) or in culture, with or without S9 (Abbondandolo *et al.*, 1980). 2-Methoxyethanol did not produce unscheduled DNA synthesis in cultured human embryo fibroblasts with or without S9 (McGregor *et al.*, 1983). It did, however, induce sperm abnormalities in mice (McGregor *et al.*, 1983; Anderson *et al.*, 1987) and rats (Anderson *et al.*, 1987). No increase in dominant lethal mutations was observed in mice treated with 2-methoxyethanol (Anderson *et al.*, 1987); similar tests in rats yielded somewhat conflicting results. Some laboratories reported small, inconclusive effects in either CD (McGregor *et al.*, 1983) or F344 (Chapin *et al.*, 1985) rats, while other laboratories found no evidence of dominant lethal mutations in either CD (Anderson *et al.*, 1987) or Sprague-Dawley (Rao *et al.*, 1983) rats treated with 2-methoxyethanol. The severe effect of 2-methoxyethanol on male fertility confounded the interpretation of the dominant lethal data in the two studies that noted an increase in the number of post-implantation losses.

2-Ethoxyethanol was not mutagenic in *Escherichia coli* (Shimizu *et al.*, 1985) with or without S9, and it did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila* treated by feeding or by injection (Valencia *et al.*, 1985). No induction of gene mutations was noted in mouse lymphoma L5178Y cells (Myhr *et al.*, 1986) or Chinese hamster ovary (CHO) cells (Guzzie *et al.*, 1986) after treatment with 2-ethoxyethanol.

However, increased frequencies of both chromosomal aberrations and sister chromatid exchanges (SCEs) were observed in CHO cells treated with 2-ethoxyethanol in the absence of S9; SCE frequencies were also increased in these cells in the presence of S9 (Guzzie *et al.*, 1986; Galloway *et al.*, 1987). No increase in the number of micronucleated polychromatic erythrocytes was reported in peripheral blood samples of Swiss-Webster mice administered a single intraperitoneal injection of 2-ethoxyethanol at doses of 25% to 80% of the LD_{50} (2589 mg/kg) (Guzzie *et al.*, 1986).

Study Rationale and Design

2-Methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol were nominated for testing by the United Auto Workers International Union, NIOSH, and the Consumer Product Safety Commission based on their current and increasing patterns of usage, their prevalence in waste sites, the gaps in toxicity data identified in a 1984 review of glycol ethers (Lucier and Hook, 1984), and the concern about carcinogenic potential.

Since occupational exposure to glycol alkyl ethers would normally occur dermally or by inhalation, these were considered to be the most appropriate routes of administration. However, preliminary studies indicated that most of a dermal dose of the labile glycol alkyl ethers would evaporate if unoccluded. The difficulties and uncertainties of dose application in chronic dermal studies and concerns about the general applicability of the findings of such studies caused the abandonment of this route of application. Because preliminary disposition studies showed that maximum systemic exposure could be readily achieved by oral administration, dosed drinking water was used in the prechronic studies to determine the relative toxicity of the three glycol alkyl ethers and to subsequently select one of the three compounds for further investigation.

Thus, toxicity studies were conducted in male and female F344/N rats and $B6C3F_1$ mice by the drinking water route to compare the toxicities of the three ethylene glycol ethers in 2-week and 13-week studies and determine the appropriate doses for long-term tests in the event that they are performed; the genotoxicity of the three glycol alkyl ethers was also assessed during these studies. The data from additional stop-exposure and leukemia inhibition studies of the glycol alkyl ethers in male F344/N rats are also included in this report.

MATERIALS AND METHODS

Procurement and Characterization of Ethylene Glycol Ethers

2-Methoxyethanol (CAS Number 109-86-4) and 2-ethoxyethanol (CAS Number 110-80-5) were obtained from Kodak Laboratory Chemicals (Rochester, NY). 2-Butoxyethanol (CAS Number 111-76-2) was obtained from Aldrich Chemical Company (Milwaukee, WI). Lot E16 of 2-methoxyethanol, Lot D16 of 2-ethoxyethanol, and Lot BT00504LP of 2-butoxyethanol were used in the 2-week and 13-week studies in rats and mice and in the stop-exposure studies in male rats.

Identity and purity analyses were conducted on all three isomers at Midwest Research Institute (MRI, Kansas City, MO). The clear, colorless liquids were identified as 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The spectra were consistent with the structures of the chemicals, with available literature references (*Sadtler Standard Spectra*), and with previous analyses of 2-ethoxyethanol and 2-butoxyethanol performed at MRI. Ultraviolet/visible spectroscopy for 2-butoxyethanol gave a spectrum consistent with the structure. Elemental analyses of 2-methoxyethanol and 2-ethoxyethanol for carbon and hydrogen agreed with theoretical values. Elemental analysis of 2-butoxyethanol for hydrogen was slightly low; analysis for carbon was in agreement with theoretical values. Karl Fischer water analysis indicated the presence of 0.080% ± 0.015% water for 2-methoxyethanol, 0.051% ± 0.007% water for 2-ethoxyethanol, and 0.079% ± 0.009% water for 2-butoxyethanol. Potentiometric titration indicated less than 0.005 mEq acid/g sample for both 2-methoxyethanol and 2-ethoxyethanol. Potentiometric titration of 2-butoxyethanol indicated 0.0011 ± 0.0001 mEq acid/g of compound. Functional group (hydroxyl) titration indicated a purity of 98.3% ± 0.05% for 2-methoxyethanol and 101.1% ± 0.5% for 2-butoxyethanol. Oxidation/reduction titration of 2-ethoxyethanol indicated a purity of 100.3% ± 0.7%. Gas chromatography by two separate systems indicated a purity of 100.2% ± 1.2% for 2-methoxyethanol, 100.5% ± 0.7% for 2-ethoxyethanol, and 100.5% ± 0.3% for 2-butoxyethanol relative to frozen reference standards. Thin-layer chromatography of 2-butoxyethanol by two systems indicated no impurities in the chemical used for the 13-week and stop-exposure studies. Overall purity was approximately 98% for 2-methoxyethanol and approximately 99% for 2-ethoxyethanol and 2-butoxyethanol.

Subsequent reanalyses of the bulk compounds were performed at EG&G Mason Research Institute. Results of analyses for peroxide content and by functional group titration (2-methoxyethanol and 2-butoxyethanol) or oxidation/reduction titration (2-ethoxyethanol) indicated that the purity of the chemicals relative to the reference standards remained unchanged throughout the studies.

Dose Formulations

Dose formulations were prepared by mixing the appropriate amount of each isomer with deionized water to achieve the desired concentrations. Dose formulations for the studies were prepared as needed and were used within 3 weeks of preparation.

For the 2-week studies, target doses were established based on published data on the acute and short-term oral toxicities of the chemicals. Drinking water solutions of the glycol ethers were formulated at concentrations estimated to deliver the target doses. These concentrations were changed during the second week of the studies to account for changes in water consumption and weight gain. The actual doses achieved differed widely from the target doses because of poor palatability.

Stability studies conducted by MRI on dose formulations indicated that doses of 20,000 ppm 2-methoxyethanol and 10,000 ppm 2-butoxyethanol were stable for up to 3 weeks when stored in the dark at 5° C in sealed glass containers. 2-Methoxyethanol- or 2-butoxyethanol-dosed water stored in rodent drinking bottles was also found to be stable for at least 4 days. Dose formulations of 4000 ppm 2-ethoxyethanol were found to be stable for 3 weeks in the dark at room temperature in sealed glass containers. Dose formulations for all studies were stored in the dark at 4° ± 3° C. Results of all dose formulation analyses were within 10% of theoretical concentrations with one exception that led to a dosing error. The 3000 ppm stop-exposure study dose of 2-methoxyethanol mixed on 23 August 1988 was analyzed on 6 September 1988 and found to contain 5820 ppm 2-methoxyethanol. All cages in this dose group were presumed to have been misdosed for 3 days.

Toxicity Study Designs

BASE STUDIES

Male and female F344/N rats and B6C3F$_1$ mice used in these studies were obtained from Taconic Farms (Germantown, NY). Rats and mice were shipped to the study laboratory at approximately 4 to 5 weeks of age, quarantined for 1 to 2 weeks, and then placed on study at about 5 to 7 weeks of age. Rats for the 13-week base studies were received in two shipments (one for the base studies and one for the clinical pathology studies). In the 2-week studies, two animals per sex per species were examined for disease and parasites; no abnormalities were found. In the 13-week base studies, blood samples were collected from rats and mice and analyzed for viral antibody titers (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). In all 13-week studies but the 2-methoxyethanol study in mice, blood samples were collected from five males and five females at the start of the studies; in the 2-methoxyethanol study in mice, samples were collected from five males at the start of the study and from four females at study end. Blood samples were also collected from five males and five females at the end of the 2-ethoxyethanol and 2-butoxyethanol studies in rats. The results of all analyses were negative. Additional details concerning study design and performance are listed in Table 2.

Rats were housed five animals per cage and mice were housed individually for the 2-week and 13-week studies. Animals were kept in polycarbonate cages lined with heat-treated hardwood chips and covered with polyester fiber cage-top filters. Cages were rotated within racks and racks were rotated within rooms on a weekly schedule. Animal rooms were maintained at 60° to 77° F and 20% to 70% relative humidity with at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day. Feed and drinking water solutions were available *ad libitum.*

In the 2-week studies of each isomer, groups of five rats and five mice per sex per dose level were administered the study chemical in drinking water available *ad libitum.* Target dose levels selected for rats and mice receiving 2-methoxyethanol were 0, 200, 400, 600, 1000, or 1200 mg/kg body weight. Target dose levels for rats and mice receiving 2-ethoxyethanol were 0, 300, 600, 900, 1500, or 2500 mg/kg body weight. Target dose levels for rats and mice receiving 2-butoxyethanol were 0, 100, 150, 250, 400, or 650 mg/kg body weight. Sufficient chemical was added to the drinking water solutions to achieve these doses based on historical water consumption data.

Dose selections for each 13-week study were based on the results of the respective 2-week studies. Due to a dose-related decrease in water consumption in the 2-week studies, the test articles were administered at a constant concentration (ppm) in the 13-week studies rather than on a mg/kg body weight basis. In the 13-week studies of each isomer, 10 rats and 10 mice per sex per dose level were administered test articles in drinking water. In the 2-methoxyethanol studies, rats received 0, 750, 1500, 3000, 4500, or 6000 ppm and mice received 0, 2000, 4000, 6000, 8000, or 10,000 ppm. In the 2-ethoxyethanol studies, rats received 0, 1250, 2500, 5000, 10,000, or 20,000 ppm and mice received 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm. In the 2-butoxyethanol studies, rats and mice received 0, 750, 1500, 3000, 4500, or 6000 ppm. Drinking water was available *ad libitum* for 13 weeks.

Complete necropsies were performed on all base-study animals in the 2-week and 13-week studies. The following organs from rats and mice were weighed: heart, right kidney, liver, lung, thymus, and right testis. Organs and tissues were examined for gross lesions and were fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

For animals in the 2-week studies, complete histopathologic examinations were performed only on those organs showing gross evidence of lesions. For animals in the 13-week studies, complete histopathologic examinations of protocol-required tissues were performed on all control animals, all animals in the highest dose group with at least 60% survivors at the time of sacrifice, and all animals in higher dose groups inclusive of early deaths and survivors. Gross lesions and selected tissues were examined in the lower dose groups to a no-observed-effect level. Tissues examined microscopically are listed in Table 2.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. The results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

SUPPLEMENTAL EVALUATIONS

Clinical Pathology

In the 13-week studies of ethylene glycol ethers, hematology and clinical chemistry evaluations were performed on supplemental rats at Weeks 1 and 3 (10 males and 10 females per dose group per time point for each chemical) and on base-study rats at study termination (Week 13). Urine samples were collected from base-study rats for evaluation at the end of the study. Animals were administered 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol in drinking water available *ad libitum*. Dose levels were 0, 750, 1500, 3000, 4500, or 6000 ppm for rats receiving 2-methoxyethanol or 2-butoxyethanol and 0, 1250, 2500, 5000, 10,000, or 20,000 ppm for rats receiving 2-ethoxyethanol.

At all time points, rats were anesthetized with 70% CO_2:30% O_2, and blood samples were collected from the retroorbital sinus using capillary tubes. Blood samples were placed in EDTA tubes for hematologic analyses and in plain tubes devoid of an anticoagulant for clinical chemistry analyses. After blood samples were collected, bone marrow cells were collected from the right femur of rats for determination of total nucleated cell counts (Thompson *et al.*, 1991). On Day 90, rats were placed individually in metabolism cages for the collection of 16-hour urine samples. During this period, animals had access to feed but not water. Samples were collected in tubes that were immersed in an ice water bath.

Hematologic determinations were performed with a Series 7000 cell counter and a Series 810 whole blood platelet analyzer (Baker Instruments, Allentown, PA). Reticulocyte counts were determined by microscopic examination of blood smears that had been incubated with new methylene blue. Leukocyte differentials were calculated from percentages of cell types determined from microscopic examination of Wright's-stained blood smears. Methemoglobin concentrations were measured using a spectrophotometric method (Evelyn and Malloy, 1938). Clinical chemistry variables were measured with a Cobas Fara chemistry analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ). Clinical pathology variables evaluated in the 13-week studies are listed in Table 2.

Sperm Morphology and Vaginal Cytology in Rats and Mice

Vaginal cytology and sperm morphology evaluations were performed on rats (10 animals per sex per dose level) and mice (10 animals per sex per dose level) from the 13-week studies. Male rats receiving 2-methoxyethanol at dose levels of 0, 750, 1500, or 3000 ppm and female rats receiving 2-methoxyethanol at dose levels of 0, 1500, 3000, or 4500 ppm were evaluated. Male mice receiving 0, 2000, 4000, or 6000 ppm 2-methoxyethanol and female mice receiving 0, 6000, 8000, or 10,000 ppm 2-methoxyethanol were evaluated. Rats administered 2-ethoxyethanol at dose levels of 0, 2500, 5000, or 10,000 ppm and mice administered 2-ethoxyethanol at dose levels of 0, 5000, 10,000, or 20,000 ppm were evaluated. Also, rats and mice administered 0, 3000, 4500, or 6000 ppm 2-butoxyethanol were evaluated. Methods were those described by Morrissey *et al.* (1988). Briefly, for the 7 days prior to sacrifice, the vaginal vaults of 10 females of each species per dose group were lavaged and the aspirated lavage fluid and cells were stained with Toluidine Blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (*i.e.*, diestrus, proestrus, estrus, and metestrus).

Sperm morphology was evaluated at necropsy in the following manner. The left epididymis was isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the epididymal tail. The sperm effluxing from the incision were dispersed in the buffer on the slides and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide.

Following completion of sperm motility estimates, each cauda epididymis was placed in buffered saline solution (0.9%). Cauda were gently minced and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were enumerated using a hemacytometer.

STOP-EXPOSURE STUDIES IN MALE RATS

Dose selections for the stop-exposure studies were based on the results of the 2-week studies of 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol. In each stop-exposure study, 30 male rats per dose group were administered 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol in drinking water. Dose levels for rats receiving 2-methoxyethanol were 0, 1500, 3000, or 6000 ppm (Note: During Week 5 of the stop-exposure study of 2-methoxyethanol, rats in the 3000 ppm dose group received 5820 ppm 2-methoxyethanol for approximately 3 days). Dose levels for rats receiving 2-ethoxyethanol were 0, 5000, 10,000, or 20,000 ppm. Dose levels for rats receiving 2-butoxyethanol were 0, 1500, 3000, or 6000 ppm (Note: During Week 6 of the stop-exposure study of 2-butoxyethanol, rats in the 1500 ppm dose group received 2500 ppm 2-ethoxyethanol). Test articles were administered daily for 60 days in drinking water that was available *ad libitum*. At the end of the treatment period, 10 rats per dose group were killed, except in the case of early deaths. If lesions were found at the 60-day necropsy, half of the remaining animals were killed after a 30-day recovery period, and the other half were killed after a 56-day recovery period. Animals were housed five per cage in the same room as the animals in the 13-week studies. At necropsy, the testes and epididymides were removed. The right testis and epididymis were weighed, and the testes and the caput and cauda of the left epididymis were examined microscopically. Organs for rats in the 30- and 56-day recovery groups in the 2-butoxyethanol stop-exposure study were not processed for histology because no microscopic lesions attributable to chemical exposure were found after the 60-day exposure period.

TABLE 2 Experimental Design and Materials and Methods in the Drinking Water Studies of Ethylene Glycol Ethers

EXPERIMENTAL DESIGN

Study Laboratory

EG&G Mason Research Institute (Worcester, MA)

Size of Study Groups

2-Week Studies: five males and five females per species per dose group
13-Week Studies:
Base Studies: 10 males and 10 females per species per dose group
Clinical Pathology Study: 20 male and 20 female rats per dose group
Stop-Exposure Studies:
30 male rats per dose group

Route of Administration

Drinking water

Doses/Duration of Dosing

2-Week Studies:
2-Methoxyethanol:
Rats and mice: 0, 200, 400, 600, 1000, or 1200 mg/kg daily for 14 days
2-Ethoxyethanol:
Rats and mice: 0, 300, 600, 900, 1500, or 2500 mg/kg daily for 14 days
2-Butoxyethanol:
Rats and mice: 0, 100, 150, 250, 400, or 650 mg/kg daily for 14 days
13-Week Studies:
Base Studies:
2-Methoxyethanol:
Rats: 0, 750, 1500, 3000, 4500, or 6000 ppm daily for 13 weeks
Mice: 0, 2000, 4000, 6000, 8000, or 10,000 ppm daily for 13 weeks
2-Ethoxyethanol:
Rats: 0, 1250, 2500, 5000, 10,000, or 20,000 ppm daily for 13 weeks
Mice: 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm daily for 13 weeks
2-Butoxyethanol:
Rats and mice: 0, 750, 1500, 3000, 4500, or 6000 ppm daily for 13 weeks
Clinical Pathology Studies:
Same as 13-week base studies; daily for 21 days
Stop-Exposure Studies:
2-Methoxyethanol: 0, 1500, 3000, or 6000 ppm daily for 60 days
2-Ethoxyethanol: 0, 5000, 10,000, or 20,000 ppm daily for 60 days
2-Butoxyethanol: 0, 1500, 3000, or 6000 ppm daily for 60 days

Date of First Dose

2-Week Studies:
2-Methoxyethanol:
Rats: 21 March 1988 (males), 22 March 1988 (females)
Mice: 23 March 1988 (males), 24 March 1988 (females)
2-Ethoxyethanol:
Rats: 18 January 1988 (males), 19 January 1988 (females)
Mice: 20 January 1988 (males), 21 January 1988 (females)
2-Butoxyethanol:
Rats: 22 February 1988 (males), 23 February 1988 (females)
Mice: 24 February 1988 (males), 25 February 1988 (females)
13-Week Studies:
Base Studies:
2-Methoxyethanol:
Rats: 19 July 1988 (males), 21 July 1988 (females)
Mice: 12 July 1988 (males), 14 July 1988 (females)
2-Ethoxyethanol:
Rats: 3 May 1988 (males), 5 May 1988 (females)
Mice: 26 April 1988 (males), 28 April 1988 (females)
2-Butoxyethanol:
Rats: 14 June 1988 (males), 16 June 1988 (females)
Mice: 21 June 1988 (males), 23 June 1988 (females)
Clinical Pathology Studies:
2-Methoxyethanol: 5 October 1988 (males), 6 October 1988 (females)
2-Ethoxyethanol: 4 August 1988 (males), 5 August 1988 (females)
2-Butoxyethanol: 31 August or 1 September 1988 (males), 1 or 2 September 1988 (females)

TABLE 2 Experimental Design and Materials and Methods in the Drinking Water Studies of Ethylene Glycol Ethers (continued)

Date of First Dose (continued)	Stop-Exposure Studies: *2-Methoxyethanol*: 22 July 1988 *2-Ethoxyethanol*: 6 May 1988 *2-Butoxyethanol*: 17 June 1988
Date of Last Dose	2-Week Studies: *2-Methoxyethanol*: Rats: 4 April 1988 (males), 5 April 1988 (females) Mice: 6 April 1988 (males), 7 April 1988 (females) *2-Ethoxyethanol*: Rats: 1 February 1988 (males), 2 February 1988 (females) Mice: 3 February 1988 (males), 4 February 1988 (females) *2-Butoxyethanol*: Rats: 7 March 1988 (males), 8 March 1988 (females) Mice: 9 March 1988 (males), 10 March 1988 (females) 13-Week Studies: Base Studies: *2-Methoxyethanol*: Rats: 18-19 October 1988 (males), 20-21 October 1988 (females) Mice: 11-12 October 1988 (males), 13-14 October 1988 (females) *2-Ethoxyethanol*: Rats: 2-3 August 1988 (males), 4-5 August 1988 (females) Mice: 26-27 July 1988 (males), 28-29 July 1988 (females) *2-Butoxyethanol*: Rats: 13-14 September 1988 (males), 15-16 September 1988 (females) Mice: 20-21 September 1988 (males), 22-23 September 1988 (females) Clinical Pathology Studies: *2-Methoxyethanol*: 10 or 26 October 1988 (males), 11 or 27 October 1988 (females) *2-Ethoxyethanol*: 9 or 25 August 1988 (males), 10 or 26 August 1988 (females) *2-Butoxyethanol*: 6 or 21 September 1988 (males), 7 or 22 September 1988 (females) Stop-Exposure Studies: *2-Methoxyethanol*: 20 September 1988 *2-Ethoxyethanol*: 5 July 1988 *2-Butoxyethanol*: 16 August 1988
Necropsy Dates	2-Week Studies: *2-Methoxyethanol*: Rats: 4 April 1988 (males), 5 April 1988 (females) Mice: 6 April 1988 (males), 7 April 1988 (females) *2-Ethoxyethanol*: Rats: 1 February 1988 (males), 2 February 1988 (females) Mice: 3 February 1988 (males), 4 February 1988 (females) *2-Butoxyethanol*: Rats: 7 March 1988 (males), 8 March 1988 (females) Mice: 9 March 1988 (males), 10 March 1988 (females) 13-Week Studies: Base Studies: *2-Methoxyethanol*: Rats: 18-19 October 1988 (males), 20-21 October 1988 (females) Mice: 11-12 October 1988 (males), 13-14 October 1988 (females) *2-Ethoxyethanol*: Rats: 2-3 August 1988 (males), 4-5 August 1988 (females) Mice: 26-27 July 1988 (males), 28-29 July 1988 (females) *2-Butoxyethanol*: Rats: 13-14 September 1988 (males), 15-16 September 1988 (females) Mice: 20-21 September 1988 (males), 22-23 September 1988 (females)

TABLE 2 Experimental Design and Materials and Methods in the Drinking Water Studies of Ethylene Glycol Ethers (continued)

Necropsy Dates (continued)	Stop-Exposure Studies: *2-Methoxyethanol*: 20 September, 20 October, or 15 November 1988 *2-Ethoxyethanol*: 5 July, 4 August, or 30 August 1988 *2-Butoxyethanol*: 16 August or 15 September 1988
Type and Frequency of Observation	2-Week Studies: Animals were observed twice daily and were weighed at the start of the studies, at the end of Week 1, and at necropsy. Clinical observations were recorded daily. Water consumption by cage was measured two times per week. 13-Week Studies: Base Studies: Animals were observed twice daily and were weighed at the start of the studies, weekly thereafter, and at necropsy. Clinical observations were recorded weekly. Water consumption by cage was measured two times per week. Clinical Pathology Studies: Animals were observed twice daily. Stop-Exposure Studies: Same as 13-week base studies.
Necropsy and Histologic Examinations	2-Week and 13-Week Base Studies: Complete necropsies were performed on all animals in the base studies. The protocol for the 2-week studies required that only organs showing evidence of gross lesions be examined microscopically. The protocol for the 13-week studies required that tissues be examined microscopically in all control animals, all animals in the highest dose group with at least 60% survivors, and all animals in the higher dose groups (inclusive of early deaths and survivors). These tissues included: adrenal glands, bone (femur) with marrow, brain (three sections), esophagus, eyes, gallbladder (mice), gross lesions, heart/aorta, intestines (large: cecum, colon, rectum; small: duodenum, jejunum, ileum), kidneys, larynx, liver, lung/mainstem bronchi, lymph nodes (mandibular, mesenteric), mammary gland, nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pituitary gland, pharynx, preputial or clitoral glands, prostate gland, salivary glands, seminal vesicles, skin, spinal cord/sciatic nerve, spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thigh muscle, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, and vagina (SMVCE animals only). Tissues examined in the lower dose groups in the 2-week studies in rats were the testis and epididymis. In the 2-week studies in mice, no tissues were designated for examination in the lower dose groups. In the 13-week studies of 2-methoxyethanol and 2-ethoxyethanol in rats, tissues examined in the lower dose groups were bone (2-methoxyethanol), bone marrow, the epididymis (2-ethoxyethanol), liver, ovary, preputial or clitoral gland, prostate gland, seminal vesicle, spleen, stomach, testis (2-ethoxyethanol), thymus, uterus, and vagina (2-ethoxyethanol). In the 13-week study of 2-butoxyethanol in rats, bone marrow and the epididymis, liver, spleen, testis, and uterus were examined in the lower dose groups. Tissues examined for mice in the lower dose groups in the 13-week studies of 2-methoxyethanol and 2-ethoxyethanol were the adrenal gland (females), ovary (2-methoxyethanol), spleen, testis, thymus (2-methoxyethanol), and uterus (2-methoxyethanol). In the 13-week study of 2-butoxyethanol in mice, no tissues were designated for examination in the lower dose groups. Stop-Exposure Studies: Tissues examined microscopically were the testes and caput and cauda of the left epididymis.

TABLE 2 Experimental Design and Materials and Methods in the Drinking Water Studies of Ethylene Glycol Ethers (continued)

Supplemental Evaluations	Clinical Pathology Studies: 13-Week Base Studies: On Days 5 and 21, blood samples were collected from the retroorbital sinuses of rats designated for the clinical pathology studies. Week 13 analyses were conducted on samples obtained from rats in the base studies. Urinalysis was done on Week 13 samples collected overnight from the base study animals. Hematology parameters evaluated included hematocrit (HCT), hemoglobin (HGB), erythrocytes (RBCs), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets, reticulocytes, leukocyte count and differential, nucleated erythrocytes, methemoglobin, and total bone marrow cellularity. Clinical chemistry parameters evaluated included urea nitrogen (UN), creatinine, total protein, albumin, alkaline phosphatase (AP), alanine aminotransferase (ALT), creatine kinase, and bile acids. Urinalysis parameters evaluated included volume, specific gravity, and pH. Sperm Morphology and Vaginal Cytology Evaluations (13-Week Base Studies): Males were evaluated for necropsy body and reproductive tissue weights and spermatozoal data. Females were evaluated for necropsy body weight, estrous cycle length, and the percent of cycle spent in the various stages. Animals in the following dose groups were evaluated. *2-Methoxyethanol*: Rats: males, 0, 750, 1500, or 3000 ppm (10 animals per dose group), females, 0, 1500, 3000, or 4500 ppm (10 animals per dose group) Mice: males, 0, 2000, 4000, or 6000 ppm (10 animals per dose group), females, 0, 6000, 8000, or 10,000 ppm (10 animals per dose group) *2-Ethoxyethanol*: Rats: 0, 2500, 5000, or 10,000 ppm (10 animals per sex per dose group) Mice: 0, 5000, 10,000, or 20,000 ppm (10 animals per sex per dose group) *2-Butoxyethanol*: Rats and mice: 0, 3000, 4500, or 6000 ppm (10 animals per sex per dose level)
ANIMALS AND ANIMAL MAINTENANCE	
Strain and Species	F344/N Rats B6C3F$_1$ Mice
Animal Source	Taconic Farms (Germantown, NY)
Time Held Before Study	2-Week Studies: Rats: 1½ weeks Mice: 2 weeks 13-Week Studies: *2-Methoxyethanol* and *2-Butoxyethanol*: approximately 2 weeks *2-Ethoxyethanol*: rats, approximately 2 weeks; mice, approximately 1 week Stop-Exposure Studies: 2 weeks
Age When Placed on Study	2-Week Studies: 6-7 weeks 13-Week Studies: Base Studies: 5-6 weeks Clinical Pathology Studies: *2-Methoxyethanol* and *2-Butoxyethanol*: approximately 7 weeks *2-Ethoxyethanol*: 19 weeks Stop-Exposure Studies: Approximately 6 weeks
Age When Killed	2-Week Studies: *2-Methoxyethanol*: 9 weeks *2-Ethoxyethanol* and *2-Butoxyethanol*: 8 weeks 13-Week Studies: Base Studies: 18-19 weeks Stop-Exposure Studies: 15, 19, or 21 weeks

TABLE 2 Experimental Design and Materials and Methods in the Drinking Water Studies of Ethylene Glycol Ethers (continued)

Method of Animal Distribution	Animals were weighed and were randomized using a computer program.
Diet	NIH-07 Open Formula Pellets (Zeigler Brothers, Inc., Gardners, PA) and deionized water (filtered and untreated) available *ad libitum*
Animal Room Environment	Rats were housed five animals per cage and mice housed individually for all base studies. Temperature was maintained at 60° to 77° F and relative humidity at 20% to 70%, with at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day.

Genetic Toxicity Studies

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing of 2-ethoxyethanol was performed as reported by Zeiger *et al.* (1985), and testing of 2-methoxyethanol and 2-butoxyethanol was performed as reported by Zeiger *et al.* (1992). The chemicals were sent to the testing laboratories as coded aliquots. They were incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, TA1537, and TA97) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat and Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C. All of the tests were repeated using either the same or different S9 concentrations.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of the test chemical. High dose was limited by experimental design to 10,000 µg/plate. Varied concentrations of S9 were used in the tests with 2-methoxyethanol and 2-butoxyethanol.

MOUSE LYMPHOMA MUTAGENICITY TEST PROTOCOL

The experimental protocol is presented in detail by Myhr *et al.* (1985). 2-Ethoxyethanol was supplied as a coded aliquot. The high dose of 2-ethoxyethanol was limited by experimental design to 5 µL/mL. Mouse lymphoma L5178Y cells were maintained at 37° C as suspension cultures in Fischer's medium supplemented with *l*-glutamine, sodium pyruvate, pluronic F68, antibiotics, and heat-inactivated horse serum; normal cell cycling

time was approximately 10 hours. To reduce the number of spontaneously occurring trifluorothymidine-resistant cells, subcultures were exposed once to medium containing THMG (thymidine, hypoxanthine, methotrexate, glycine) for 1 day, to medium containing THG for 1 day, and to normal medium for 3 to 5 days. For cloning, horse serum content was increased and Noble agar was added.

All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in 10 mL of medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with 2-ethoxyethanol continued for 4 hours, at which time the medium plus 2-ethoxyethanol was removed and the cells were resuspended in fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine (TFT) for selection of TFT-resistant cells ($TK^{-/-}$); 600 cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C in 5% CO_2 for 10 to 12 days. This assay was initially performed without S9; if a clearly positive response was not obtained, the experiment was repeated using freshly prepared S9 from the livers of Aroclor 1254-induced Fischer 344 male rats.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1987). 2-Ethoxyethanol and 2-butoxyethanol were sent to the laboratory as coded aliquots. They were tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs) both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and at least three doses of the particular test chemical. In the SCE test, the highest testable dose of 2-butoxyethanol, in the absence of S9, was limited by toxicity to 3000 (Trial 1) and 3500 µg/mL (Trial 2); with S9, no toxicity was observed and the high dose was limited to 5000 µg/mL. In the Abs test with 2-butoxyethanol, high dose was limited to 5000 µg/mL. For 2-ethoxyethanol, the high dose was not limited by excessive toxicity or lack of solubility and reached 9510 µg/mL in both the SCE and Abs tests. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

In the standard SCE test without S9, CHO cells were incubated for approximately 26 hours with the test chemical in McCoy's 5A medium supplemented with fetal bovine serum, *l*-glutamine, and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After about 26 hours (25.5 hours for 2-ethoxyethanol), the medium containing the test chemical was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with the test chemical, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no test chemical and incubation proceeded for an additional 26 hours (25.5 hours for 2-ethoxyethanol), with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level. Because significant chemical-induced cell cycle delay was seen with 2-butoxyethanol in the absence of S9, incubation time was lengthened to ensure a sufficient number of scorable (second-division metaphase) cells.

In the Abs test without S9, cells were incubated in McCoy's 5A medium with the test chemical for 8.5 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with the test chemical and S9 for 2 hours, after which the treatment medium was removed and the cells incubated for 8.5 to 10.5 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test: because cell cycle delay was anticipated for 2-butoxyethanol in the absence of S9, the incubation period was extended in two of the three trials.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. One or two hundred first-division metaphase cells were scored at each dose level. Classes of aberrations included "simple" (breaks and terminal deletions), "complex" (rearrangements and translocations), and "other" (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

DROSOPHILA MELANOGASTER SEX-LINKED RECESSIVE LETHAL TEST PROTOCOL

The assays for induction of sex-linked recessive lethal (SLRL) mutations were performed with adult flies as described in Valencia *et al.* (1985) and Mason *et al.* (1992). 2-Ethoxyethanol was supplied as a coded aliquot. It was assayed in the SLRL test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because no positive response was obtained, it was retested by injection into adult males.

To administer 2-ethoxyethanol by injection, a glass Pasteur pipette was drawn out in a flame to a microfine filament and the tip was broken off to allow delivery of the test solution. Injection was performed either manually, by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution (0.2 to 0.3 µL) to slightly distend the abdomen of the fly, or by attaching the pipette to a microinjector which automatically delivers a calibrated volume. Flies were anaesthetized with ether and immobilized on a strip of tape. Injection into the thorax, under the wing, was performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of 2-ethoxyethanol at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. For the SLRL test, oral exposure was achieved by allowing Canton-S males to feed for 72 hours on a solution of 2-ethoxyethanol in 5% sucrose. In the injection experiments, 24- to 72-hour old Canton-S males were treated with a solution of 2-ethoxyethanol dissolved in saline and allowed to recover for 24 hours. Treated males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case, sample sperm from successive matings were treated at successively earlier post-meiotic stages). F_1 heterozygous females were mated with their siblings and then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event, and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution.) A cluster was identified in the feeding experiment in test 2 and all data from the male in question were discarded. Presumptive lethal mutations were identified as vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

Statistical Methods

ANALYSIS OF CONTINUOUS VARIABLES

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) or Dunnett (1955). Clinical chemistry and hematology data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value. The extreme values chosen by the statistical test were subject to approval by NTP personnel. In addition, values indicated by the laboratory report as being inadequate due to technical problems were eliminated from the analysis.

ANALYSIS OF VAGINAL CYTOLOGY DATA

Because the data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels.

ANALYSIS OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

A positive response in the *Salmonella typhimurium* assay was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response was defined as an increase in revertants that was not dose related, not reproducible, or of insufficient magnitude to support a

determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

ANALYSIS OF MOUSE LYMPHOMA MUTAGENICITY DATA

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Caspary *et al.* (1988). All data were evaluated statistically for both trend and peak responses. Both responses had to be significant ($P \leq 0.05$) for a chemical to be considered capable of inducing TFT resistance; a single significant response led to a "questionable" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call.

ANALYSIS OF CHO CELL CYTOGENETICS DATA

For the SCE data, statistical analyses were conducted on the slopes of the dose-response curves (Galloway *et al.*, 1985). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose, along with a trend P-value less than 0.025, was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.05$) in the absence of any responses reaching 20% above background led to a call of equivocal (Galloway *et al.*, 1985).

Chromosomal aberration data are presented as percentage of cells with aberrations. Statistical analyses were conducted on both the dose-response curve and individual dose points (Galloway *et al.*, 1985). For a single trial, a statistically significant ($P<0.05$) difference for one dose point and a significant trend ($P<0.005$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend, in the absence of a statistically significant increase at any one dose point, led to a conclusion of equivocal activity.

ANALYSIS OF *DROSOPHILA MELANOGASTER* DATA

Sex-linked recessive lethal data were analyzed by simultaneous comparison with the concurrent and historical controls using a normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered positive if the P-value was less than or equal to 0.01 and the mutation frequency in the tested group was greater than 0.10%, or if the P-value was less than or equal to 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if (a) the P-value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or (b) the P-value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if the P-value was greater than or equal to 0.10 or if the frequency in the treatment group was less than 0.10%.

Quality Assurance

The animal studies of the ethylene glycol ethers were performed in compliance with United States FDA Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of EG&G Mason Research Institute performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

RESULTS

2-Week Drinking Water Studies in F344/N Rats

No rats in the 2-week study of 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol died or were killed before the end of the study (Table 3). The final mean body weights and mean body weight changes of males and females receiving target doses of 600, 1000, or 1200 mg/kg 2-methoxyethanol were notably lower than those of the control group. In the 2-ethoxyethanol study, the final mean body weights and mean body weight changes of male rats in the treated groups were variably lower than those of the control group. For female rats receiving target doses of 1500 or 2500 mg/kg 2-ethoxyethanol for 2 weeks, mean final body weights and mean body weight changes were notably lower than those of the control group. In the 2-butoxyethanol study, the final mean body weights and body weight gains of male rats in all treated groups were similar to those of the control group. However, the final mean body weight of females receiving a target dose of 650 mg/kg 2-butoxyethanol was lower than that of the control group.

In the 2-week studies of ethylene glycol ethers, dose-related decreases in mean water consumption were noted for male and female rats treated with 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol (Table 3). Average compound consumption increased in a dose-related manner for male and female rats treated with the ethylene glycol ethers. However, because of reduced water consumption, doses were below targeted levels for males and females treated with 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol.

TABLE 3 Survival, Weight Gain, Water Consumption, and Compound Consumption in F344/N Rats in the 2-Week Drinking Water Studies of Ethylene Glycol Ethers

Target Dose (mg/kg)	Survival[1]	Mean Body Weight (grams) Initial	Final	Change[2]	Final Weight Relative to Controls (%)[3]	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
MALE							
2-Methoxyethanol							
0	5/5	167	204	37		23.2	
200	5/5	167	212	45	104	20.4	116
400	5/5	168	201	34	99	17.7	206
600	5/5	168	180	12	88	14.7	273
1000	5/5	172	148	-24	73	11.6	393
1200	5/5	170	135	-35	66	9.9	418
2-Ethoxyethanol							
0	5/5	107	167	60		17.4	
300	5/5	110	152	42	91	16.5	200
600	5/5	108	148	40	89	14.9	357
900	5/5	108	156	48	93	16.2	572
1500	5/5	107	159	52	95	15.8	919
2500	5/5	107	135	28	81	13.7	1582
2-Butoxyethanol							
0	5/5	105	169	64		19.1	
100	5/5	108	171	63	101	18.6	73
150	5/5	108	167	59	99	18.3	108
250	5/5	108	175	67	104	18.1	174
400	5/5	107	178	70	105	16.4	242
650	5/5	108	173	65	102	13.8	346

TABLE 3 Survival, Weight Gain, Water Consumption, and Compound Consumption in F344/N Rats in the 2-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

Target Dose (mg/kg)	Survival	Mean Body Weight (grams)			Final Weight Relative to Controls (%)	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change			
FEMALE							
2-Methoxyethanol							
0	5/5	133	156	23		18.6	
200	5/5	132	150	18	96	14.8	113
400	5/5	132	147	15	94	11.3	175
600	5/5	132	130	–2	83	9.4	231
1000	5/5	133	110	–23	71	6.7	297
1200	5/5	134	111	–23	71	6.2	326
2-Ethoxyethanol							
0	5/5	108	139	31		18.1	
300	5/5	109	135	27	97	15.7	192
600	5/5	109	130	21	94	14.5	360
900	5/5	109	136	27	98	14.8	526
1500	5/5	112	130	18	94	13.6	824
2500	5/5	111	115	4	82	11.8	1281
2-Butoxyethanol							
0	5/5	95	129	34		15.3	
100	5/5	93	133	40	103	15.9	77
150	5/5	93	137	44	106	14.5	102
250	5/5	93	135	41	104	12.8	152
400	5/5	93	136	43	105	11.1	203
650	5/5	92	116	23	89	7.8	265

[1] Number surviving at 2 weeks/number of animals per dose group.
[2] Mean weight change of the survivors.
[3] (Dosed group mean/control group mean) × 100.

Dehydration, abnormal posture, and thin appearance were noted for males treated with the two highest concentrations of 2-methoxyethanol, and all females in the three highest 2-methoxyethanol dose groups were dehydrated. Abnormal posture and thin appearance were observed in all females in the two highest dose groups, and all females receiving the highest dose of 2-methoxyethanol were emaciated by the end of the study. No clinical signs of toxicity were observed for males or females treated with 2-ethoxyethanol or 2-butoxyethanol.

2-Methoxyethanol: In the 2-week study of 2-methoxyethanol in rats, most changes in absolute and relative organ weights were related to low final body weights, excluding changes in thymus and testis weights. Absolute and relative thymus weights decreased in a dose-related fashion for males and females as did absolute and relative testis weights for males (Table 4).

In the 2-methoxyethanol study, chemical-related gross lesions were present only in rats in the two highest dose groups. Gross lesions were observed in the forestomach and mesenteric lymph nodes of male and female rats receiving the highest concentration of 2-methoxyethanol and in female rats receiving the targeted dose of 1000 mg/kg. Microscopic changes in the forestomach that corresponded to the gross lesions included hemorrhage and edema of the mucosa and focal necrosis and ulceration of the squamous epithelium. Mild hyperplasia of the forestomach squamous mucosa was also present and was generally associated with the focal areas of necrosis or ulceration. Sinusoidal congestion, hemorrhage, and erythrophagocytosis were present in the mesenteric lymph nodes, which appeared enlarged or reddened at necropsy. In addition to chemical-related gross lesions, the testis and epididymis from all dosed and control rats were examined microscopically. Degeneration was clearly present in the testis of male rats in all but the lowest dose group. This degeneration consisted of moderate to marked loss of germinal epithelium and the presence of multinucleated spermatid giant cells and cell debris in the lumen of seminiferous tubules. In male rats in the three highest dose groups, the lumen of the epididymis contained necrotic cells and cell debris and only a few spermatozoa. Degeneration was of mild severity at the targeted 400 mg/kg dose level, and in one of five rats administered the lowest dose of 2-methoxyethanol, there was minimal degeneration of the testes.

TABLE 4 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 2-Week Drinking Water Study of 2-Methoxyethanol[1]

	Target Dose (mg/kg)					
	0	200	400	600	1000	1200
MALE						
n	5	5	5	5	5	5
Necropsy body wt	204	212	201	180**	148**	135**
Right testis						
Absolute	1.235	1.182	0.667**	0.429**	0.372**	0.316**
Relative	6.07	5.59	3.29**	2.38**	2.51**	2.35**
Thymus						
Absolute	0.362	0.193**	0.095**	0.097**	0.059**	0.059**
Relative	1.77	0.91**	0.48**	0.54**	0.40**	0.43**
FEMALE						
n	5	5	5	5	5	5
Necropsy body wt	156	150	147**	130**	110**	111**
Thymus						
Absolute	0.320	0.153**	0.089**	0.066**	0.066**	0.051**
Relative	2.05	1.02**	0.60**	0.51**	0.61**	0.46**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.
** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

2-Ethoxyethanol: Excluding changes in thymus and testis weights, the majority of changes in absolute and relative organ weights for rats in the 2-week study of 2-ethoxyethanol were related to low final body weights. Dose-related decreases were noted for the absolute and relative thymus weights of males and females and the absolute and relative testis weights of males (Table 5).

There were no chemical-related gross lesions in male or female rats in the 2-week study of 2-ethoxyethanol. At the end of the study, the testis and epididymis from all male rats were evaluated microscopically. Degeneration of the seminiferous tubules was present in males in the two highest dose groups. Morphologic features of testicular degeneration were similar to those described for the 2-methoxyethanol study. At the highest dose, the severity of degeneration ranged from moderate to marked; at the next dose level, the severity ranged from minimal to mild. No testicular effects were seen in animals in the three lowest dose groups.

TABLE 5 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 2-Week Drinking Water Study of 2-Ethoxyethanol[1]

	Target Dose (mg/kg)					
	0	300	600	900	1500	2500
MALE						
n	5	5	5	5	5	5
Necropsy body wt	167	152**	148**	156**	159**	135**
Right testis						
Absolute	1.019	0.983	0.958	0.995	0.785**	0.395**
Relative	6.09	6.46	6.47	6.39	4.95**	2.93**
Thymus						
Absolute	0.404	0.370	0.273**	0.294**	0.229**	0.089**
Relative	2.41	2.43	1.84*	1.87*	1.44**	0.66**
FEMALE						
n	5	5	5	5	5	5
Necropsy body wt	139	135	130	136	130*	115**
Thymus						
Absolute	0.407	0.370	0.286**	0.247**	0.158**	0.075**
Relative	2.94	2.73	2.20**	1.83**	1.21**	0.65**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.
* Significantly different (P≤0.05) from the control group by Williams' test.
** Significantly different (P≤0.01) from the control group by Williams' test.

2-Butoxyethanol: Changes in organ weights were minimal in the 2-week study of 2-butoxyethanol in rats. Slight decreases were noted for the absolute and relative thymus weights of high-dose female rats, but the absolute and relative thymus and testis weights of male rats were not affected by 2-butoxyethanol treatment (Table 6). In the 2-week study of 2-butoxyethanol, there were no chemical-related gross lesions in male or female rats. Microscopic examination was limited to the testis and epididymis of dosed and control rats; there were no chemical-related microscopic lesions.

TABLE 6 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 2-Week Drinking Water Study of 2-Butoxyethanol[1]

	Target Dose (mg/kg)					
	0	100	150	250	400	650
MALE						
n	5	5	5	5	5	5
Necropsy body wt	169	171	167	175	178	173
Right testis						
Absolute	1.057	1.063	1.078	1.042	1.080	1.043
Relative	6.26	6.22	6.45	5.96	6.07	6.05
Thymus						
Absolute	0.425	0.394	0.408	0.422	0.425	0.393
Relative	2.52	2.31	2.44	2.42	2.39	2.28
FEMALE						
n	5	5	5	5	5	5
Necropsy body wt	129	133	137	135	136	116**
Thymus						
Absolute	0.356	0.376	0.396	0.354	0.357	0.292
Relative	2.75	2.83	2.91	2.64	2.63	2.54

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight. Absolute and relative organ weights are not significant by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test.

For the 13-week studies of the ethylene glycol ethers in rats, chemical administration was changed from a mg/kg basis to a constant ppm in the drinking water. The maximum concentrations used for 2-ethoxyethanol and 2-butoxyethanol were somewhat higher than the doses that were found to affect water consumption and cause minimal toxicity in the 2-week studies. For 2-methoxyethanol, the highest doses chosen (4500 and 6000 ppm) were too high when considering the marked body weight effects seen in the 2-week studies. A high dose of 3000 ppm would have been more appropriate.

13-Week Drinking Water Studies in F344/N Rats

In the 2-methoxyethanol study in rats, eight males and five females in the 4500 ppm groups and all males and females in the 6000 ppm groups died or were killed prior to scheduled termination (Table 7). In the 2-ethoxyethanol study, five males and seven females in the 20,000 ppm groups died or were killed early; due to the high mortality at this exposure level, the remaining male and female rats in the 20,000 ppm groups were removed from treatment during Week 9 of the study. No rats treated with 2-butoxyethanol died or were killed before the end of the 13-week study.

The final mean body weights for males and females receiving 1500 to 4500 ppm 2-methoxyethanol were notably lower than values for the control group. Body weight analyses were not performed for male or female rats in the 6000 ppm groups due to 100% mortality. In the 13-week study of 2-ethoxyethanol, males dosed with 10,000 or 20,000 ppm and females dosed with 5000 to 20,000 ppm had notably decreased final mean body weights when compared to the control group values. Mean body weight gains for males and females receiving 5000 to 20,000 ppm 2-ethoxyethanol were also notably lower than those of the control groups (Figures 1-3). The final mean body weights and mean weight gains for male and female rats treated with 4500 or 6000 ppm 2-butoxyethanol were notably less than the control values.

In the 13-week study of 2-methoxyethanol, decreases in mean water consumption were noted for males and females in the 3000 and 6000 ppm groups as well as for females in the 1500 ppm group (Table 7). For male and female rats treated with 2-ethoxyethanol or 2-butoxyethanol in the drinking water, average daily water consumption decreased, with a dose-related decrease occurring in females administered 2-butoxyethanol.

Average compound consumption increased in a dose-related manner for male and female rats treated with the ethylene glycol ethers for 13 weeks. However, in rats treated with 2-butoxyethanol, compound consumption generally decreased over the course of the study because of a decrease in water consumption.

TABLE 7 Survival, Weight Gain, Water Consumption, and Compound Consumption in F344/N Rats in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers

Dose (ppm)	Survival[1]	Mean Body Weight (grams) Initial	Final	Change[2]	Final Weight Relative to Controls (%)[3]	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
MALE							
2-Methoxyethanol							
0	10/10	129	311	182		21.2	
750	10/10	132	294	163	95	20.8	71
1500	10/10	127	259	132	81	21.4	165
3000	10/10	132	218	86	70	18.9	324
4500	2/10[4]	130	136	16	44	21.5	715
6000	0/10[4]	124	—	—	—	16.5	806
2-Ethoxyethanol							
0	10/10	142	333	191		21.2	
1250	10/10	142	331	189	99	20.7	109
2500	10/10	146	325	179	98	19.4	205
5000	10/10	144	315	171	95	18.3	400
10,000	10/10	142	268	127	80	16.6	792
20,000	5/10[5]	143	204	61	61	18.4	2240
2-Butoxyethanol							
0	10/10	137	297	160		22.3	
750	10/10	139	306	167	103	20.9	69
1500	10/10	135	308	173	104	19.6	129
3000	10/10	138	295	157	99	20.5	281
4500	10/10	137	277	140	93	17.7	367
6000	10/10	138	260	122	88	16.4	452

TABLE 7 Survival, Weight Gain, Water Consumption, and Compound Consumption in F344/N Rats in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

Dose (ppm)	Survival	Mean Body Weight (grams)			Final Weight Relative to Controls (%)	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change			
FEMALE							
2-Methoxyethanol							
0	10/10	114	194	79		15.6	
750	10/10	116	194	78	100	14.9	70
1500	10/10	114	174	60	90	13.5	135
3000	10/10	114	148	34	76	13.4	297
4500	5/10[6]	115	153	37	79	16.3	546
6000	0/10[7]	115	—	—	—	13.2	785
2-Ethoxyethanol							
0	10/10	123	197	74		17.9	
1250	10/10	123	194	71	98	16.3	122
2500	10/10	124	190	66	96	16.2	247
5000	10/10	127	186	59	94	14.8	466
10,000	10/10	126	171	45	89	12.4	804
20,000	3/10[8]	126	185	59	94	14.6	2061
2-Butoxyethanol							
0	10/10	110	187	77		18.8	
750	10/10	110	188	78	101	17.1	82
1500	10/10	109	185	76	99	15.5	151
3000	10/10	107	180	73	96	15.2	304
4500	10/10	112	164	52	88	11.8	363
6000	10/10	103	150	47	80	10.7	470

[1] Number surviving at 13 weeks/number of animals per dose group.
[2] Mean weight change of the survivors.
[3] (Dosed group mean/control group mean) × 100.
[4] All deaths occurred during the first 5 weeks of dosing.
[5] Week of death: 8, 8, 9, 9, 9.
[6] Week of death: unavailable.
[7] All deaths occurred during the first 7 weeks of dosing.
[8] Week of death: 5, 5, 6, 6, 7, 8, 9.

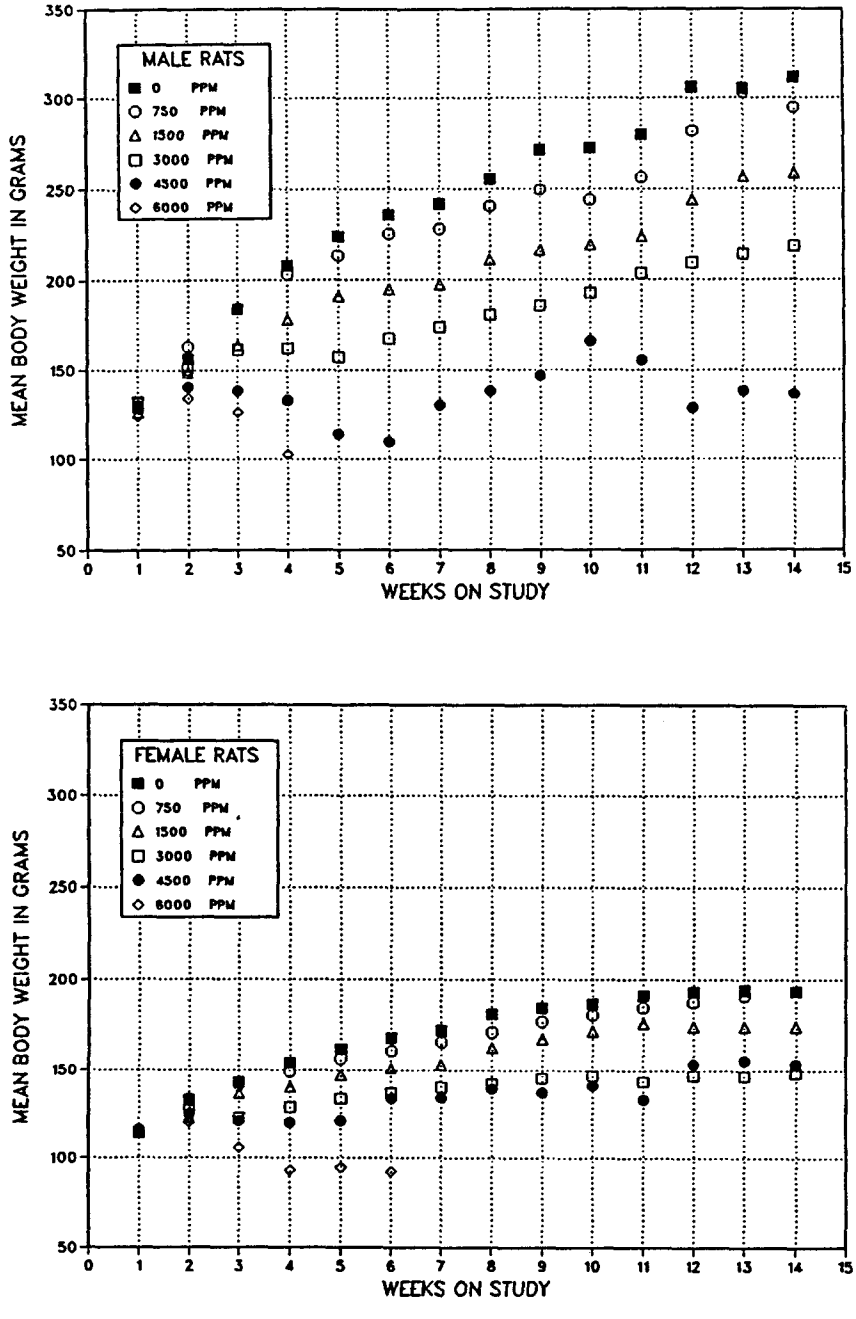

FIGURE 1 Body Weights of F344/N Rats Administered 2-Methoxyethanol in Drinking Water for 13 Weeks

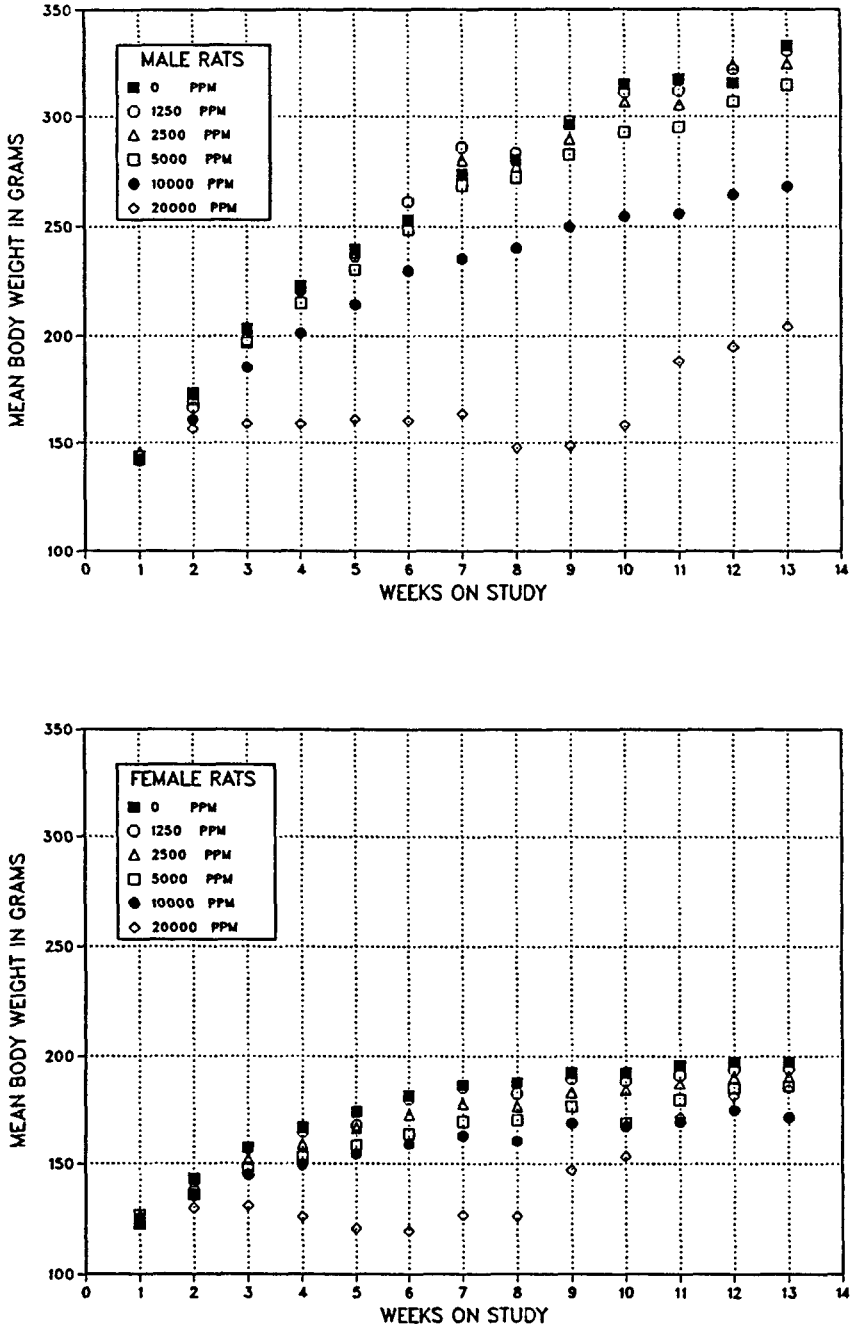

FIGURE 2 **Body Weights of F344/N Rats Administered 2-Ethoxyethanol in Drinking Water for 13 Weeks**

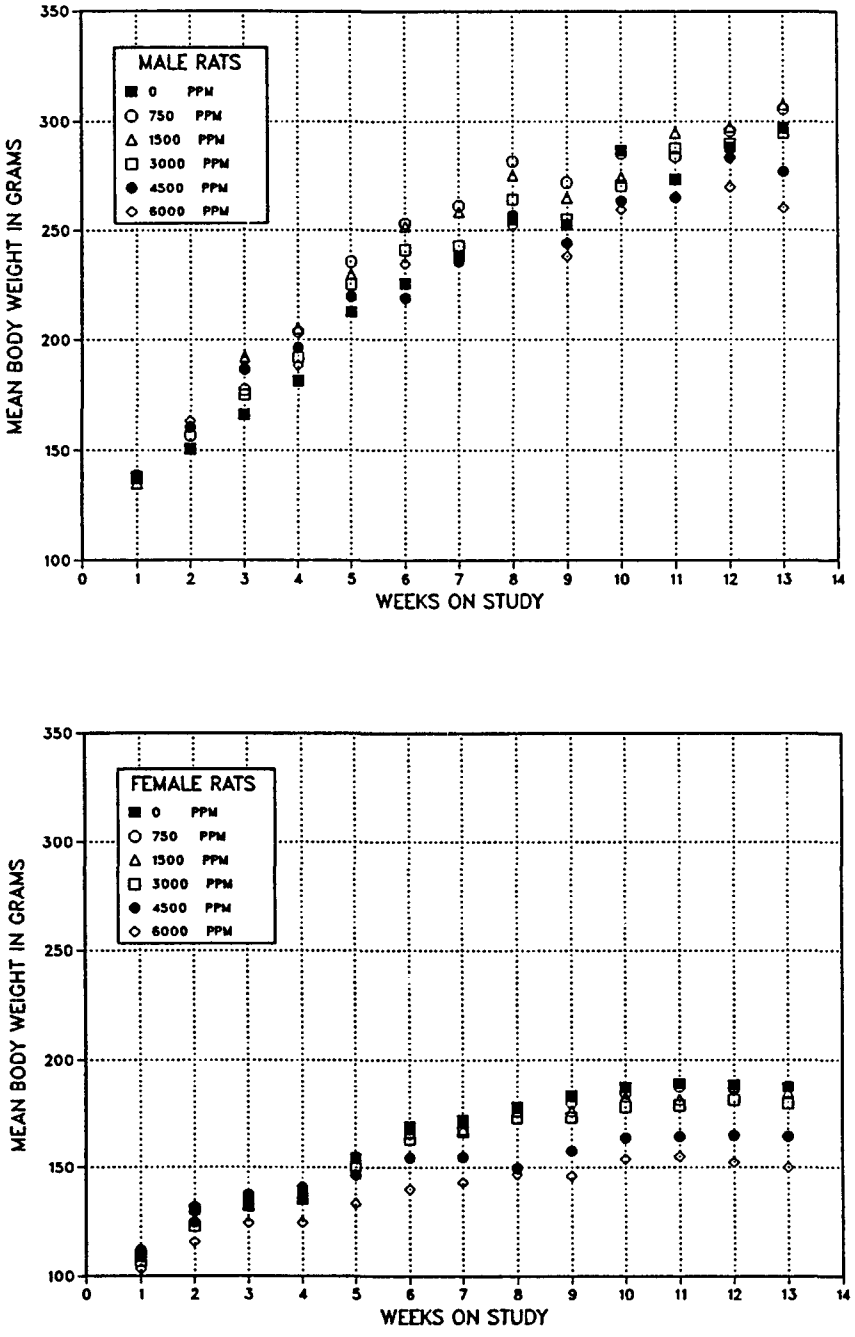

FIGURE 3 Body Weights of F344/N Rats Administered 2-Butoxyethanol in Drinking Water for 13 Weeks

For male and female rats dosed with 2-methoxyethanol, clinical signs of toxicity considered to be chemical related included tremors, diarrhea, emaciation, abnormal posture, pallor, tachypnea, hypoactivity, and comatose state. Clinical signs noted for male and female rats treated with 2-ethoxyethanol were emaciation, diarrhea, abnormal posture, and tremors. The only clinical sign noted for male and female rats treated with 2-butoxyethanol was diarrhea.

2-Methoxyethanol: At Week 1 in the hematologic evaluations of 2-methoxyethanol, mild anemia, moderate leukopenia, and moderate thrombocytopenia were present in male rats in the higher dose groups. These animals had decreases in hematocrit (HCT) and hemoglobin (HGB) concentrations and in erythrocyte (RBC), platelet, and total leukocyte counts (Appendix D, Table D1). The anemia was normocytic (no change in mean cell volume), normochromic (no change in mean cell hemoglobin concentration), and poorly regenerative (indicated by a decrease in reticulocyte count). Leukopenia was produced by decreases in neutrophils and lymphocytes. There were moderate decreases in bone marrow cellularity counts in rats in the higher dose groups. At Weeks 3 and 13, the anemia was moderate, progressive, normocytic, and normochromic, with inadequate regeneration (no increase in reticulocyte count). Moderate leukopenia (lymphopenia and neutropenia) and thrombocytopenia were present at each time point, and bone marrow cellularity counts were decreased in male rats in the higher dose groups at Week 13.

Changes in clinical chemistry variables at the various time points for male rats included decreases in creatinine, total protein, albumin, and alkaline phosphatase (AP) (all consistent with decreased food intake) and mild increases in concentrations of bile acids at Weeks 1 and 3.

At Week 1, female rats had a mild normocytic, normochromic, poorly regenerative anemia. At Weeks 3 and 13, the anemia remained mild but, unlike that in male rats, was slightly microcytic (Weeks 3 and 13). Reticulocyte counts were unchanged in the presence of anemia at Weeks 3 and 13 (Appendix D, Table D1). Moderate thrombocytopenia and leukopenia (lymphopenia and neutropenia) occurred at all time points in numerous dose groups. Bone marrow cellularity was decreased by treatment at Weeks 1 and 3 but was unchanged at Week 13.

Clinical chemistry effects in female rats included decreases in AP activity and total protein and albumin concentrations in numerous dose groups at all time points. These findings are consistent with the decreased feed consumption of these animals. Additionally, there were mild increases in concentrations of bile acids in animals in multiple dose groups at Weeks 1 and 3.

For males and females, treatment-related changes in urinalysis parameters consisted of decreases in urine volume and increases in specific gravity.

With the exception of changes in thymus and testis weights, changes in absolute and relative organ weights in the 13-week study of 2-methoxyethanol could be attributed to low final mean body weights. Dose-related decreases were noted for the absolute and relative testis weights of male rats and the absolute and relative thymus weights of male and female rats (Table 8). Complete organ weight data for rats treated with 2-methoxyethanol for 13 weeks are presented in Appendix C, Tables C1 and C2.

Almost all observed gross lesions in the 13-week study of 2-methoxyethanol were considered to be secondary to the marked reduction in body weight gain and the overall smaller size of rats administered the higher exposure concentrations of 2-methoxyethanol. The only gross lesion attributed directly to the toxicity of 2-methoxyethanol was a reduction in testis size in males administered 2-methoxyethanol at concentrations of 1500 ppm and greater.

TABLE 8 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	Dose (ppm)					
	0	750	1500	3000	4500	6000
MALE						
n	10	10	10	10	2	0
Necropsy body wt	316	295	260**	214**	136**	–
Right testis						
Absolute	1.398	1.411	0.603**	0.442**	0.254**	–
Relative	4.44	4.81	2.31**	2.07**	1.89*	–
Thymus						
Absolute	0.268	0.198*	0.160**	0.095**	0.072**	–
Relative	0.85	0.67	0.61	0.45**	0.53	–
FEMALE						
n	10	10	10	10	5	0
Necropsy body wt	189	189	170**	145**	151**	–
Thymus						
Absolute	0.224	0.180*	0.125**	0.084**	0.099**	–
Relative	1.19	0.95**	0.74**	0.57**	0.66**	–

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

Histopathologic changes in the testes consisted of a minimal to marked degeneration of germinal epithelium in the seminiferous tubules; in more severely affected rats, the atrophic seminiferous tubules contained only Sertoli cells and a few spermatogonia. The presence of cell debris and a decrease in sperm within the lumen of the epididymis were associated with these changes. Degeneration was present at all dose levels but was only minimal in 7 of 10 rats in the 750 ppm group (Table 9).

TABLE 9 Incidence and Severity of Selected Histopathologic Lesions in F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	Dose (ppm)					
	0	750	1500	3000	4500	6000
MALE						
n	10	10	10	10	10	10
Bone marrow						
Cellular depletion	0	0	0	0	8 (2.6)	10 (3.0)
Spleen						
Atrophy	0	0	0	0	7[2] (2.4)	10 (2.8)
Capsular fibrosis	0	1 (1.0)	4 (1.5)	10 (2.2)	5[2] (1.2)	1 (1.0)
Thymus						
Atrophy	0	0	3[2] (2.0)	2 (1.5)	9[2] (3.1)	9[2] (3.6)
Testes						
Degeneration	0	7 (1.0)	10 (2.6)	10 (4.0)	9 (4.0)	10 (4.0)
Prostate						
Atrophy	0	0	0	0	9 (2.2)	10 (2.7)
Preputial gland						
Atrophy	0	0	0	1 (1.0)	9 (2.1)	8[3] (2.8)
Bone, metaphysis						
Atrophy	0	–[4]	0[5]	0	9[2] (3.0)	10 (3.0)
FEMALE						
n	10	10	10	10	10	10
Bone marrow						
Cellular depletion	0	0	1 (1.0)	7 (1.6)	6 (1.8)	9 (3.6)
Spleen						
Atrophy	0	0	1 (2.0)	1 (1.0)	5 (1.8)	10 (2.3)
Capsular fibrosis	0	0	3 (1.0)	5 (1.2)	0	0
Thymus						
Atrophy	0	0	1 (1.0)	9 (1.4)	7[2] (2.3)	10 (3.6)
Uterus						
Atrophy	0	0	0	8 (2.6)	9 (2.7)	10 (2.9)
Ovary						
Atrophy	0	0	0	6 (1.5)	10 (2.3)	10 (3.1)
Clitoral gland						
Atrophy	0	0	0	4[3] (1.8)	8 (2.6)	8[3] (2.8)
Bone, metaphysis						
Atrophy	0	–	–	0	10 (3.0)	10 (3.0)

[1] Incidences are given as the number of animals with lesions. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, 4=marked.

[2] n=9.

[3] n=8.

[4] Not applicable; tissue not examined for animals in this dose group.

[5] n=2.

Additionally, a chemical-related fibrosis of the splenic capsule was present in male and female rats (Plates 1 and 2) and was most prominent in animals in the 1500 to 4500 ppm groups. This fibrosis was characterized by focal areas in which there was thickening of the splenic capsule by fibrous connective tissue and a minimal mixed inflammatory cell infiltrate; inflammation and fibrosis of the serosal surfaces of other abdominal organs did not occur.

Other microscopic changes were associated with the marked reduction in body weight gain or stress-related physiological changes typically seen in animals that die during study or are killed moribund. Specifically, these changes included atrophy of the clitoral/preputial glands, uterus, ovary, salivary glands, and prostate (Table 9). Atrophic changes included not only an overall reduction in the size of the organs but a depletion of secretory product in the lumen of glands, decreased height of the secretory epithelium, and an increased number of degenerative and apoptotic cells. Lymphoid depletion (atrophy) in lymph nodes, thymus, and spleen, bone marrow depletion, absence of metaphyseal bone growth, focal erosion/ulcerations of the glandular stomach, and focal proliferation of bacterial or fungal organisms were also seen in animals that died or were killed moribund during the study; these lesions were considered to be secondary to the marked generalized toxicity and reduction in body weight gain seen in the 4500 and 6000 ppm 2-methoxyethanol groups.

A summary of lesions in rats in the 13-week drinking water study of 2-methoxyethanol is presented in Appendix A, Tables A1 and A2.

In the 13-week study of 2-methoxyethanol, sperm morphology evaluations were performed on male rats treated with 0, 750, 1500, or 3000 ppm, and vaginal cytology evaluations were performed on female rats treated with 0, 1500, 3000, or 4500 ppm. Testicular and epididymal weights were significantly lower than control values for males receiving 1500 or 3000 ppm 2-methoxyethanol (Appendix E, Table E1). Also, spermatozoal measurements were significantly decreased for males in the two highest dose groups (1500 or 3000 ppm). There were no significant differences from control in estrous cycle length for females treated with 2-methoxyethanol (Appendix E, Table E2). However, there was evidence to suggest that animals in the 1500 and 3000 ppm groups differed from the control animals in the relative frequency of time spent in estrous stages. The lack of significance at the 4500 ppm dose level may have been due to increased variability and/or the small sample size (five females).

2-Ethoxyethanol: At Week 1 in the hematologic evaluations of 2-ethoxyethanol, male rats exhibited a mild anemia, as indicated by decreases in RBC count and HGB concentration, that was macrocytic (increase in mean cell volume), hypochromic (decrease in mean cell hemoglobin concentration), and poorly regenerative. Hypochromia resulted from an increase in cell size, which kept HCT high relative to HGB concentration; the hypochromia did not result from an increase in numbers of large, young RBCs (reticulocytes), which, in fact, were markedly reduced and are typically normochromic (Appendix D, Table D2). Mild thrombocytopenia and leukopenia, produced by moderate lymphopenia and mild neutrophilia, were present, and a moderate decrease in bone marrow cellularity occurred in males in the 10,000 ppm group. There were mild decreases in total protein and albumin concentrations, as well as a moderate decrease in AP activity.

At Weeks 3 and 13, the anemia in male rats was moderate to marked, as indicated by decreases in HCT and HGB concentrations and RBC count, and was macrocytic, normochromic, and regenerative. Mild thrombocytopenia was present at Week 3 but absent at Week 13. Moderate leukopenia produced by lymphopenia and neutropenia persisted at Week 3, but marked leukocytosis (lymphocytosis and neutrophilia) appeared to be present at Week 13. Bone marrow cellularity was unchanged at Week 3 and increased in males in the 10,000 ppm group at Week 13. Clinical chemistry findings at these time points consisted of mild decreases in total protein and albumin concentrations and moderate decreases in AP activity. Concentrations of total bile acids increased significantly in males in the two highest dose groups (10,000 and 20,000 ppm) at Week 3 but were unchanged at Week 13.

As in male rats, a mild anemia, as indicated by decreases in RBC count and HGB concentration, was noted in female rats at Week 1; the anemia was macrocytic (increase in mean cell volume), hypochromic (decrease in mean cell hemoglobin concentration), and poorly regenerative (decrease in reticulocyte count) (Appendix D, Table D2). These rats had a moderate to marked thrombocytopenia and moderate leukopenia (lymphopenia). Bone marrow cellularity counts were not affected. Clinical chemistry findings consisted of mild decreases in total protein and albumin concentrations and in AP activity.

At Weeks 3 and 13, the anemia progressed from mild to moderate and remained macrocytic (marked at 13 weeks), regenerative (marked at 13 weeks), and mildly hypochromic. Thrombocytopenia was moderate at each time point, and the moderate

leukopenia (lymphopenia and neutropenia) at Week 3 appeared to be replaced by marked leukocytosis (neutrophilia and lymphocytosis) at Week 13. Bone marrow cellularity counts did not change at Week 3 but were significantly increased in animals in multiple dose groups at Week 13. Decreases in total protein concentration and AP activity were similar to those noted in male rats. At Week 3, alanine aminotransferase activity and concentrations of total bile acids were significantly increased in females in the three highest dose groups (5000, 10,000, and 20,000 ppm), and creatinine kinase activity was significantly increased in females in the four highest dose groups (2500, 5000, 10,000, and 20,000 ppm). Mild hepatocellular alterations were present at Week 3, but these effects were not detected at Week 13.

For rats treated with 2-ethoxyethanol, treatment-related changes in urinalysis parameters, when present, involved decreases in urine volume and increases in specific gravity.

In the 13-week study of 2-ethoxyethanol, no organ weight analyses were performed for male or female rats in the 20,000 ppm groups due to the high mortality at this exposure level. For the remaining dose groups, changes in absolute and relative organ weights could probably be attributed to low final mean body weights, excluding decreases noted in absolute and relative thymus and testis weights. Absolute and relative thymus weights decreased in a dose-related fashion for males and females, and absolute and relative testis weights for males in the 10,000 ppm 2-ethoxyethanol group were significantly lower than those of the control group (Table 10). Complete organ weight data for rats treated with 2-ethoxyethanol for 13 weeks are presented in Appendix C, Tables C1 and C2.

TABLE 10 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	Dose (ppm)					
	0	1250	2500	5000	10,000	20,000
MALE						
n	10	10	10	10	10	0
Necropsy body wt	315	309	296**	295*	236**	—
Right testis						
Absolute	1.394	1.431	1.443	1.342	0.618**	—
Relative	4.43	4.64	4.89	4.56	2.62*	—
Thymus						
Absolute	0.299	0.270	0.213**	0.258**	0.154**	—
Relative	0.95	0.87	0.72**	0.87*	0.65**	—
FEMALE						
n	10	10	10	10	10	0
Necropsy body wt	185	183	177	173**	149**	—
Thymus						
Absolute	0.214	0.210	0.221	0.186	0.069**	—
Relative	1.16	1.15	1.25	1.07	0.47**	—

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

In the 13-week study of 2-ethoxyethanol, the only chemical-related gross lesion noted in rats was a reduction in testis size in males in the 10,000 and 20,000 ppm groups. Microscopic changes in the testis were morphologically similar to those seen in the 2-methoxyethanol study in rats and consisted of a minimal to marked degeneration of germinal epithelium in the seminiferous tubules. In more severely affected animals, the atrophic tubules contained only Sertoli cells and a few spermatogonia. At the highest dose (20,000 ppm), there was a decrease in the size of the interstitial cells compared to those of the control group. Testicular degeneration was present in all male rats administered 2-ethoxyethanol at concentrations of 5000 ppm or greater for 13 weeks (Table 11). At the 5000 ppm exposure level, the severity of degeneration was minimal; although degeneration was present in a few tubules throughout the testes, there was no apparent histopathologic effect on the majority of seminiferous tubules. At the two highest exposure levels (10,000 and 20,000 ppm), the severity of degeneration was moderate to marked.

TABLE 11 Incidence and Severity of Selected Histopathologic Lesions in F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	Dose (ppm)					
	0	1250	2500	5000	10,000	20,000
MALE						
n	10	10	10	10	10	5
Liver						
Degeneration	0	0	0	0	0	5 (2.4)
Pigmentation	0	0	0	0	10 (1.0)	5 (1.0)
Hematopoiesis	0	0	0	0	9 (1.7)	0
Bone marrow						
Cellular depletion	0	0	0	0	0	5 (3.6)
Hyperplasia	0	0	0	0	10 (2.7)	0
Spleen						
Hematopoiesis	0	0	0	10 (2.0)	10 (3.2)	0
Pigmentation	0	0	0	0	0	5 (2.6)
Atrophy	0	0	0	0	0	4 (2.3)
Thymus						
Atrophy	0	0[2]	0[3]	0	4 (2.0)	2[4] (4.0)
Testes						
Degeneration	0	0	0	10 (1.1)	10 (3.5)	5 (4.0)
Prostate						
Atrophy	0	0	6 (1.3)	7 (1.4)	10 (2.0)	5 (3.4)
FEMALE						
n	10	10	10	10	10	7
Liver						
Degeneration	0	0	0	0	0	6 (1.8)
Pigmentation	0	0	0	0	10 (1.0)	7 (1.0)
Hematopoiesis	0	0	0	0	9 (2.0)	0
Bone marrow						
Cellular depletion	0	0	0	0	0	7 (3.3)
Hyperplasia	0	0	0	0	10 (3.0)	0
Spleen						
Hematopoiesis	0	0	0	0	10 (2.5)	0
Pigmentation	0	0	0	0	0	7 (2.7)
Atrophy	0	0	0	0	0	6 (2.2)
Thymus						
Atrophy	0	–[5]	–	0	10 (1.3)	6[6] (4.0)
Uterus						
Atrophy	0	0	0	0	9 (2.7)	7 (3.7)

[1] Incidences are given as the number of animals with lesions. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, 4=marked.
[2] n=1.
[3] n=2.
[4] n=3.
[5] Not applicable; tissue not examined for animals in this dose group.
[6] n=6.

Chemical-related lesions at other sites that were related to hematologic toxicity included increased hematopoiesis and hemosiderin pigmentation in the spleen (Plate 3), increased bone marrow hematopoiesis, and increased hemosiderin pigmentation in Kupffer's cells of the liver (Table 11). Other microscopic changes in rats were associated with the marked reduction in body weight gain or physiological stress-related changes typically seen in animals that die or are killed moribund. These changes, present almost exclusively at the highest dose, included atrophy of the clitoral/preputial glands, uterus, ovary, salivary glands, seminal vesicle, and prostate. Lymphoid depletion in the lymph nodes, thymus, and spleen was also noted.

A summary of lesions in rats in the 13-week drinking water study of 2-ethoxyethanol is presented in Appendix A, Tables A3 and A4.

Sperm morphology and vaginal cytology evaluations were performed on rats receiving 0, 2500, 5000, or 10,000 ppm 2-ethoxyethanol. Testicular weights were significantly lower than the control value for males in the highest dose group (10,000 ppm), and epididymal weights were significantly lower than those of the control group for males receiving 5000 or 10,000 ppm 2-ethoxyethanol (Appendix E, Table E3). All spermatozoal measurements were significantly less than those of the control group for males in the 10,000 ppm group, and sperm concentration was also significantly less than that of the control group for males treated with 2500 or 5000 ppm 2-ethoxyethanol. There was a significant decrease in estrous cycle length compared to the control value for females receiving 10,000 ppm 2-ethoxyethanol (Appendix E, Table E4). Evidence suggested that animals in this dose group differed significantly from the controls in the relative frequency of time spent in estrous stages, with females in the 10,000 ppm group spending more time in diestrus and less time in proestrus and estrus than did control animals.

2-Butoxyethanol: At all time points in the hematologic evaluations of 2-butoxyethanol, mild anemia indicated by a decrease in RBC counts was present in male rats in the three highest dose groups (3000, 4500, and 6000 ppm), and thrombocytopenia was present in males in the two highest dose groups (4500 and 6000 ppm). Decreases in HGB concentration were mild at Weeks 1 and 13 and sporadic at Week 3 (Appendix D, Table D3). There were no consistent changes in HCT. The anemia was markedly macrocytic and mildly hypochromic at each time point, and reticulocyte counts were moderately increased at Weeks 1 and 13. Leukocyte counts were mildly to markedly

increased (lymphocytosis and neutrophilia) at Week 1 in male rats in the three highest dose groups and unchanged at successive time points. Bone marrow cellularity was mildly increased in the two highest dose groups at Week 1. Clinical chemistry effects included mild increases in total protein and albumin in males in multiple dose groups at Week 1 and decreases of similar magnitude at Week 13. AP activity was increased in male rats in multiple groups at Week 1 and in the highest dose group (6000 ppm) at Week 3. Increased AP activity is consistent with mild cholestasis.

In female rats, there was mild to moderate anemia, as indicated by decreases in RBC counts and, less consistently, HCT and HGB concentrations, in most dose groups at each time point (Appendix D, Table D3). The anemia was markedly macrocytic, mildly to moderately hypochromic (normochromic at Week 1), and regenerative, with the exception of Week 3 reticulocyte counts, which were not increased. Platelet counts were mildly increased in animals in the higher dose groups at Week 1 but were decreased at Weeks 3 and 13. Marked leukocytosis (neutrophilia and lymphocytosis) was present at Week 1. There were mild increases in bone marrow cellularity in female rats in the higher dose groups at Weeks 1 and 13. Changes in clinical chemistry variables included moderate, consistent increases in concentrations of urea nitrogen and creatinine (mild, less prevalent) at Weeks 3 and 13 and mild decreases in concentrations of total protein and albumin at these same time points. AP activity was mildly increased in rats in the high-dose group at Week 1 and in the two highest dose groups at Week 13.

For male and female rats treated with 2-butoxyethanol, treatment-related changes in urinalysis parameters consisted of decreases in urine volume and increases in specific gravity.

In the 13-week study of 2-butoxyethanol in rats, the absolute thymus weights of males in the 4500 ppm group and males and females in the 6000 ppm groups were significantly lower than those of the control groups. Other changes noted in absolute and relative organ weights were considered to be secondary to changes in body weight. Complete organ weight data for rats treated with 2-butoxyethanol for 13 weeks are presented in Appendix C, Tables C1 and C2.

In the 2-butoxyethanol study, the only gross lesion considered to be chemical related was a reduction in the size of the uterus of female rats in the 4500 and 6000 ppm groups. Microscopically, there was minimal to mild uterine atrophy characterized by a decreased thickness of the muscular wall and uterine mucosa. This was considered to be secondary to the reduction in body weight gain rather than a direct chemical effect of 2-butoxyethanol.

Chemical-related histopathologic lesions occurred in the liver, spleen, and bone marrow of male and female rats. Liver lesions included cytoplasmic alteration, hepatocellular degeneration, and pigmentation. All of these lesions were present in the majority of dosed rats, but they were more prominent in the three highest dose groups (3000, 4500, and 6000 ppm); lesions were slightly more severe in females (Table 12). Cytoplasmic alteration in the liver of 2-butoxyethanol-dosed rats was characterized by hepatocytes that stained more eosinophilic and lacked the amphophilic to basophilic granularity of the cytoplasm typically present in controls. Hepatocellular degeneration was primarily centrilobular and was characterized by a variety of changes, including the occasional intensely eosinophilic-stained hepatocyte and hepatocytes that appeared shrunken with angular cytoplasmic borders and a densely stained nucleus (Plate 4). Pigmentation was present in Kupffer's cell cytoplasm, primarily in the centrilobular region. This brown to green granular pigment stained strongly positive for iron; some of the pigment granules also stained weakly positive by the PAS method. Hyperplasia of the bone marrow in dosed rats consisted of increased cellularity of hematopoietic cells in the mid shaft of the femur with a decrease in the amount of marrow fat cells relative to that seen in controls. A corresponding increase in hematopoiesis and hemosiderin pigment was also present in the spleen.

A summary of lesions in rats in the 13-week drinking water study of 2-butoxyethanol is presented in Appendix A, Tables A5 and A6.

TABLE 12 Incidence and Severity of Selected Histopathologic Lesions in F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

	Dose (ppm)					
	0	750	1500	3000	4500	6000
MALE						
n	10	10	10	10	10	10
Liver						
Cytoplasmic alteration	0	4 (1.0)	8 (1.0)	7 (1.1)	10 (2.0)	10 (1.8)
Degeneration	0	0	0	8 (1.0)	8 (1.0)	10 (1.0)
Pigmentation	0	0	0	0	0	7 (1.0)
Bone marrow						
Hyperplasia	0	0	0	2 (1.0)	2 (2.0)	8 (2.0)
Spleen						
Hematopoiesis	0	0	0	0	2 (1.0)	2 (1.0)
Pigmentation	0	0	2 (1.0)	10 (1.1)	8 (1.4)	10 (2.0)
FEMALE						
n	10	10	10	10	10	10
Liver						
Cytoplasmic alteration	0	5 (1.4)	9 (2.0)	10 (2.2)	10 (3.0)	10 (3.0)
Degeneration	0	0	0	10 (1.3)	10 (1.3)	10 (1.1)
Pigmentation	0	0	2 (1.0)	10 (1.2)	10 (1.9)	10 (1.9)
Bone marrow						
Hyperplasia	0	0	0	0	4 (2.0)	3 (2.0)
Spleen						
Hematopoiesis	0	0	0	0	6 (1.2)	10 (1.0)
Pigmentation	0	0	1 (2.0)	9 (2.0)	10 (2.0)	9 (2.0)
Uterus						
Atrophy	0	0	0	1 (1.0)	9 (1.2)	8 (2.0)

[1] Incidences are given as the number of animals with lesions. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, 4=marked.

Male and female rats treated with 0, 3000, 4500, or 6000 ppm 2-butoxyethanol were evaluated for sperm morphology and vaginal cytology. Decreases were noted for the left epididymal weights of males in the 4500 and 6000 ppm groups; however, these weights were appropriate for the reduced body weights (Appendix E, Table E5). The only spermatozoal measurement that showed a significant change relative to the control group was sperm concentration, which was decreased in all groups of treated males. There were no significant differences from the control group in estrous cycle length for treated females (Appendix E, Table E6). However, evidence suggested that animals in the 4500 and 6000 ppm groups differed significantly from the controls in the amount of time spent in estrous stages, with females in these two groups spending more time in diestrus and less time in proestrus, metestrus, and estrus than did control animals.

Stop-Exposure Drinking Water Studies in Male F344/N Rats

In the stop-exposure drinking water studies, male rats were treated with the ethylene glycol ethers for 60 days; rats were evaluated at the end of the treatment period and 30 and 56 days after treatment was stopped. All rats treated with 6000 ppm 2-methoxyethanol died by Week 6 of the study. For rats treated with 2-ethoxyethanol, 20 of 30 animals in the 20,000 ppm group died or were killed before the scheduled 60-day evaluation. One death each in the 10,000 and 20,000 ppm groups occurred after treatment with 2-ethoxyethanol was discontinued (Table 13). Due to the excessive mortality in males receiving 20,000 ppm 2-ethoxyethanol in both the stop-exposure and 13-week base studies, the five surviving rats in the 20,000 ppm base-study group were combined with the 10 surviving rats in the 20,000 ppm stop-exposure group at Day 60 of the stop-exposure study. No rats treated with 2-butoxyethanol died or were killed prior to the scheduled terminations.

Due to 100% mortality in the 6000 ppm 2-methoxyethanol group, mean body weights and weight changes were not determined for rats in this dose group after Week 6 of the study. However, at the Day 60 evaluation, mean body weights for rats in the 1500 and 3000 ppm 2-methoxyethanol groups were notably lower than those of the control group (Table 13). Although rats in these dose groups gained more weight than controls from Day 60 to the end of the recovery period, final mean body weights for rats in the 1500 and 3000 ppm 2-methoxyethanol groups remained at least 9% less than the control value (Figure 4).

In the 2-ethoxyethanol stop-exposure study, Day 60 mean body weights were at least 6% lower than the control value for rats in all treated groups, and the Day 60 mean body weight of rats in the 20,000 ppm group was 48% lower than the control value (Table 13). Mean body weight changes at Day 60 were also markedly lower in rats treated with 10,000 or 20,000 ppm 2-ethoxyethanol. During the recovery period, rats in the 10,000 and 20,000 ppm groups gained more weight than controls. However, final mean body weights in all treated groups were still at least 7% lower than that of the control group; the final mean body weight of rats in the 20,000 ppm group was 29% lower than the control value (Figure 5).

TABLE 13 Survival, Weight Gain, Water Consumption, and Compound Consumption in Male F344/N Rats in the Stop-Exposure Drinking Water Studies of Ethylene Glycol Ethers

Dose (ppm)	Survival[1]	Mean Body Weight (grams)				Weight Relative to Controls (%)[3]		Water Consumption (g/day)[4]	Compound Consumption (mg/kg/day)[5]
		Initial	Day 60	Final	Change[2]	Day 60	Final		
2-Methoxyethanol									
0	10/30	142	303	379	237			20.7	
1500	10/30	136	253	346	210	83	91	20.3	123
3000	10/30	144	223	329	185	74	87	17.5	255
6000	0/30[6]	143	—	—	—	—	—	16.5	745
2-Ethoxyethanol									
0	10/30	164	302	388	224			21.2	
5000	10/30	164	284	361	197	94	93	19.3	407
10,000	9/30[7]	165	255	353	188	84	91	17.5	792
20,000	5/35[8]	161	157	277	116	52	71	19.9	2390
2-Butoxyethanol									
0	10/30	147	289	356	209			21.1	
1500	10/30	144	295	363	219	102	102	20.2	124
3000	10/30	150	284	342	192	98	96	19.8	234
6000	10/30	147	261	329	182	90	92	19.7	443

[1] Number surviving at the end of the recovery period/number of rats per group. Number surviving does not include animals killed after 60 days of treatment or 30 days of recovery.
[2] Mean weight change from study start to study end.
[3] (Dose group mean/control group mean) x 100.
[4] Average water consumed per dose group from study start to study end.
[5] Average compound consumption during Days 0 to 60 of study.
[6] All rats in this group died before Day 60.
[7] One rat in this group died after Day 60.
[8] Twenty rats in this group died at or before Day 60; one rat died after Day 60. Because of the excessive mortality of rats administered 20,000 ppm 2-ethoxyethanol in both the stop-exposure and 13-week base studies, the five surviving base-study rats were moved to the 20,000 ppm stop-exposure group at Day 60.

In the 2-butoxyethanol stop-exposure study, the mean body weight of rats in the 6000 ppm group at Day 60 and at the study end was lower than that of the control group; however, the mean body weight of rats in this dose group remained within 10% of the control value at both time points (Table 13). The mean body weights of rats in the 1500 and 3000 ppm groups were similar to the control value at Day 60 and study end (Figure 6).

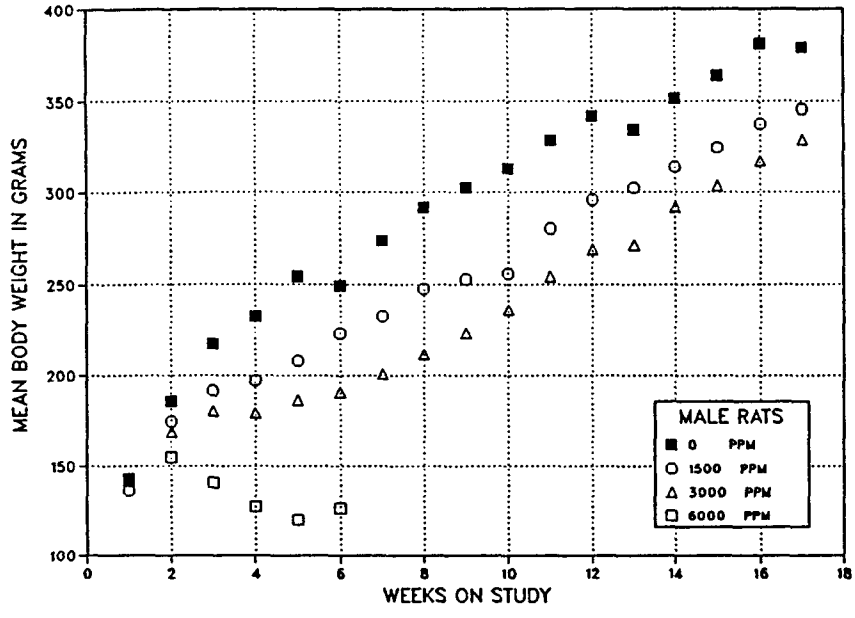

FIGURE 4 Body Weights of Male F344/N Rats Administered 2-Methoxyethanol in Drinking Water for 60 Days

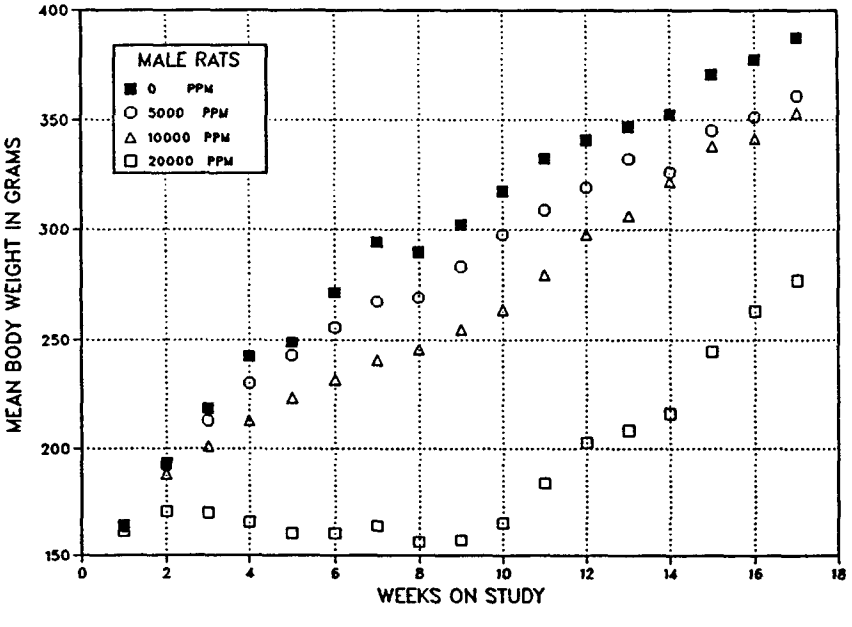

FIGURE 5 Body Weights of Male F344/N Rats Administered 2-Ethoxyethanol in Drinking Water for 60 Days

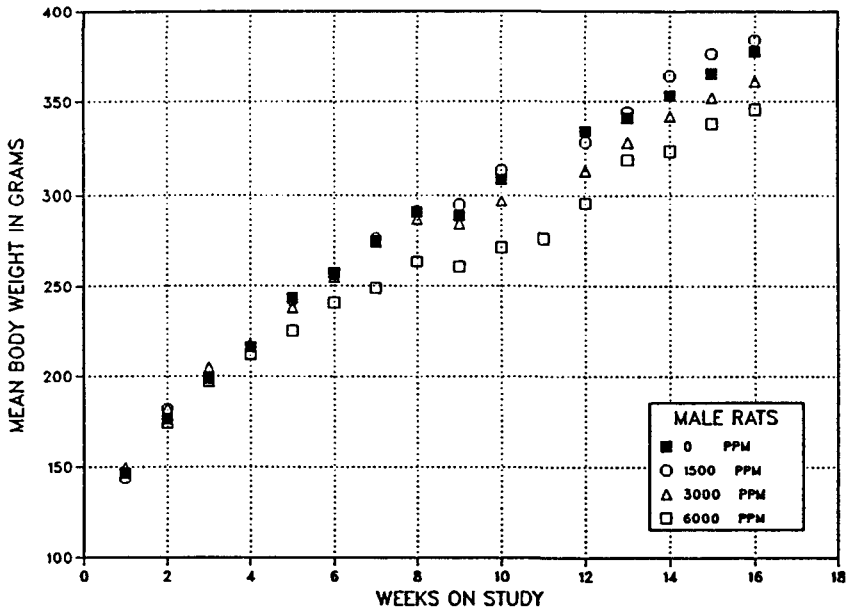

FIGURE 6 Body Weights of Male F344/N Rats Administered 2-Butoxyethanol in Drinking Water for 60 Days

For male rats treated with 2-methoxyethanol or 2-butoxyethanol, a dose-related decrease was noted in mean daily water consumption. Mean daily water consumption was also decreased for rats in all 2-ethoxyethanol dose groups. Average compound consumption increased with dose for rats treated with the ethylene glycol ethers for 60 days (Table 13). Over the course of the 60-day exposure period, compound consumption decreased slightly in rats in the 1500 and 3000 ppm 2-methoxyethanol groups, the 5000 and 10,000 ppm 2-ethoxyethanol groups, and in all 2-butoxyethanol-treated groups.

Clinical observations for rats treated with 2-methoxyethanol included abnormal posture, emaciation, and tachypnea. Clinical observations noted for animals treated with 2-ethoxyethanol included abnormal posture, diarrhea, emaciation, and polyuria. Clinical observations noted for animals treated with 2-butoxyethanol were sporadic and did not appear to be treatment related.

2-Methoxyethanol: For male rats treated with 1500 or 3000 ppm 2-methoxyethanol, absolute and relative testis and epididymal weights were significantly lower than those of the control group after 60 days of treatment and 30 and 56 days of recovery (Appendix C, Table C3).

In the stop-exposure study of 2-methoxyethanol, microscopic evaluations were performed on rats after 60 days of exposure to 1500 or 3000 ppm 2-methoxyethanol and after recovery periods of 30 and 56 days; a 6000 ppm group was initially included in the stop-exposure study, but all rats died prior to the end of the 60-day exposure period. Degeneration of the seminiferous tubules was present in rats in the 1500 and 3000 ppm groups at the end of the 60-day exposure period (Plates 5-8). Degeneration of the seminiferous tubules was also present in rats in the 6000 ppm group that died before the end of the exposure period. The severity of degeneration was marked in all rats from the 3000 and 6000 ppm groups and mild to moderate in rats in the 1500 ppm group (Table 14). In the two highest dose groups, the seminiferous tubules contained only a few spermatogonia and Sertoli cells; there was no evidence of active spermatogenesis in the seminiferous tubules. The lumen of the epididymis contained degenerative cells from the seminiferous tubules and only a few spermatozoa relative to controls. In rats in the 3000 ppm group, there was no evidence of recovery from the testicular degeneration after 30 days of recovery; after 56 days of recovery, all rats had degenerative lesions (mild to marked severity), but some tubules appeared relatively normal, and the lumen contained mature spermatids. In the 1500 ppm group, there was some recovery from the degenerative lesion in the testis after 30 days, but minimal to mild lesions were still present in all rats. After 56 days, there was no evidence of further recovery; all rats had minimal to mild degenerative lesions.

TABLE 14 Incidence and Severity of Testicular Degeneration in Male F344/N Rats in the Stop-Exposure Drinking Water Studies of 2-Methoxyethanol and 2-Ethoxyethanol[1]

	Dose (ppm)			
	0	1500	3000	6000
2-Methoxyethanol				
60-day treatment period	0/10	10/10 (2.4)	10/10 (4.0)	30/30 (4.0)
30-day recovery period	0/10	10/10 (1.2)	10/10 (3.9)	–[2]
56-day recovery period	0/10	10/10 (1.3)	10/10 (3.0)	–
	Dose (ppm)			
	0	**5000**	**10,000**	**20,000**
2-Ethoxyethanol				
60-day treatment period	0/10	0/10	10/10 (2.9)	24/24 (4.0)
30-day recovery period	0/10	6/10 (1.0)	11/11 (2.7)	5/5 (4.0)
56-day recovery period	0/10	7/10 (1.0)	9/9 (2.7)	5/5 (4.0)

[1] Incidences are given as the number of animals with lesions/number of animals examined microscopically. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, 4=marked.
[2] Not applicable; all animals died during the 60-day exposure period.

2-Ethoxyethanol: For rats that were treated with 10,000 or 20,000 ppm 2-ethoxyethanol, absolute and relative right testis and epididymal weights were significantly lower than those of the control group after 60 days of treatment and 30 and 56 days of recovery. Also, the absolute testis weight of males treated with 5000 ppm 2-ethoxyethanol was significantly lower than that of the control group after 56 days of recovery (Appendix C, Table C3).

In the stop-exposure study of 2-ethoxyethanol, moderate to marked testicular degeneration was present in rats in the 10,000 and 20,000 ppm groups, but not in the 5000 ppm group, after the 60-day exposure period (Table 14). At the 30 and 56 day recovery periods, there was no evidence of recovery from the testis lesions in these groups. Although no degeneration was evident in the testis of rats from the 5000 ppm group when the exposure was stopped (Day 60), minimal degeneration, similar to that seen at this dose level in the base study, was present in most male rats at the 30 and 56 day recovery periods.

2-Butoxyethanol: In the 2-butoxyethanol stop-exposure study, organ weights appeared appropriate for body weights at the end of the 60-day treatment period (Appendix C, Table C3). Organ weights were not evaluated at the end of the 56-day recovery period for rats treated with 2-butoxyethanol. No chemical-related microscopic lesions were noted in the testis or epididymis of rats in the stop-exposure study of 2-butoxyethanol.

2-Week Drinking Water Studies in B6C3F$_1$ Mice

No male or female mice treated with 2-methoxyethanol or 2-butoxyethanol and no female mice treated with 2-ethoxyethanol died or were killed before the end of the studies. One male mouse receiving the targeted dose of 900 mg/kg 2-ethoxyethanol died on Day 10 of the 2-week study (Table 15). The final mean body weights and mean body weight changes of males and females treated with 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol were variable and did not differ from those of the control groups (Table 15).

Average water consumption decreased for all males treated with 2-methoxyethanol and for females receiving targeted doses of 200, 400, 1000, or 1200 mg/kg 2-methoxyethanol (Table 15). In the 2-ethoxyethanol study, average water consumption was similar or somewhat increased for males in all treated groups excluding the 2500 mg/kg treatment group; average water consumption for males in this dose group and females in all 2-ethoxyethanol dose groups was decreased. In the 2-butoxyethanol study, there were no clear treatment-related changes in the water consumption of male mice. The average water consumption of female mice in the 2-butoxyethanol study was decreased at all dose levels excluding the 650 mg/kg level, where consumption was slightly increased. Spillage was not taken into consideration in any of these measurements.

As shown in Table 15, average compound consumption increased with dose in male and female mice treated with 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol; the actual doses achieved during the 2-week study in mice were much closer to targeted doses than those achieved in the 2-week study in rats.

TABLE 15 Survival, Weight Gain, Water Consumption, and Compound Consumption in B6C3F$_1$ Mice in the 2-Week Drinking Water Studies of Ethylene Glycol Ethers

Target Dose (mg/kg)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change[2]			
MALE							
2-Methoxyethanol							
0	5/5	24.2	25.3	1.1		5.2	
200	5/5	24.7	26.2	1.5	104	3.9	181
400	5/5	24.3	25.9	1.6	102	4.2	380
600	5/5	24.6	25.7	1.1	102	4.2	603
1000	5/5	25.4	25.5	0.1	101	3.6	865
1200	5/5	24.4	23.6	−0.8	93	3.8	1269
2-Ethoxyethanol							
0	5/5	22.1	24.6	2.5		4.5	
300	5/5	22.0	24.7	2.7	100	5.7	415
600	5/5	21.7	24.0	2.3	98	4.6	850
900	4/5[4]	22.2	25.6	3.4	104	5.8	1140
1500	5/5	22.2	25.0	2.8	102	4.6	1633
2500	5/5	22.6	25.0	2.4	102	4.0	2583
2-Butoxyethanol							
0	5/5	25.4	24.9	−0.5		4.3	
100	5/5	26.0	26.8	0.8	108	4.3	93
150	5/5	26.2	26.4	0.2	106	4.6	148
250	5/5	26.4	27.3	0.9	110	3.8	210
400	5/5	26.9	24.9	−2.0	100	4.6	370
650	5/5	27.4	26.2	−1.2	105	4.3	627

TABLE 15 Survival, Weight Gain, Water Consumption, and Compound Consumption in B6C3F$_1$ Mice in the 2-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

Target Dose (mg/kg)	Survival	Mean Body Weight (grams)			Final Weight Relative to Controls (%)	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change			
FEMALE							
2-Methoxyethanol							
0	5/5	20.3	20.6	0.3		8.1	
200	5/5	21.0	19.6	−1.4	95	6.9	255
400	5/5	21.5	21.7	0.2	105	7.7	544
600	5/5	21.0	20.9	−0.1	101	9.2	971
1000	5/5	21.3	22.0	0.7	107	6.0	1094
1200	5/5	21.2	22.5	1.3	109	4.9	1124
2-Ethoxyethanol							
0	5/5	18.3	19.4	1.1		8.7	
300	5/5	18.9	19.6	0.7	101	8.0	403
600	5/5	19.1	19.7	0.6	102	7.1	793
900	5/5	18.6	20.2	1.6	104	6.6	1069
1500	5/5	18.5	20.0	1.5	103	6.9	1966
2500	5/5	18.7	20.3	1.6	105	5.8	2815
2-Butoxyethanol							
0	5/5	20.5	20.4	−0.1		8.3	
100	5/5	20.8	20.9	0.1	102	6.1	150
150	5/5	20.5	20.4	−0.1	100	6.0	237
250	5/5	20.6	20.8	0.2	102	6.3	406
400	5/5	20.7	20.7	0.0	101	6.6	673
650	5/5	20.7	19.3	−1.4	95	8.9	1364

[1] Number surviving at 2 weeks/number of animals per dose group.
[2] Mean weight change of the survivors.
[3] (Dosed group mean/control group mean) × 100.
[4] Day of death: 10.

The only clinical observation noted for male mice treated with 2-methoxyethanol was dehydration in two of five males in the 1200 mg/kg group. Dehydration was also noted in one female each in the 0, 1000, and 1200 mg/kg 2-methoxyethanol groups and in two females in the 600 mg/kg group. In the 2-ethoxyethanol study, one male in the 900 mg/kg group that died was hypoactive and dehydrated prior to death. No other clinical signs of toxicity were reported in mice treated with 2-ethoxyethanol. In the 2-week study of 2-butoxyethanol, three of five males in the 400 mg/kg group and two of five males in the 650 mg/kg group were dehydrated. Dehydration was also noted in one female treated with 400 mg/kg and three females treated with 650 mg/kg. One female receiving 650 mg/kg 2-butoxyethanol was thin on Day 14 and hunched and moribund on Day 15.

2-Methoxyethanol: In the 2-week study of 2-methoxyethanol in mice, changes in organ weights were minimal. For male mice, absolute and relative testis and thymus weights decreased in a dose-related fashion, and for female mice in the two highest dose groups (1000 and 1200 mg/kg), absolute and relative thymus weights were lower than those of the control group (Table 16).

2-Ethoxyethanol: As with the 2-methoxyethanol study, changes in organ weights for mice in the 2-week study of 2-ethoxyethanol were minimal. For males in the high-dose (2500 mg/kg) group, relative testis weight was significantly lower than that of the control group (Table 17). The absolute and relative thymus weights of treated male and female mice were similar to those of the controls.

2-Butoxyethanol: For male mice treated with 400 or 650 mg/kg 2-butoxyethanol for 2 weeks, absolute and relative thymus weights were significantly lower than those of the control group (Table 18). The thymus weights of females and the testis weights of males receiving 2-butoxyethanol were not markedly different from those of the control groups.

No chemical-related gross lesions were noted in male or female mice in the 2-week study of 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol; microscopic evaluation of tissues was not performed.

TABLE 16 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 2-Week Drinking Water Study of 2-Methoxyethanol[1]

	Target Dose (mg/kg)					
	0	200	400	600	1000	1200
MALE						
n	5	5	5	5	5	5
Necropsy body wt	25.3	26.2	25.9	25.7	25.5	23.6
Right testis						
Absolute	0.107	0.105	0.095	0.089**	0.056**	0.054**
Relative	4.25	4.02	3.67*	3.47**	2.21**	2.33**
Thymus						
Absolute	0.053	0.069	0.056	0.052	0.029**	0.026**
Relative	2.11	2.63	2.15	2.02	1.14**	1.06**
FEMALE						
n	5	4	5	5	5	5
Necropsy body wt	20.6	19.6	21.7	20.9	22.0	22.5
Thymus						
Absolute	0.077	0.061	0.082	0.075	0.067	0.060
Relative	3.75	2.90	3.80	3.58	3.03*	2.68**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

TABLE 17 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 2-Week Drinking Water Study of 2-Ethoxyethanol[1]

	Target Dose (mg/kg)					
	0	300	600	900	1500	2500
MALE						
n	5	5	5	4	5	5
Necropsy body wt	24.6	24.7	24.0	25.6	25.0	25.0
Right testis						
Absolute	0.102	0.101	0.099	0.099	0.100	0.092
Relative	4.16	4.12	4.17	3.88	4.01	3.69*
Thymus						
Absolute	0.054	0.047	0.055[2]	0.040	0.057	0.051
Relative	2.19	1.91	2.19[2]	1.58	2.26	2.06
FEMALE						
n	5	5	5	5	5	5
Necropsy body wt	19.4	19.6	19.7	20.2	20.0	20.3
Thymus						
Absolute	0.079	0.070	0.078	0.071	0.079	0.069
Relative	4.08	3.59	3.95	3.53	3.93	3.38

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight. Necropsy body weights are not significant by Williams' or Dunnett's test.
[2] n=4.
* Significantly different (P≤0.05) from the control group by Williams' test.

TABLE 18 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 2-Week Drinking Water Study of 2-Butoxyethanol[1]

	Target Dose (mg/kg)					
	0	100	150	250	400	650
MALE						
n	5	5	5	5	5	5
Necropsy body wt	24.9	26.8	26.4	27.3	24.9	26.2
Right testis						
Absolute	0.110	0.110	0.106	0.112	0.108	0.109
Relative	4.44	4.08	4.03	4.10	4.39	4.16
Thymus						
Absolute	0.060	0.060	0.059	0.051	0.037*	0.048*
Relative	2.39	2.24	2.24	1.87	1.46*	1.85*
FEMALE						
n	5	5	5	5	5	5
Necropsy body wt	20.4	20.9	20.4	20.8	20.7	19.3
Thymus						
Absolute	0.077	0.075	0.074	0.066	0.069	0.062
Relative	3.76	3.59	3.62	3.19	3.35	3.04

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight. Necropsy body weights are not significant by Dunnett's test.
* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

During the 13-week studies of the ethylene glycol ethers in mice, fixed concentrations were administered. The maximum doses chosen for 2-methoxyethanol and 2-butoxyethanol were approximately equal to the doses that caused a measurable decrease in water consumption in the 2-week studies. The highest dose chosen for 2-butoxyethanol was inadvertently set about 4-fold higher than the appropriate high dose based on the 2-week study data, although this high dose did not result in mortality or marked toxicity.

13-Week Drinking Water Studies in B6C3F$_1$ Mice

No male or female mice receiving 2-methoxyethanol, 2-ethoxyethanol, or 2-butoxyethanol died or were killed before the end of the studies. The mean body weight gains of male mice receiving 10,000 ppm 2-methoxyethanol and female mice receiving 8000 or 10,000 ppm 2-methoxyethanol were notably lower than those of the control groups. For male and female mice in the 20,000 and 40,000 ppm 2-ethoxyethanol groups, body weight gains were lower than those of the control groups. Male and female mice receiving 3000 to 6000 ppm 2-butoxyethanol had slightly lower mean body weight gains than those of the control groups (Table 19; Figures 7-9).

In the 13-week study of ethylene glycol ethers, average water consumption was variable, and no clear treatment-related patterns were evident (Table 19). Average compound consumption increased with dose for male and female mice treated with the glycol ethers (Table 19).

There were no significant clinical observations in male or female mice during the 13-week studies of 2-methoxyethanol and 2-butoxyethanol. The only treatment-related clinical sign of toxicity noted for mice treated with 2-ethoxyethanol was emaciation, which was observed in males and females in the 20,000 and 40,000 ppm groups.

TABLE 19 Survival, Weight Gain, Water Consumption, and Compound Consumption in B6C3F$_1$ Mice in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers

Dose (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change[2]			
MALE							
2-Methoxyethanol							
0	10/10	24.0	39.3	15.3		4.5	
2000	10/10	24.0	40.2	16.2	102	4.9	295
4000	10/10	24.8	41.2	16.4	105	4.5	529
6000	10/10	24.4	38.1	13.7	97	4.1	765
8000	10/10	25.2	38.0	12.8	97	4.0	992
10,000	10/10	24.5	30.5	6.0	78	3.9	1367
2-Ethoxyethanol							
0	10/10	22.7	39.2	16.5		6.7	
2500	10/10	23.7	41.7	18.0	106	7.6	587
5000	10/10	23.5	43.1	19.6	110	6.5	971
10,000	10/10	22.8	41.0	18.2	105	6.3	2003
20,000	10/10	23.4	33.2	9.8	85	7.8	5123
40,000	10/10	23.9	32.5	8.6	83	5.2	7284
2-Butoxyethanol							
0	10/10	24.7	40.9	16.2		5.1	
750	10/10	24.9	40.0	15.1	98	5.2	118
1500	10/10	24.5	40.5	16.0	99	4.9	223
3000	10/10	24.8	38.0	13.2	93	6.0	553
4500	10/10	24.7	39.0	14.3	95	4.8	676
6000	10/10	24.5	38.2	13.7	93	3.7	694

TABLE 19 Survival, Weight Gain, Water Consumption, and Compound Consumption in B6C3F$_1$ Mice in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

Dose (ppm)	Survival	Mean Body Weight (grams)			Final Weight Relative to Controls (%)	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change			
FEMALE							
2-Methoxyethanol							
0	10/10	19.9	30.7	10.8		6.3	
2000	10/10	19.1	30.6	11.5	100	6.4	492
4000	10/10	19.8	30.4	10.6	99	5.8	902
6000	10/10	19.6	29.3	9.7	95	5.1	1194
8000	10/10	20.0	27.2	7.2	89	4.7	1489
10,000	10/10	20.4	24.9	4.5	81	4.5	1839
2-Ethoxyethanol							
0	10/10	19.3	32.0	12.7		8.7	
2500	10/10	19.0	34.0	15.0	106	7.5	722
5000	10/10	18.9	34.1	15.2	107	6.9	1304
10,000	10/10	19.1	30.2	11.1	94	6.9	2725
20,000	10/10	19.1	26.4	7.3	83	8.7	7255
40,000	10/10	19.0	24.9	5.9	78	6.1	11,172
2-Butoxyethanol							
0	10/10	20.1	31.6	11.5		6.2	
750	10/10	20.3	31.9	11.6	101	6.6	185
1500	10/10	20.2	30.8	10.6	97	6.5	370
3000	10/10	20.0	28.5	8.5	90	5.6	676
4500	10/10	19.9	29.7	9.8	94	4.8	861
6000	10/10	20.0	29.0	9.0	92	5.6	1306

[1] Number surviving at 13 weeks/number of animals per dose group.
[2] Mean weight change of the animals in each dose group surviving to Week 13.
[3] (Dosed group mean/control group mean) × 100.

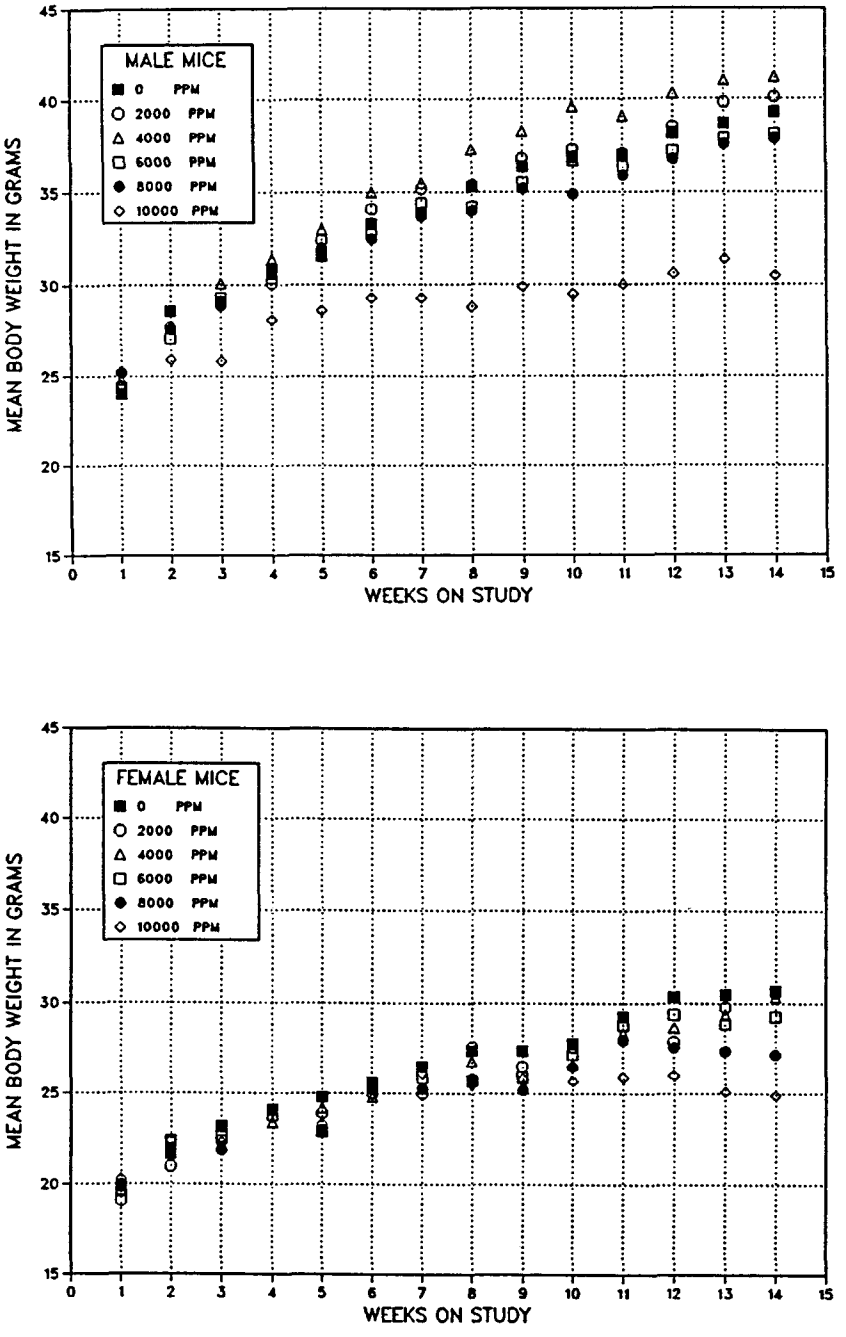

FIGURE 7 Body Weights of B6C3F$_1$ Mice Administered 2-Methoxyethanol in Drinking Water for 13 Weeks

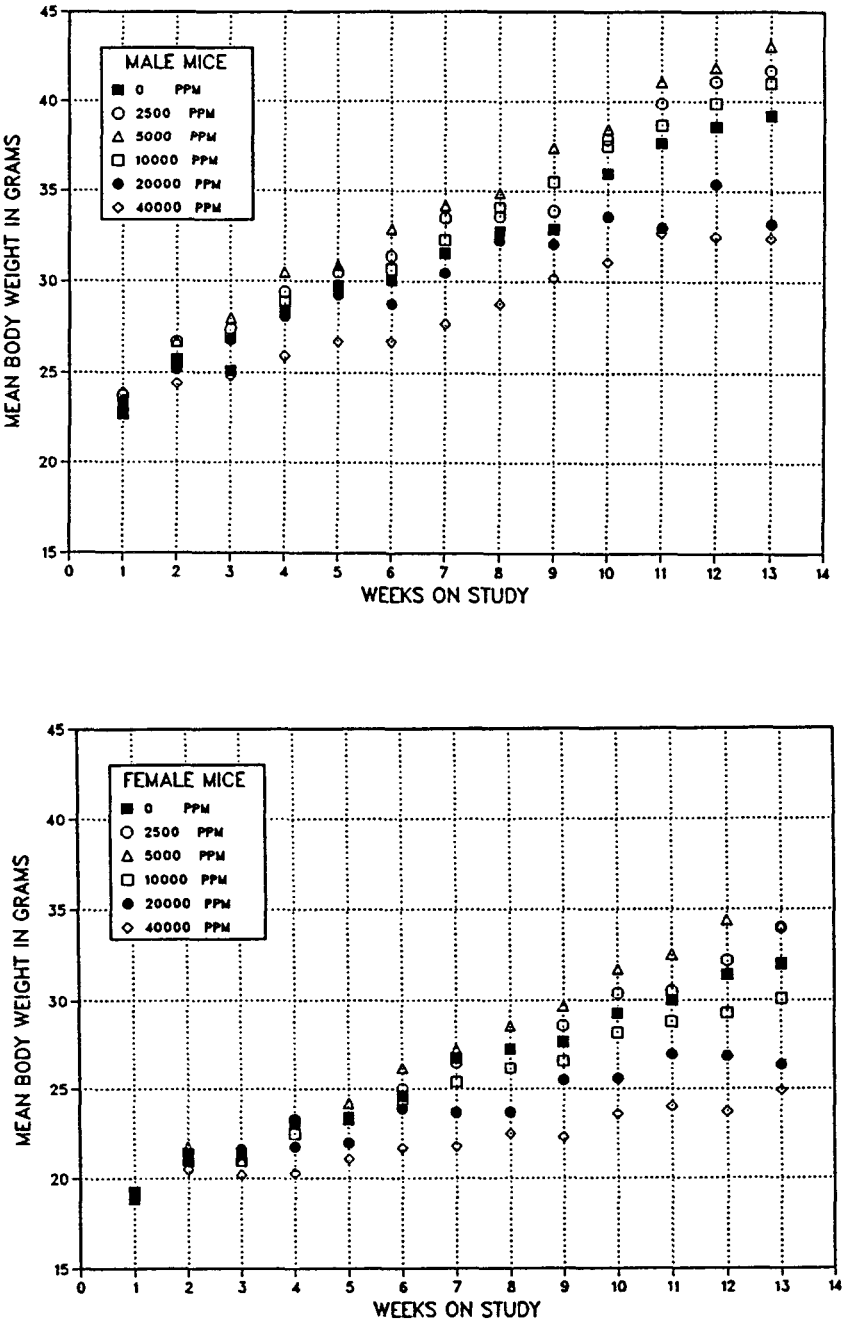

FIGURE 8 Body Weights of B6C3F$_1$ Mice Administered 2-Ethoxyethanol in Drinking Water for 13 Weeks

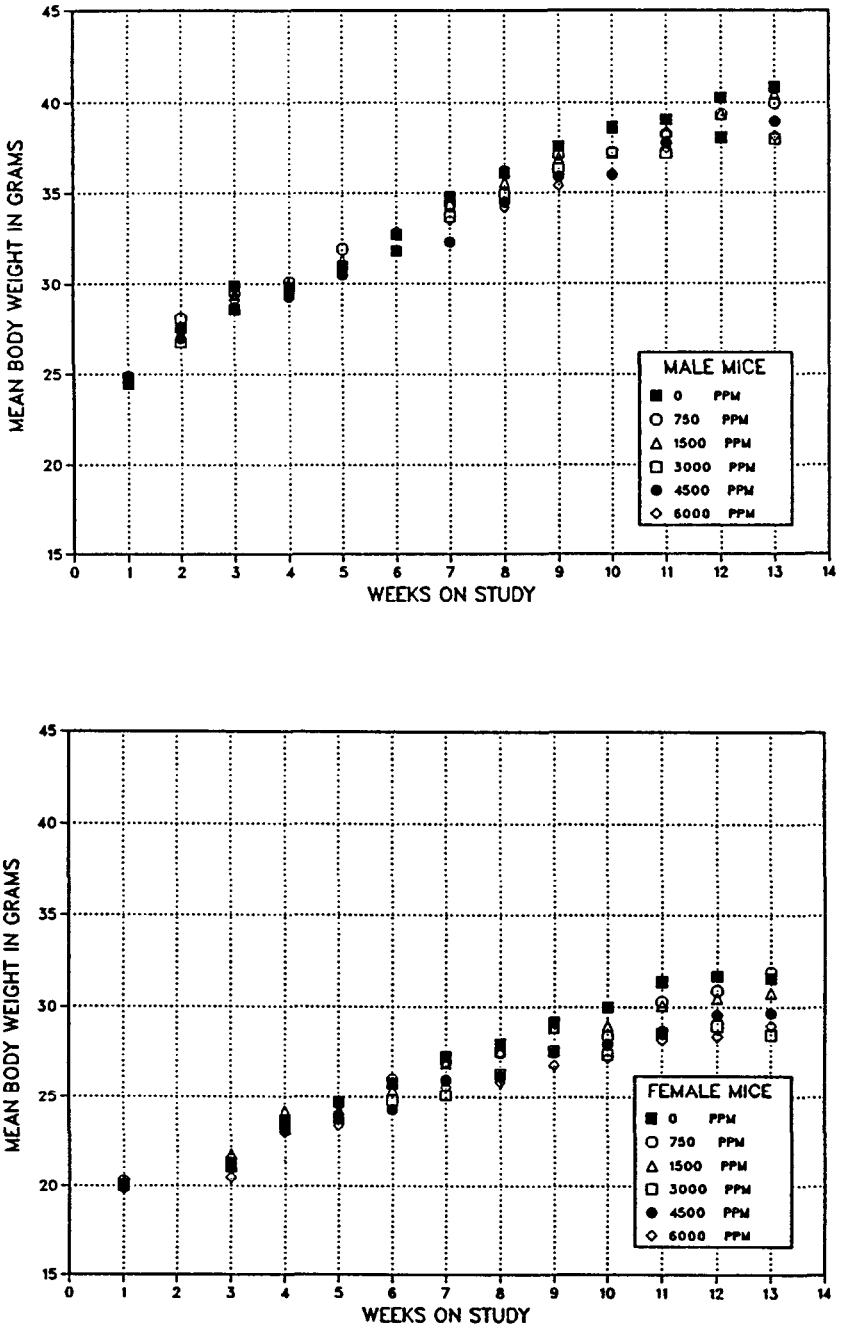

FIGURE 9 Body Weights of B6C3F$_1$ Mice Administered 2-Butoxyethanol in Drinking Water for 13 Weeks

2-Methoxyethanol: With the exception of decreases in thymus and testis weights, most changes in absolute and relative organ weights in the 13-week study of 2-methoxyethanol in mice could be attributed to low final mean body weights. Dose-related decreases were noted for the absolute and relative testis weights of male mice and the absolute and relative thymus weights of male and female mice (Table 20). Complete organ weight data for mice in the 13-week study of 2-methoxyethanol are provided in Appendix C, Tables C4 and C5.

TABLE 20 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	Dose (ppm)					
	0	2000	4000	6000	8000	10,000
MALE						
n	10	10	10	10	9	10
Necropsy body wt	39.2	39.6	40.8	37.8	37.1	30.1**
Right testis						
Absolute	0.121	0.120	0.102**	0.029**	0.026**	0.023**
Relative	3.11	3.04	2.51**	0.77**	0.69**	0.78**
Thymus						
Absolute	0.046	0.047	0.047	0.039	0.036*	0.023**
Relative	1.17	1.18	1.15	1.04	0.98*	0.76**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	29.7	29.3	29.6	27.2	26.0**	23.9**
Thymus						
Absolute	0.048	0.055	0.049	0.042	0.037*	0.026**
Relative	1.63	1.89	1.67	1.57	1.46	1.09*

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.
* Significantly different (P≤0.05) from the control group by Dunn's test or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's test or Shirley's test.

In the 13-week study of 2-methoxyethanol, chemical-related gross lesions were identified in the testis and thymus. Testes from mice in the 6000, 8000, and 10,000 ppm groups were small. The thymuses of males in the 8000 and 10,000 ppm groups and females in the 10,000 ppm (high-dose) group were also smaller than those of the control animals. In male mice, degeneration of the testis was characterized microscopically by a dose-related, minimal to marked degeneration of the germinal epithelium in seminiferous tubules (Table 21); at the higher doses, the lumen of many tubules contained only Sertoli cells (Plate 9). In the thymus of most males from the two highest dose groups and females in the high-dose group, there was minimal to mild lymphoid depletion (atrophy) consisting of a reduction in the thickness of the thymic cortex and in the number of thymocytes.

Histopathologic changes were also present in the spleen of male and female mice and in the adrenal gland of female mice (Table 21). Increased hematopoiesis was present in the spleen of mice from all dosed groups, excluding male mice in the lowest dose group (2000 ppm), and was characterized by a marked increase in the number of megakaryocytes present in the red pulp (Plates 10-12). In the adrenal gland of female mice in all dosed groups, there was hypertrophy of the X-zone. In dosed mice, there was a marked increase in the lipid vacuolization normally present in this region of the adrenal gland in young female mice (Plates 13-16).

A summary of lesions in mice treated with 2-methoxyethanol for 13 weeks is presented in Appendix B, Tables B1 and B2.

TABLE 21 Incidence and Severity of Selected Histopathologic Lesions in B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	Dose (ppm)					
	0	2000	4000	6000	8000	10,000
MALE						
n	10	10	10	10	10	10
Spleen						
Hematopoiesis	0	0	10 (1.0)	9 (1.0)	9 (1.0)	10 (1.1)
Thymus						
Atrophy	0[2]	–[3]	–	0	6 (1.5)	9 (2.0)
Testes						
Degeneration	0	0[2]	3 (1.0)	10 (3.0)	10 (4.0)	10 (4.0)
FEMALE						
n	10	10	10	10	10	10
Spleen						
Hematopoiesis	0	5 (1.0)	10 (1.0)	8 (1.1)	9 (1.0)	10 (1.0)
Thymus						
Atrophy	0[2]	–	–	–	0	4 (2.3)
Adrenal gland						
X–zone, hypertrophy	0	10 (2.1)	9[2] (2.9)	10 (3.1)	10 (3.7)	10 (3.6)

[1] Incidences are given as the number of animals with lesions. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, 4=marked.
[2] n=9.
[3] Not applicable; tissue not examined for animals in this dose group.

Sperm morphology evaluations were performed on male mice treated with 0, 2000, 4000, or 6000 ppm 2-methoxyethanol. Vaginal cytology evaluations were performed on female mice treated with 0, 6000, 8000, or 10,000 ppm 2-methoxyethanol. Results showed significant decreases in epididymal and cauda epididymal weights for males in the 6000 ppm group and in testicular weight for males in the 4000 and 6000 ppm groups (Appendix E, Table E7). The values for sperm motility were significantly less than controls for the 2000 and 6000 ppm groups, as were sperm concentration measurements for males treated with 2000 to 6000 ppm 2-methoxyethanol. Spermatid measurements were significantly lower than controls for males receiving 4000 or 6000 ppm 2-methoxyethanol. For females, all dose groups differed significantly from controls in the relative frequency of time spent in estrous stages (Appendix E, Table E8).

2-Ethoxyethanol: Most changes in absolute and relative organ weights in the 13-week study of 2-ethoxyethanol in mice could be attributed to low final mean body weights, excluding decreases in testis weights. Absolute testis weights were significantly decreased for males in the two highest dose groups (20,000 and 40,000 ppm) (Table 22). Complete organ weight data for mice in the 13-week study of 2-ethoxyethanol are provided in Appendix C, Tables C4 and C5.

In the 13-week study of 2-ethoxyethanol, chemical-related gross lesions consisted of small testes and epididymides in mice from the 40,000 ppm group. Histopathologic changes were present in the spleen and testis of male mice and the spleen and adrenal gland of female mice (Table 23). In male mice, degeneration of the testis was characterized as a marked, diffuse loss of germinal epithelium in the seminiferous tubules. Histopathologic changes were not seen in the testis of mice in the lower dose groups. In the spleen of female mice in the 20,000 ppm group and males and females from the 40,000 ppm groups, there was a minimal to mild increase in hematopoiesis; there was also a minimal increase in splenic hematopoiesis in one female mouse in the 10,000 ppm group. Splenic hematopoiesis was characterized by an increase in the number of erythroid elements and megakaryocytes and was similar to that seen in mice from the 2-methoxyethanol study. Based upon histologic sections, there was no apparent effect in the bone marrow. In the adrenal gland, hypertrophy of the X-zone was present in all dose groups and was morphologically identical to that described for mice in the 2-methoxyethanol study.

A summary of lesions in mice treated with 2-ethoxyethanol for 13 weeks is presented in Appendix B, Tables B3 and B4.

Sperm morphology and vaginal cytology evaluations were performed on mice treated with 0, 5000, 10,000, or 20,000 ppm 2-ethoxyethanol. Epididymal and testicular weights were significantly lower than control values for males in the high-dose group (20,000 ppm) (Appendix E, Table E9). Values for sperm motility, spermatid heads per testis, and spermatid count were significantly lower than control values for males receiving 20,000 ppm 2-ethoxyethanol. All treated females had significantly longer estrous cycles than did controls (Appendix E, Table E10).

TABLE 22 Testis Weights and Testis-Weight-to-Body-Weight Ratios for Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	Dose (ppm)					
	0	2500	5000	10,000	20,000	40,000
n	10	10	10	10	10	10
Necropsy body wt	38.9	40.9	43.0	40.5	33.6*	31.9**
Right testis						
Absolute	0.119	0.124	0.123	0.119	0.097**	0.019**
Relative	3.08	3.05	2.86	2.95	2.88	0.59**

[1] Testis weights and body weights are given in grams; relative testis weights (testis-weight-to-body-weight ratios) are given as mg organ weight/g body weight.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE 23 Incidence and Severity of Selected Histopathologic Lesions in B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	Dose (ppm)					
	0	2500	5000	10,000	20,000	40,000
MALE						
n	10	10	10	10	10	10
Spleen						
Hematopoiesis	0	0	0	0	0	10 (1.6)
Testes						
Degeneration	0	0	0	0	0	10 (4.0)
FEMALE						
n	10	10	10	10	10	10
Spleen						
Hematopoiesis	0	0	0	1 (1.0)	9 (1.3)	10 (1.8)
Adrenal gland						
X-zone, hypertrophy	0	0	1 (2.0)	8 (1.8)	10 (2.8)	9 (2.4)

[1] Incidences are given as the number of animals with lesions. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, 4=marked.

2-Butoxyethanol: In the 13-week study of 2-butoxyethanol in mice, all changes in organ weights were considered to be secondary to reduced body weights. Complete organ weight data for mice in the 13-week study of 2-butoxyethanol are provided in Appendix C, Tables C4 and C5.

There were no chemical-related gross or microscopic lesions in male or female mice administered 2-butoxyethanol in the drinking water for 13 weeks. A summary of lesions in mice treated with 2-butoxyethanol for 13 weeks is presented in Appendix B, Tables B5 and B6.

Sperm morphology and vaginal cytology evaluations were performed in mice treated with 0, 3000, 4500, or 6000 ppm 2-butoxyethanol. No biologically significant changes were observed in any of the reproductive parameters evaluated in male or female mice (Appendix E, Tables E11 and E12).

Genetic Toxicity Studies

2-Ethoxyethanol (Zeiger *et al.*, 1985), 2-methoxyethanol, and 2-butoxyethanol (Zeiger *et al.*, 1992) were negative in *Salmonella typhimurium* mutation tests conducted with and without induced hamster and rat liver S9 (Appendix G, Tables G1-G3). Each of the three glycol ethers was tested up to the maximum dose of 10,000 µg/plate. In the mouse lymphoma L5178Y cell mutation assay, 2-ethoxyethanol was negative without S9 but was judged to be weakly positive in two of three trials conducted in the presence of induced rat liver S9 (Table G4). Neither of the other two glycol ethers was tested in this assay.

2-Ethoxyethanol (Galloway *et al.*, 1987) and 2-butoxyethanol gave contrasting results in tests of induction of chromosomal damage in Chinese hamster ovary (CHO) cells *in vitro*. 2-Ethoxyethanol induced sister chromatid exchanges (SCEs) in CHO cells at very high concentrations (3170 and 9510 µg/mL) with and without S9 (Table G5). It also induced chromosomal aberrations (Abs) in CHO cells, but only in the absence of S9 (Galloway *et al.*, 1987; Table G7). The concentrations which produced a positive response were, as in the SCE test, very high (6830 and 9510 µg/mL). Despite these high concentrations of 2-ethoxyethanol, no cell cycle delay was observed in treated cultures. In contrast, 2-butoxyethanol induced cell cycle delay but did not induce either SCEs (Table G6) or Abs (Table G8) in CHO cells with or without S9. In the Abs test without S9,

a weakly positive response was obtained in the second trial at the highest dose tested (5000 µg/mL), but this response was not reproduced in a third trial and the chemical was concluded to be negative. Because of the cell cycle delay caused by 2-butoxyethanol in the trials conducted without S9, a delayed harvest was used to increase the number of cells available for analysis.

2-Ethoxyethanol was the only one of the three glycol ethers to be tested for induction of sex-linked recessive lethal mutations in germ cells of adult male *Drosophila melanogaster* (Valencia *et al.*, 1985; Mason *et al.*, 1992; Table G9). Two separate experiments were performed using both feeding and injection as the routes of administration; all results were negative.

PLATE 1

Spleen of a male rat exposed to 3000 ppm 2-methoxyethanol showing marked thickening (fibrosis) of capsule (arrows) compared to the spleen from a control male rat shown in Plate 2. 128×.

PLATE 2

Spleen of a control male rat for comparison with Plates 1 and 3. Note typical appearance of thin fibrous capsule (arrows) compared to the spleen of a 2-methoxyethanol-treated rat in Plate 1. Scattered, darkly stained foci of hematopoiesis (asterisks) are present in addition to periarteriolar lymphoid sheath (L). Compare to the increased hematopoiesis present in the spleen from a 2-ethoxyethanol-treated rat shown in Plate 3. 128×.

PLATE 3

Spleen of a male rat exposed to 10,000 ppm 2-ethoxyethanol showing a marked increase in darkly staining hematopoietic cells compared to the spleen from a control rat shown in Plate 2. 128×.

PLATE 4

Liver from a female rat exposed to 6000 ppm 2-butoxyethanol showing hepatocyte degeneration adjacent to a central vein (V). Note the scattered darkly stained hepatocytes which appear shrunken with angular cytoplasmic borders and a densely stained nucleus (arrows). 240×.

PLATE 5

Testis of a control male rat from the stop-exposure study at 60 days showing normal morphologic appearance of seminiferous tubules. GMA section, 64×.

PLATE 6

Higher magnification of the testis shown in Plate 5. Compare with Plate 8. GMA section, 320×.

PLATE 1

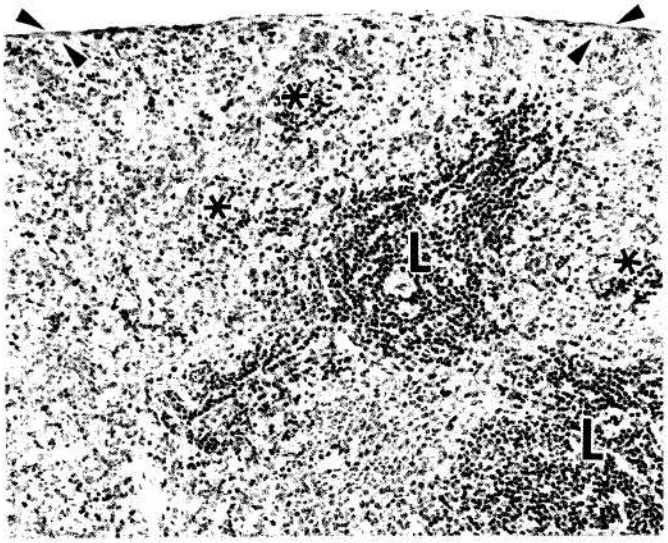

PLATE 2

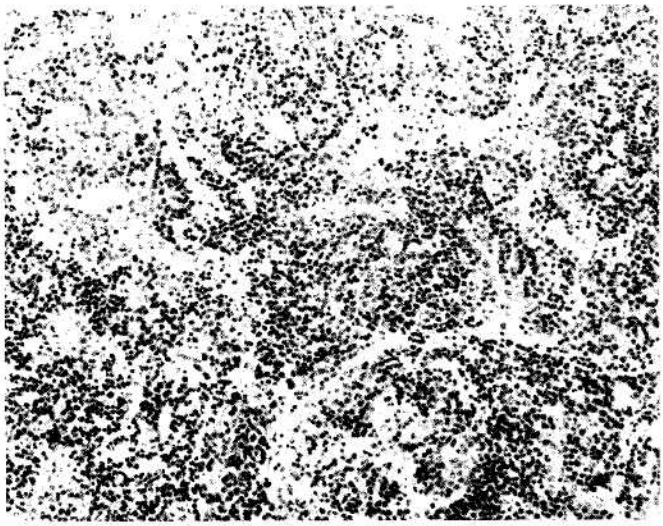

PLATE 3

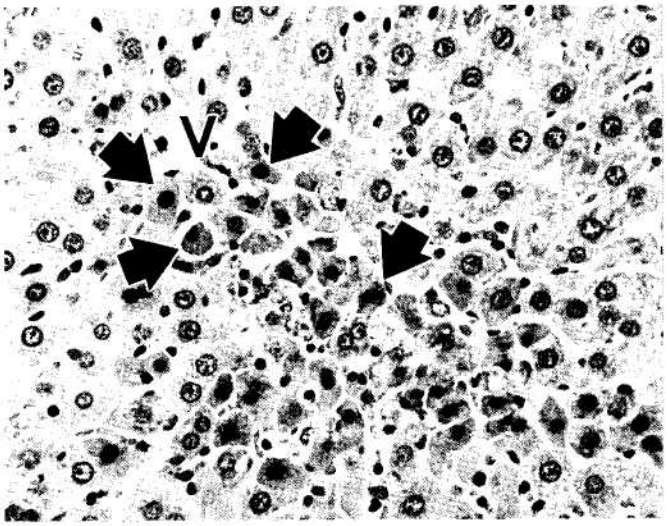

PLATE 4

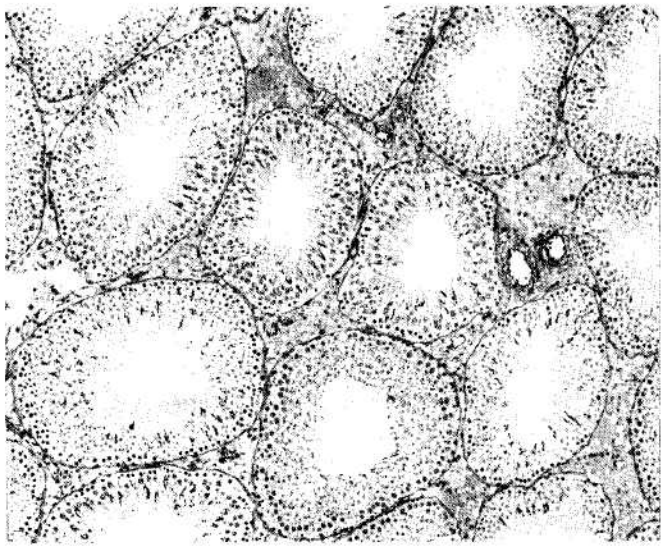

PLATE 5

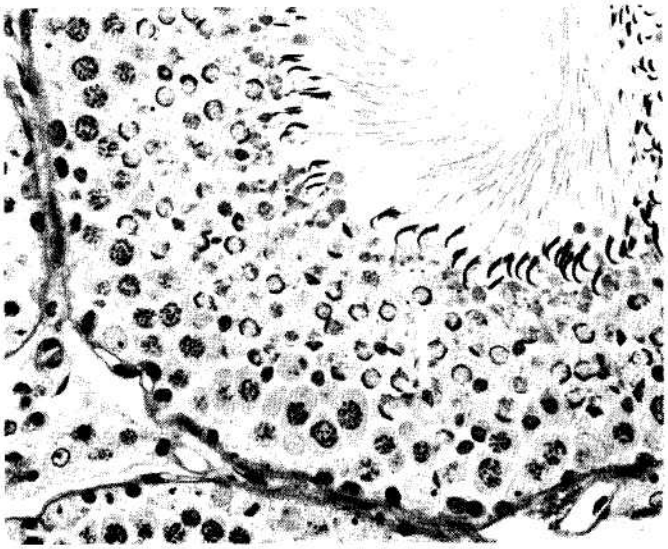

PLATE 6

PLATE 7

Testis of a male rat from the stop-exposure study exposed to 3000 ppm 2-methoxyethanol for 60 days with no recovery period. Note smaller diameter of the seminiferous tubules and marked depletion of spermatogenic cells. GMA section, 64×.

PLATE 8

Higher magnification of the testis shown in Plate 7. GMA section, 320×.

PLATE 9

Testis from a mouse exposed to 8000 ppm 2-methoxyethanol showing marked degeneration with no evidence of spermatogenesis in atrophic seminiferous tubules. 240×.

PLATE 10

Spleen from a control male mouse showing the normal appearance of the red pulp. Note the single megakaryocyte (arrow) in field. 160×.

PLATE 11

Spleen from a male mouse exposed to 10,000 ppm 2-methoxyethanol showing increased hematopoiesis characterized primarily by aggregates of megakaryocytes beneath the splenic capsule (arrows). 160×.

PLATE 12

Higher magnification of the mouse spleen in Plate 11 showing numerous multilobulated, sometimes darkly stained nuclei of megakaryocytes and foci of smaller darkly stained erythroid cell precursors. 240×.

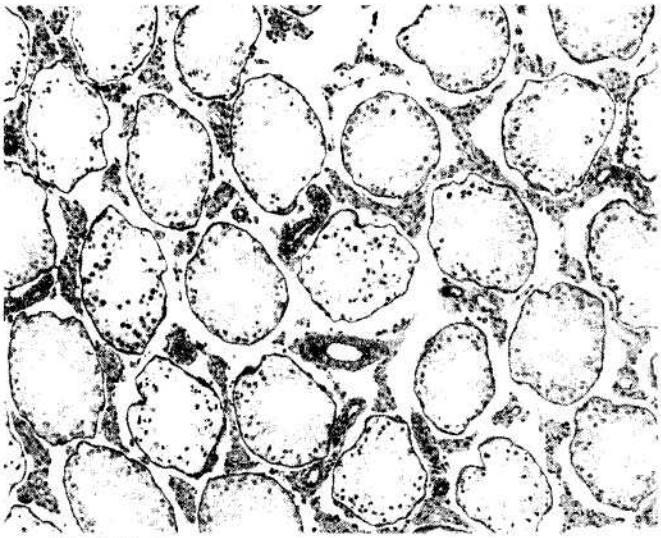

PLATE 7

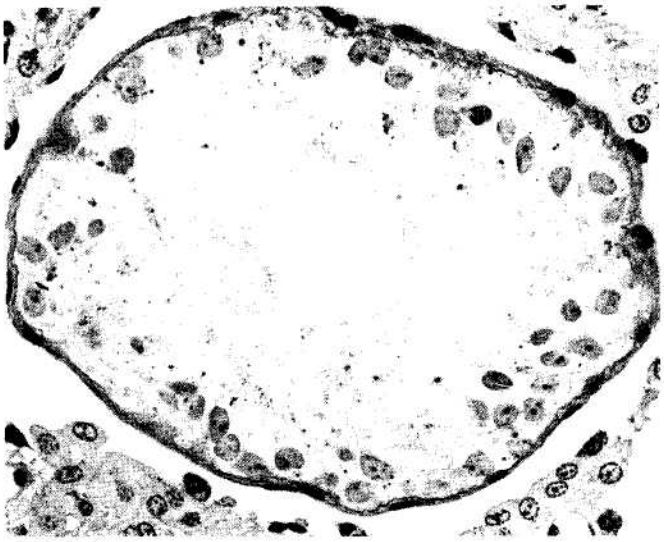

PLATE 8

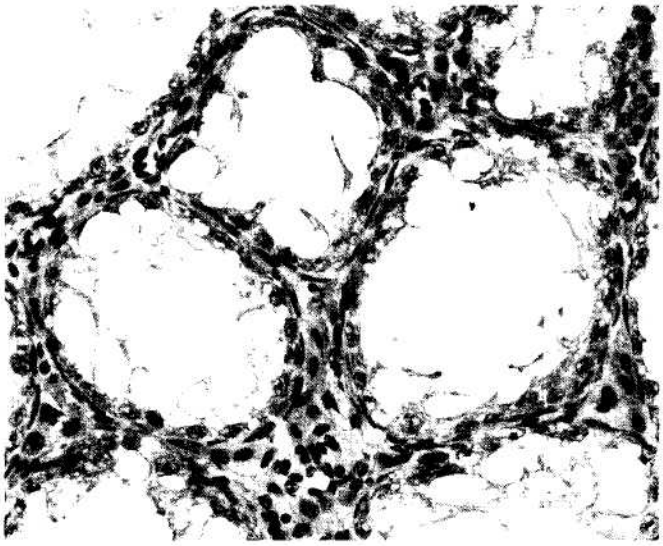

PLATE 9

PLATE 10

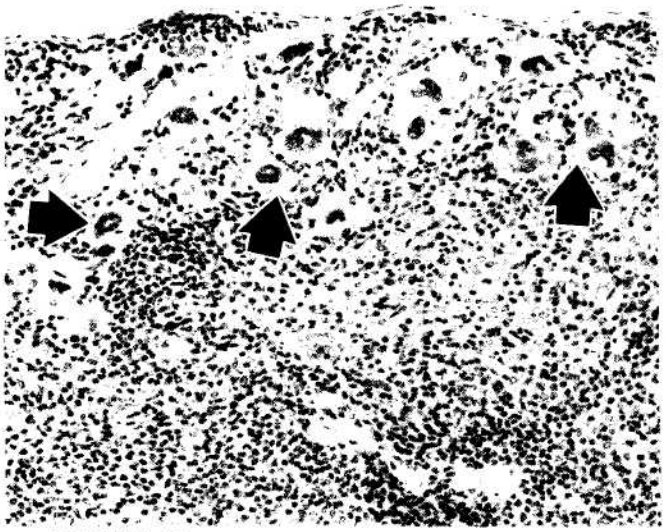

PLATE 11

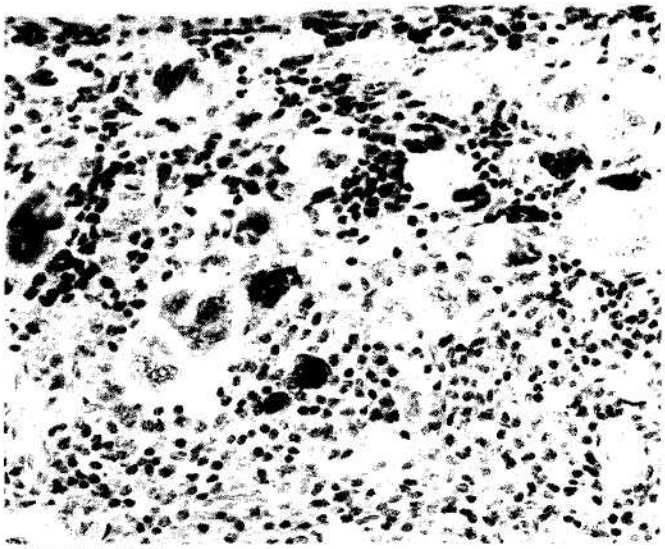

PLATE 12

PLATE 13

Adrenal gland from a control female mouse showing darkly stained X-zone between the pale staining adrenal cortex and the medulla (M). Note the scattered lipid vacuoles (arrows) present in this area. 30×.

PLATE 14

Higher magnification of the adrenal gland shown in Plate 13. 75×.

PLATE 15

Adrenal gland from a female mouse exposed to 10,000 ppm 2-methoxyethanol showing marked hypertrophy of the X-zone with slight compression of the cortex as a result of marked lipid vacuolization of the X-zone. 30×.

PLATE 16

Higher magnification of the adrenal gland shown in Plate 15. 75×.

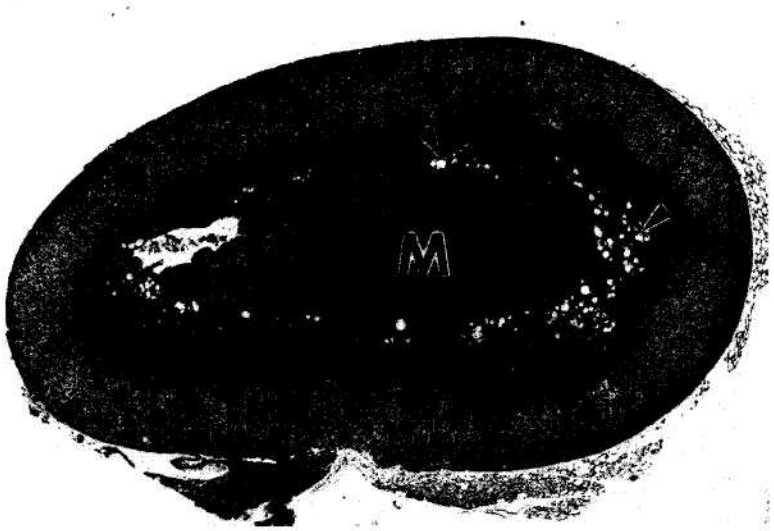

PLATE 13

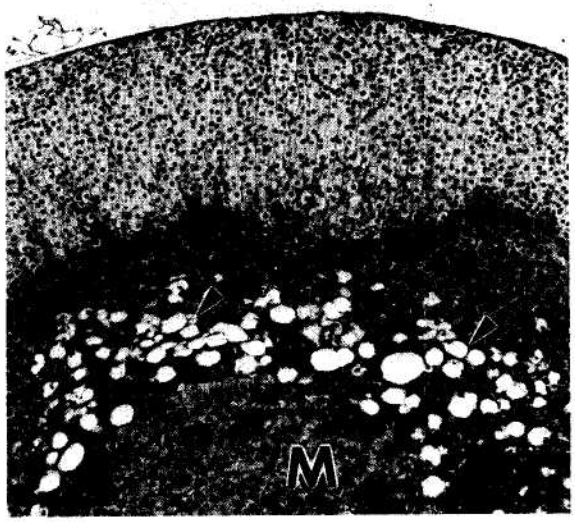

PLATE 14

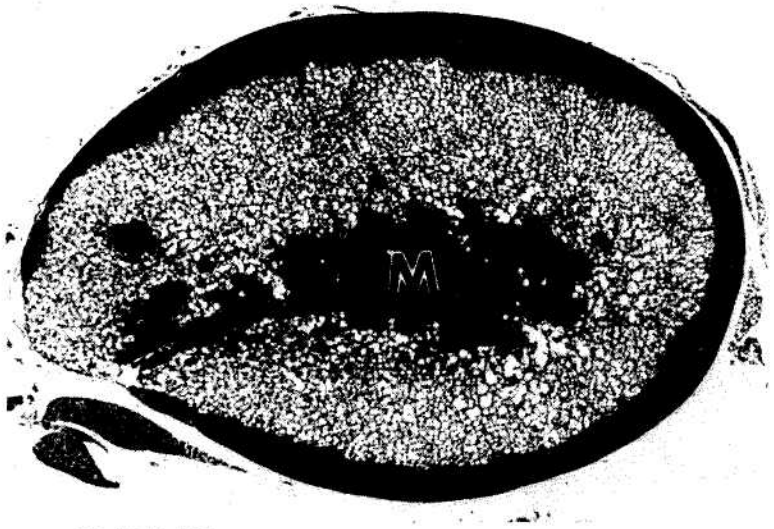

PLATE 15

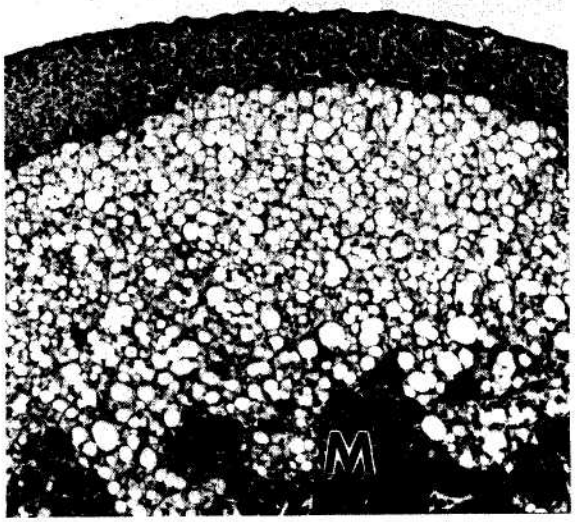

PLATE 16

DISCUSSION

The results of these comparative studies of the toxicities of 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol are generally consistent with the findings of previous studies. 2-Methoxyethanol is primarily a reproductive and developmental toxicant, inducing spermatotoxicity and teratogenicity, as is 2-ethoxyethanol to a lesser extent (NIOSH, 1991). 2-Butoxyethanol is primarily a hematotoxic agent to the erythrocyte series (NIOSH, 1990), while 2-methoxyethanol and 2-ethoxyethanol act primarily as hematotoxic agents to the leukocyte series (NIOSH, 1991).

The majority of previous studies conducted with the glycol alkyl ethers have demonstrated that the various toxic effects of these compounds result from their alkoxyacetic acid metabolites and not as a direct response to the parent compounds. For example, developmental and reproductive toxicity similar to that caused by 2-methoxyethanol and 2-ethoxyethanol occurred when methoxyacetic acid and ethoxyacetic acid were administered alone (Miller *et al.*, 1982, 1983b; Foster *et al.*, 1987; Sleet *et al.*, 1988; Clarke*etal.*, 1991). Similarly, the hematoxicity of 2-butoxyethanol was effected by butoxyacetic acid; utilization of metabolic inhibitors of alcohol and aldehyde dehydrogenase in *in vivo* (Ghanayem *et al.*, 1987b, 1990a) and *in vitro* studies (Ghanayem *et al.*, 1989) clearly demonstrated that the alkoxyacetic acid metabolite was the effector of hemolysis.

A possible reason for the differences in the toxicities of these compounds could involve variations in their mode or rate of metabolism to the respective alkoxyacetic acids. However, an examination of the metabolic data does not provide an adequate explanation to account for the different toxicities of 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol. Irrespective of the route of administration (dermal, inhalation, or oral), alkoxyacetic acids or their derivatives were the major metabolites formed from the ethylene glycol ethers (Ghanayem *et al.*, 1987a; Medinsky*etal.*, 1990; Sabourin *et al.*, 1992a,b). For example, a study in F344 rats in which comparable doses of [^{14}C]-labeled 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol were administered for 24 hours in drinking water indicated that the majority of the ^{14}C from each of the chemicals was excreted in the urine or exhaled as CO_2, with less than 5% exhaled as unmetabolized glycol ether (Medinsky *etal.*, 1990). The metabolism of the glycol alkyl ethers was dependent upon chain length and, to a lesser extent, upon dose, such that the urinary

alkoxyacetic acids excreted constituted 34% of the dose for 2-methoxyethanol, 25% to 40% of the dose for 2-ethoxyethanol, and 50% to 60% of the dose for 2-butoxyethanol; 10% to 30% of the dose for 2-methoxyethanol, 20% of the dose for 2-ethoxyethanol, and 8% to 10% of the dose for 2-butoxyethanol were eliminated in the breath as CO_2. Ethylene glycol was also excreted in the urine at 21%, 18%, and 10% of the doses for 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol, respectively (Medinsky *et al.*, 1990).

Apparently, ingestion of the glycol alkyl ethers leads to dealkylation of a significant and varying percentage of the compounds prior to oxidation to the more toxic alkoxyacetic acid metabolites. However, this alternative pathway of metabolism, decreasing the formation of the more toxic alkoxyacetic acids, was inversely proportional to chain length and therefore cannot account for the rank order of increasing toxicity demonstrated in the present studies (*e.g.*, 2-methoxyethanol > 2-ethoxyethanol > 2-butoxyethanol). A possibly unique product of 2-methoxyethanol metabolism has been identified in the urine of F344 rats treated dermally with 2-methoxyethanol. This unknown metabolite was produced in amounts (30% to 50% of the administered dose) comparable to methoxyacetic acid. By comparison, there was 5.7% to 9.1% of this unknown metabolite in the urine of 2-ethoxyethanol-treated rats and none in the urine of 2-butoxyethanol-treated rats (Sabourin *et al.*, 1992b). Based on chromatographic analyses, the unknown metabolite was not glycolic acid, glyoxylic acid, or oxalic acid, all of which are possible metabolites of ethylene glycol.

Other factors in the comparative metabolism of the three glycol ethers that may influence the general rank order of toxicity were demonstrated in dermal studies in F344 rats; in these studies, although alkoxyacetic acids were the major urinary metabolites for all three compounds, only 2-butoxyethanol was metabolized to detectable amounts of a glucuronide conjugate (Sabourin *et al.*, 1992b). A gavage study in F344 rats identified a third metabolite, the sulfate conjugate of 2-butoxyethanol, which was present in the urine of animals dosed with 125 mg/kg 2-butoxyethanol but not in the urine of those dosed with 500mg/kg (Ghanayem *et al.*, 1987a). Inhalation studies of 2-butoxyethanol in F344 rats indicated that formation of the alkoxyacetic acid metabolite was linearly related to exposure concentration up to doses that were toxic (Sabourin *et al.*, 1992a). These data are consistent with the toxicokinetic data from Ghanayem *et al.* (1990a), which showed blood levels of butoxyacetic acid were linearly related to the doses of 2-butoxyethanol administered. Thus, 2-butoxyethanol can be metabolized to butoxyacetic acid, the

glucuronide conjugate of 2-butoxyethanol, and the sulfate conjugate of 2-butoxyethanol, whereas the minor metabolic products of 2-methoxyethanol and 2-ethoxyethanol are different.

In the 13-week studies, toxic and other changes were noted in the liver of rats receiving 2-butoxyethanol. A minimal change in the staining characteristics of the cytoplasm was termed cytoplasmic alteration. This may be related to enzyme induction associated with production of the glucuronide and sulfate conjugates of 2-butoxyethanol. However, there was no evidence of the hepatocellular hypertrophy or increased liver weight that commonly accompany marked enzyme induction. The accumulation of pigment in Kupffer cells was more prominent in treated females than in males and was likely a secondary change associated with the hematotoxicity of 2-butoxyethanol. A third effect was hepatocellular degeneration. In males, this effect was seen at doses lower than those associated with significant hematopoietic toxicity or pigment accumulation in Kupffer cells; thus, this effect may represent a primary toxicity of 2-butoxyethanol or a metabolite.

Hypertrophy of the X-zone of the adrenal gland, a rare lesion, occurred in female mice treated with 2-methoxyethanol or 2-ethoxyethanol for 13 weeks. This change was most prominent in the 2-methoxyethanol study, where a no-effect level was not achieved. The X-zone is a portion of the adrenal gland between the medulla and outer cortex that normally undergoes an age-related degeneration and atrophy (Dunn, 1970). Typically associated with this atrophy is a variable amount of lipid vacuolization in the X-zone. Experimentally, the X-zone degeneration and atrophy have been shown to occur more rapidly with dietary restriction or with the administration of some chemicals or corticosteroid hormones (Dunn,1970). In the present studies, hypertrophy of the X-zone in treated mice was the result of a marked lipid vacuolization rather than a chemical-related change in the spontaneously occurring atrophy. Similar findings have also been reported in female mice treated with other compounds, including thyroxine and methanol (Ribelin, 1984).

Comparable absolute or molar equivalent doses were not used in the present studies, but a general barometer to approximate the comparative toxicity of 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol was provided by an examination of relative thymus weights (thymus-weight-to-body-weight ratios). In the 2-week studies in rats, decreases in relative thymus weights were noted for males and females at all dose levels of

2-methoxyethanol and at all but the lowest dose level of 2-ethoxyethanol; changes in relative thymus weights were not significant for rats treated with 2-butoxyethanol. Generally, male and female mice treated with the ethylene glycol ethers for 2 weeks also exhibited decreases in relative thymus weights. In the 13-week studies, thymic atrophy was greater in rats administered 2-methoxyethanol than in those given 2-ethoxyethanol, and it was much less severe in rats receiving 2-butoxyethanol. Similar responses in thymic weight reflected the comparative toxicity of 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol in mice treated for 13weeks. Thymic effects may not represent a direct toxic action of the glycol ethers on the thymus in all cases but, rather, may be secondary effects related to stress and other toxicities associated with the exposures.

The rank order of testicular toxicity in male rats and mice was analogous to that demonstrated in the thymus. Decreases in testicular and epididymal weights were greatest in animals treated with 2-methoxyethanol and were also significant in animals treated with 2-ethoxyethanol; in animals receiving 2-butoxyethanol, there was no significant evidence to indicate toxicity to the testis. Testicular atrophy was accompanied by lesions characterized by degeneration of the germinal epithelium in the seminiferous tubules of the testes, abnormal sperm morphology, and reduced sperm counts.

In separate stop-exposure studies conducted to determine the persistence of the testicular toxicity, it was evident that 2-methoxyethanol exerted a greater toxic effect than 2-ethoxyethanol. Rats treated with 1500 or 3000 ppm 2-methoxyethanol for 60 days had greater degeneration of the seminiferous tubules of the testes than did rats treated with 5000 ppm 2-ethoxyethanol for 60 days. 2-Methoxyethanol also caused persistent degenerative lesions after 30 and 56 days of recovery. At the 5000 ppm exposure level, 2-ethoxyethanol caused no initial lesions during the 60-day exposure period but elicited minimal degeneration in the testes in most male rats at both time points in the recovery period. In the stop-exposure study of 2-butoxyethanol, there was no testicular toxicity in rats in any of the treatment groups during the exposure or recovery periods. Additional studies to investigate the mechanism of action of 2-methoxyethanol for spermatotoxicity suggested that calcium deregulation in testicular cells by 2-methoxyethanol may be directly or indirectly responsible for the toxicity. In these studies, one, two, three, or four doses of the calcium channel blockers verapamil or diltiazem, given in combination with a single gavage dose of 300 mg/kg 2-methoxyethanol, partially prevented testicular damage in male F344 rats (Ghanayem and Chapin, 1990).

The teratogenicity of 2-methoxyethanol has been well characterized in a series of studies using the CD-1 mouse (Sleet *et al.*, 1988; Clarke *et al.*, 1991); abnormalities in cranial development and limb bud morphogenesis occurred after pregnant mice were exposed to 2-methoxyethanol on gestation Days 7 and 11, respectively. Subsequently, it was shown that teratogenesis correlated with peak concentrations of methoxyacetic acid in the blood of the dam, embryo, and surrounding extraembryonic fluids following subcutaneous administration of 250 mg/kg 2-methoxyethanol. The urinary elimination half-life of methoxyacetic acid from both maternal and embryonic compartments was determined to be 5 to 6 hours, and the embryonic exposure was estimated at 60 to 70mmol/hour perkg (Clarke *et al.*, 1991). The pharmacokinetics of 2-methoxyethanol/methoxyacetic acid were similar in the pregnant rat, with a calculated half-life of about 12 hours for methoxyacetic acid (Scott *et al.*, 1989). By comparison, the half-life of methoxyacetic acid in pregnant macaque monkeys dosed with 12 to 36 mg/kg 2-methoxyethanol was about 20 hours, compared to a half-life of greater than 70 hours in humans (Groeseneken *et al.*, 1989).

It was evident that common cellular targets for glycol alkyl ether toxicity were undifferentiated, rapidly dividing cells, such as those that occur in the embryo or in the hematopoietic system of adult animals (Nagano *et al.*, 1981, 1984; Tyler, 1984). Because of this demonstrable cell sensitivity, studies were conducted to determine the potential anti-tumorigenicity of the glycol alkyl ethers. Previous toxicity studies showed that administration of 2-methoxyethanol or 2-ethoxyethanol resulted in a decrease in white blood cells and in bone marrow cellularity (Hong *et al.*, 1988, 1989). Initial studies demonstrated that 2-methoxyethanol and 2-ethoxyethanol prevented mortality in mice challenged with L1210 leukemia cells in an allogeneic tumor model (Houchens*et al.*, 1984). Additional investigations with an F344 rat transplanted leukemia cell tumor model showed that 2-ethoxyethanol would inhibit the progression of leukemia in syngeneic transplant recipients (Dieter *et al.*, 1989) and that the degree of inhibition was about one-half as effective as that from an equivalent dosage of 2-methoxyethanol (Dieter*etal.*,1990). These findings were confirmed in the present independent study (AppendixF). Further, it was found that among nine different glycol ethers, including 2-methoxyethanol, 2-ethoxyethanol, 2-butoxyethanol, ethylene glycol, and diethylene glycol, only 2-methoxyethanol and 2-ethoxyethanol inhibited the progression of leukemia (Dieter*etal.*, 1990).

Subsequent immunotoxicity investigations in Sprague-Dawley rats with 2-methoxyethanol and 2-butoxyethanol examined the relationship between thymic atrophy and immune parameters such as natural killer cell function, specific antibody production, splenocyte production of _-interferon, and spleen cell counts (Exon *et al.*, 1991). The results of these investigations provide one possible explanation for the specificity of the anti-tumorigenic activities of 2-methoxyethanol and 2-ethoxyethanol. The paucity of immune effects from 2-butoxyethanol is also in concert with the hypothesis that the anti-leukemic effects of 2-methoxyethanol and 2-ethoxyethanol were a result of an indirect effect of the glycol ethers on host immune effectiveness. Male and female rats were exposed to concentrations of 1600 to 6000 ppm 2-methoxyethanol or 2-butoxyethanol in drinking water for 21 days; rats exposed to 2-methoxyethanol exhibited the expected decreases in body weights and thymus and testis weights. There were dose-related increases in natural killer cell cytotoxic activity and decreases in specific antibody production in both sexes of rats treated with 2-methoxyethanol. Splenocyte production of _-interferon was decreased in males exposed to 2000 or 6000 ppm 2-methoxyethanol and in females treated with the high dose. Spleen cell numbers were reduced in males given the high dose of 2-methoxyethanol and in female rats in both dose groups. One immune parameter was affected by 2-butoxyethanol treatment; natural killer cell activity was marginally increased in the low-dose groups but not in the high-dose groups of both sexes. These data provide further credibility for the anti-tumorigenic activity of 2-methoxyethanol and also provide one explanation for the unusual specificity exhibited by only 2 of the 11 glycol ether compounds investigated (Dieter *et al.*, 1990).

The mode of action of 2-methoxyethanol, and to a lesser extent 2-ethoxyethanol, may be to effect an *in vivo* stimulation of natural killer cell tumoricidal activity in the immune defense system of the host. It is unlikely that 2-methoxyethanol acts directly as a cytotoxic agent based on the following: (1) data showing that the spermatotoxic and teratotoxic metabolite of 2-methoxyethanol, methoxyacetic acid, was ineffective in reducing the number of rodent leukemia cells after *in vitro* exposure (Dieter *et al.*, 1990) and (2) the *in vivo* data from Houchens *et al.* (1984), which showed that B6C3F_1 mice given allogeneic L1210 tumor cells and treated with 2-methoxyethanol or 2-ethoxyethanol were protected from mortality while syngeneic CD2F_1 mice were not.

In the present studies, treatment with the ethylene glycol ethers produced two different hematologic profiles that are consistent with distinct mechanisms. 2-Methoxyethanol produced pancytopenia characterized by a poorly regenerative, normochromic, normocytic to microcytic anemia, leukopenia, and thrombocytopenia. These findings indicate a treatment-related effect at the level of a pluripotent stem cell or a disruption of the hematopoietic microenvironment necessary for maintenance of normal hematopoiesis. In contrast, the regenerative, macrocytic, normochromic to hypochromic anemias produced by 2-ethoxyethanol and 2-butoxyethanol are consistent with an appropriate response to hemolysis of circulating erythrocytes (RBCs) accompanied by cellular swelling.

In previous *in vivo* experiments with 2-butoxyethanol in F344 rats, swelling of circulating RBCs preceded the onset of intravascular hemolysis (Ghanayem*etal.*, 1990b). Incubation of rat RBCs with butoxyacetic acid, an active metabolite of 2-butoxyethanol, also produced swelling of the cells (increased hematocrit and mean cell volume) shortly before lysis occurred (Ghanayem *et al.*, 1992). At most time points during the current studies, anemias produced by treatment with 2-butoxyethanol and 2-ethoxyethanol were generally regenerative (increase in reticulocyte counts), macrocytic, and hypochromic (occasionally, normochromic). Therefore, in addition to macrocytosis related to increased numbers of reticulocytes (which would be normochromic), the hypochromic nature of these anemias (produced by an increase in cell size resulting in a decreased ratio of hemoglobin concentration to hematocrit) indicates that a component of this effect was produced by cellular swelling.

In summary, the rank order of toxicity for the three glycol alkyl ethers in these studies was 2-methoxyethanol > 2-ethoxyethanol > 2-butoxyethanol. This is in agreement with the toxicities attributed to the respective glycol ethers or their alkoxyacetic acids in previous studies. Although the metabolism of the three chemicals was similar (resulting in production of their respective alkoxyacetic acids), dissimilar, minor metabolites were reported to be produced at different rates by each of the chemicals, and these minor metabolites may also partially account for the specificity of the toxicity exerted by 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol. The major target organs for toxicity were the testes in males of both species and the hematopoietic system in both sexes and species. 2-Methoxyethanol appeared to act primarily as a spermatotoxic and immunotoxic agent. 2-Ethoxyethanol was intermediate as a toxic agent and was effective

only in the highest dose ranges, while 2-butoxyethanol was relatively nontoxic at the doses tested and affected only the erythroid series in the hematopoietic system.

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APPENDIX A

Summary of Nonneoplastic Lesions in Rats

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Natural death					7	8
Moribund sacrifice					1	2
Survivors						
Terminal sacrifice	10	10	10	10	2	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine large, cecum	(10)			(10)	(5)	(5)
Lymphoid tissue, hyperplasia, reticulum cell				1 (10%)		
Intestine small, ileum	(10)			(10)	(5)	(5)
Peyer's patch, hyperplasia, reticulum cell				1 (10%)		
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Bacterium						4 (40%)
Hepatodiaphragmatic nodule				2 (20%)	2 (20%)	
Necrosis					1 (10%)	2 (20%)
Pancreas	(10)			(10)	(9)	(10)
Acinus, atrophy					1 (11%)	
Pharynx					(4)	
Palate, bacterium					4 (100%)	
Palate, ulcer					4 (100%)	
Salivary glands	(10)			(10)	(9)	(10)
Atrophy					2 (22%)	10 (100%)
Stomach, glandular	(10)	(2)	(10)	(10)	(8)	(10)
Erosion					2 (25%)	6 (60%)
Mineralization					1 (13%)	2 (20%)
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(9)	(10)
Bacterium						1 (10%)
Cardiomyopathy	2 (20%)	2 (20%)	6 (60%)	1 (10%)	2 (22%)	2 (20%)
Endocrine System						
Adrenal gland, cortex	(10)			(10)	(9)	(10)
Bacterium					1 (11%)	
Hemorrhage					3 (33%)	8 (80%)
General Body System						
None						

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Genital System						
Epididymis	(10)	(9)	(10)	(10)	(10)	(10)
Aspermia			10 (100%)	10 (100%)	9 (90%)	10 (100%)
Fat, inflammation, chronic active				1 (10%)		
Preputial gland	(10)	(10)	(10)	(10)	(10)	(8)
Atrophy				1 (10%)	9 (90%)	8 (100%)
Prostate	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy					9 (90%)	10 (100%)
Inflammation, chronic active					2 (20%)	
Metaplasia, squamous					1 (10%)	
Seminal vesicle	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy					8 (80%)	9 (90%)
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy		7 (70%)	10 (100%)	10 (100%)	9 (90%)	10 (100%)
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Depletion cellular					8 (80%)	10 (100%)
Lymph node	(10)	(10)	(10)	(10)	(9)	(9)
Mediastinal, angiectasis				1 (10%)	1 (11%)	
Mediastinal, depletion lymphoid					1 (11%)	1 (11%)
Mediastinal, infiltration cellular, lymphocyte		1 (10%)				
Pancreatic, angiectasis		1 (10%)				
Lymph node, mandibular	(10)	(10)	(10)	(10)	(9)	(9)
Angiectasis		1 (10%)	1 (10%)			
Depletion lymphoid					9 (100%)	9 (100%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(7)	(9)
Angiectasis					2 (29%)	2 (22%)
Depletion lymphoid				1 (10%)	6 (86%)	9 (100%)
Spleen	(10)	(10)	(10)	(10)	(9)	(10)
Depletion lymphoid					7 (78%)	10 (100%)
Capsule, fibrosis		1 (10%)	4 (40%)	10 (100%)	5 (56%)	1 (10%)
Thymus	(10)	(10)	(9)	(10)	(9)	(9)
Depletion lymphoid			3 (33%)	2 (20%)	9 (100%)	9 (100%)
Integumentary System						
None						
Musculoskeletal System						
Bone	(10)		(2)	(10)	(9)	(10)
Metaphysis, atrophy					9 (100%)	10 (100%)
Skeletal muscle	(10)			(10)	(9)	(10)
Mineralization					1 (11%)	
Nervous System						
None						

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(9)	(10)
Bacterium					1 (11%)	
Fungus						1 (10%)
Hemorrhage	1 (10%)	2 (20%)			1 (11%)	2 (20%)
Inflammation, chronic active	8 (80%)	6 (60%)	7 (70%)	9 (90%)	6 (67%)	2 (20%)
Mediastinum, bacterium						1 (10%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(9)	(10)
Bacterium					2 (22%)	2 (20%)
Infarct					1 (11%)	
Inflammation, acute					1 (11%)	
Cortex, mineralization					3 (33%)	1 (10%)
Papilla, mineralization						1 (10%)
Renal tubule, regeneration	6 (60%)	6 (60%)	5 (50%)			1 (10%)
Urinary bladder	(10)	(1)	(1)	(9)	(7)	(9)
Calculus gross observation		1 (100%)	1 (100%)			
Calculus micro observation only		1 (100%)	1 (100%)			
Artery, mineralization	1 (10%)					

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund sacrifice					3	6
Natural death					2	4
Survivors						
Terminal sacrifice	10	10	10	10	5	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Bacterium						1 (10%)
Hepatodiaphragmatic nodule	3 (30%)	1 (10%)	3 (30%)		1 (10%)	1 (10%)
Inflammation, chronic active					1 (10%)	
Necrosis						1 (10%)
Mesentery	(1)					
Fat, necrosis	1 (100%)					
Pharynx					(1)	
Palate, bacterium					1 (100%)	
Palate, fungus					1 (100%)	
Palate, ulcer					1 (100%)	
Salivary glands	(10)			(10)	(10)	(10)
Atrophy					2 (20%)	8 (80%)
Stomach, forestomach	(10)			(10)	(10)	(10)
Hemorrhage						1 (10%)
Hyperplasia						1 (10%)
Stomach, glandular	(10)			(10)	(10)	(10)
Erosion					3 (30%)	2 (20%)
Mineralization						1 (10%)
Cardiovascular System						
Heart	(10)			(10)	(10)	(10)
Cardiomyopathy				1 (10%)		2 (20%)
Endocrine System						
Adrenal gland, cortex	(10)			(10)	(10)	(10)
Hemorrhage					3 (30%)	8 (80%)
Pituitary gland	(10)	(1)		(10)	(10)	(10)
Pars distalis, cyst		1 (100%)				
General Body System						
None						

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Genital System						
Clitoral gland	(10)	(10)	(10)	(8)	(10)	(8)
Atrophy				4 (50%)	8 (80%)	8 (100%)
Ovary	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy				6 (60%)	10 (100%)	10 (100%)
Cyst	2 (20%)	4 (40%)	2 (20%)			
Uterus	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy				8 (80%)	9 (90%)	10 (100%)
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Depletion cellular			1 (10%)	7 (70%)	6 (60%)	9 (90%)
Fibrosis, focal	2 (20%)	3 (30%)	5 (50%)	3 (30%)	1 (10%)	
Lymph node	(10)	(10)	(10)	(10)	(10)	(10)
Mediastinal, angiectasis						4 (40%)
Mediastinal, depletion lymphoid						5 (50%)
Pancreatic, angiectasis						1 (10%)
Pancreatic, infiltration cellular, histiocyte		1 (10%)				
Lymph node, mandibular	(10)	(10)	(10)	(10)	(10)	(10)
Angiectasis						1 (10%)
Depletion lymphoid					2 (20%)	5 (50%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(10)	(9)
Angiectasis					2 (20%)	5 (56%)
Depletion lymphoid					5 (50%)	8 (89%)
Infiltration cellular, histiocyte			2 (20%)			
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid			1 (10%)	1 (10%)	5 (50%)	10 (100%)
Fibrosis						1 (10%)
Capsule, fibrosis			3 (30%)	5 (50%)		
Thymus	(10)	(10)	(10)	(10)	(9)	(10)
Depletion lymphoid			1 (10%)	9 (90%)	7 (78%)	10 (100%)
Integumentary System						
None						
Musculoskeletal System						
Bone	(10)			(10)	(10)	(10)
Metaphysis, atrophy					10 (100%)	10 (100%)
Nervous System						
None						

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Edema						1 (10%)
Fungus						1 (10%)
Hemorrhage		3 (30%)		1 (10%)		2 (20%)
Inflammation, chronic active	1 (10%)	6 (60%)	4 (40%)	4 (40%)	4 (40%)	3 (30%)
Nose	(10)			(10)	(10)	(10)
Inflammation, acute					1 (10%)	
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Bacterium					1 (10%)	
Inflammation, acute					1 (10%)	1 (10%)
Cortex, mineralization	10 (100%)	8 (80%)	8 (80%)	8 (80%)	9 (90%)	4 (40%)
Renal tubule, regeneration		4 (40%)	2 (20%)		2 (20%)	

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund sacrifice						5
Survivors						
Terminal sacrifice	10	10	10	10	10	
Aborted						5
Animals examined microscopically	10	10	10	10	10	5
Alimentary System						
Intestine large, cecum	(10)				(10)	(5)
Parasite metazoan	2 (20%)				1 (10%)	
Intestine large, colon	(10)				(10)	(5)
Parasite metazoan	2 (20%)					1 (20%)
Intestine large, rectum	(10)				(10)	(5)
Parasite metazoan	1 (10%)					1 (20%)
Intestine small, ileum	(10)			(2)	(10)	(5)
Hyperplasia, lymphoid				2 (100%)		
Intestine small, jejunum	(10)			(1)	(10)	(5)
Hyperplasia, lymphoid				1 (100%)		
Liver	(10)	(10)	(10)	(10)	(10)	(5)
Hematopoietic cell proliferation					9 (90%)	
Hepatodiaphragmatic nodule	1 (10%)		1 (10%)	1 (10%)		
Inflammation, chronic, focal	1 (10%)				3 (30%)	
Hepatocyte, centrilobular, degeneration						5 (100%)
Kupffer cell, pigmentation					10 (100%)	5 (100%)
Pancreas	(10)	(1)			(10)	(5)
Acinus, atrophy	2 (20%)					
Pharynx						(1)
Palate, ulcer, acute, focal						1 (100%)
Salivary glands	(10)				(10)	(5)
Atrophy						5 (100%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(5)
Edema, focal						1 (20%)
Hyperplasia, focal, squamous						1 (20%)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(5)
Edema, focal						1 (20%)
Inflammation, focal						1 (20%)
Cardiovascular System						
Heart	(10)				(10)	(5)
Cardiomyopathy	10 (100%)				6 (60%)	
Inflammation, chronic, focal					1 (10%)	

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
Endocrine System						
Pituitary gland	(10)				(10)	(5)
Pars distalis, cyst	1 (10%)				1 (10%)	
General Body System						
None						
Genital System						
Epididymis	(10)	(10)	(10)	(10)	(10)	(5)
Aspermia					10 (100%)	5 (100%)
Granuloma sperm				1 (10%)		
Preputial gland	(9)	(10)	(10)	(10)	(10)	(5)
Atrophy					1 (10%)	5 (100%)
Cyst			1 (10%)	1 (10%)		
Dilatation	1 (11%)	1 (10%)				
Inflammation, chronic, focal	1 (11%)				1 (10%)	
Prostate	(10)	(10)	(10)	(10)	(10)	(5)
Atrophy			6 (60%)	7 (70%)	10 (100%)	5 (100%)
Seminal vesicle	(10)	(10)	(10)	(10)	(10)	(5)
Atrophy						4 (80%)
Testes	(10)	(10)	(10)	(10)	(10)	(5)
Atrophy				10 (100%)	10 (100%)	5 (100%)
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(5)
Atrophy						5 (100%)
Proliferation					10 (100%)	
Lymph node	(10)	(3)	(1)	(5)	(10)	(5)
Mediastinal, hemorrhage, acute	1 (10%)					
Pancreatic, hyperplasia, lymphoid				1 (20%)	2 (20%)	
Lymph node, mandibular	(10)	(2)	(1)	(4)	(10)	(5)
Atrophy						3 (60%)
Congestion		1 (50%)	1 (100%)	3 (75%)		
Hyperplasia, lymphoid			1 (100%)	1 (25%)		
Lymph node, mesenteric	(10)	(1)			(10)	(5)
Atrophy						5 (100%)
Pigmentation						1 (20%)
Spleen	(10)	(10)	(10)	(10)	(10)	(5)
Pigmentation, hemosiderin						5 (100%)
Capsule, mineralization, focal						4 (80%)
Lymphoid follicle, atrophy						4 (80%)
Red pulp, hematopoietic cell proliferation, diffuse				10 (100%)	10 (100%)	
Thymus	(10)	(1)	(2)	(10)	(10)	(3)
Atrophy					4 (40%)	2 (67%)
Congestion		1 (100%)	2 (100%)			

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
Integumentary System						
Skin	(10)				(10)	(5)
Prepuce, inflammation, acute						1 (20%)
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)				(10)	(5)
Inflammation, chronic, focal					2 (20%)	
Metaplasia, osseous	1 (10%)					
Alveolar epithelium, hyperplasia, focal					1 (10%)	
Alveolus, infiltration cellular, focal, histiocyte	3 (30%)					1 (20%)
Nose	(10)				(10)	(5)
Respiratory epithelium, inflammation, chronic	1 (10%)					
Respiratory epithelium, metaplasia, squamous	1 (10%)					
Special Senses System						
Harderian gland				(1)		
Hemorrhage, acute				1 (100%)		
Urinary System						
Kidney	(10)	(4)	(3)	(10)	(10)	(5)
Cyst		1 (25%)				
Cortex, mineralization	1 (10%)					4 (80%)
Renal tubule, dilatation						1 (20%)
Renal tubule, regeneration	10 (100%)	4 (100%)	3 (100%)	9 (90%)	6 (60%)	
Urinary bladder	(10)			(1)	(10)	(5)
Calculus micro observation only	1 (10%)			1 (100%)	1 (10%)	

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
Dispostion Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund sacrifice						7
Survivors						
Terminal sacrifice	10	10	10	10	10	
Aborted						3
Animals examined microscopically	10	10	10	10	10	7
Alimentary System						
Intestine large, cecum	(10)				(10)	(7)
Parasite metazoan					1 (10%)	
Intestine large, colon	(10)				(10)	(7)
Parasite metazoan					1 (10%)	
Parasite metazoan, chronic					1 (10%)	
Intestine large, rectum	(10)				(10)	(7)
Parasite metazoan	2 (20%)				2 (20%)	
Liver	(10)	(10)	(10)	(10)	(10)	(7)
Developmental malformation				1 (10%)		
Hematopoietic cell proliferation					9 (90%)	
Hepatodiaphragmatic nodule	2 (20%)		3 (30%)	3 (30%)	1 (10%)	
Bile duct, hyperplasia, focal			1 (10%)			
Hepatocyte, centrilobular, degeneration						6 (86%)
Kupffer cell, pigmentation					10 (100%)	7 (100%)
Pancreas	(10)				(10)	(7)
Acinus, atrophy, focal	1 (10%)					
Pharynx						(1)
Palate, ulcer						1 (100%)
Salivary glands	(10)				(10)	(7)
Atrophy						7 (100%)
Stomach, glandular	(10)	(10)	(9)	(10)	(10)	(7)
Ectopic tissue					1 (10%)	
Ulcer						2 (29%)
Cardiovascular System						
Heart	(10)				(10)	(7)
Cardiomyopathy	5 (50%)				2 (20%)	
Endocrine System						
None						
General Body System						
None						

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
Genital System						
Clitoral gland	(10)	(10)	(10)	(9)	(10)	(7)
Atrophy					1 (10%)	7 (100%)
Cyst	1 (10%)	2 (20%)				
Dilatation		1 (10%)				
Inflammation, chronic, focal	1 (10%)				1 (10%)	
Ovary	(10)	(10)	(10)	(10)	(10)	(7)
Atrophy						7 (100%)
Cyst	1 (10%)		1 (10%)	1 (10%)		
Uterus	(10)	(10)	(10)	(10)	(10)	(7)
Atrophy					9 (90%)	7 (100%)
Dilatation	2 (20%)					
Vagina	(10)	(10)	(10)	(10)	(10)	(7)
Epithelium, atrophy					1 (10%)	7 (100%)
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(7)
Atrophy						7 (100%)
Proliferation, diffuse					10 (100%)	
Lymph node	(10)			(3)	(10)	(7)
Mediastinal, atrophy						1 (14%)
Pancreatic, hyperplasia, lymphoid	1 (10%)					
Lymph node, mandibular	(10)			(2)	(10)	(7)
Atrophy						5 (71%)
Congestion				1 (50%)		
Lymph node, mesenteric	(10)				(9)	(7)
Atrophy						7 (100%)
Spleen	(10)	(10)	(10)	(10)	(10)	(7)
Pigmentation, hemosiderin						7 (100%)
Capsule, mineralization, focal						5 (71%)
Lymphoid follicle, atrophy						6 (86%)
Red pulp, hematopoietic cell proliferation, diffuse					10 (100%)	
Thymus	(10)			(10)	(10)	(6)
Atrophy					10 (100%)	6 (100%)
Integumentary System						
Skin	(10)	(1)		(1)	(10)	(7)
Foot, developmental malformation		1 (100%)				
Musculoskeletal System						
None						
Nervous System						
None						

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
Respiratory System						
Lung	(10)		(1)		(10)	(7)
Hemorrhage, acute, focal			1 (100%)		2 (20%)	
Inflammation, chronic, focal	4 (40%)				1 (10%)	
Alveolus, infiltration cellular, focal, histiocyte	2 (20%)				3 (30%)	2 (29%)
Special Senses System						
Eye	(1)					
Lens, cataract	1 (100%)					
Urinary System						
Kidney	(10)	(1)			(10)	(7)
Cyst, multiple		1 (100%)				
Developmental malformation					1 (10%)	
Inflammation, chronic, focal	2 (20%)				1 (10%)	
Cortex, mineralization	10 (100%)				10 (100%)	7 (100%)
Renal tubule, necrosis, focal						1 (14%)
Renal tubule, regeneration	1 (10%)				4 (40%)	

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A5 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Cytoplasmic alteration		4 (40%)	8 (80%)	7 (70%)	10 (100%)	10 (100%)
Degeneration				8 (80%)	8 (80%)	10 (100%)
Hepatodiaphragmatic nodule	1 (10%)	2 (20%)	3 (30%)	3 (30%)	1 (10%)	2 (20%)
Pigmentation						7 (70%)
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy	6 (60%)					10 (100%)
Endocrine System						
Pituitary gland	(10)					(10)
Pars distalis, cyst	1 (10%)					
General Body System						
None						
Genital System						
Seminal vesicle	(10)			(1)		(10)
Atrophy				1 (100%)		
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia				2 (20%)	2 (20%)	8 (80%)
Lymph node	(10)			(1)	(2)	(10)
Mediastinal, angiectasis				1 (100%)		
Pancreatic, infiltration cellular, histiocyte					1 (50%)	
Lymph node, mandibular	(10)			(1)	(1)	(10)
Angiectasis				1 (100%)	1 (100%)	
Pigmentation						1 (10%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation					2 (20%)	2 (20%)
Pigmentation			2 (20%)	10 (100%)	8 (80%)	10 (100%)

TABLE A5 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)					(10)
Hemorrhage	1 (10%)					2 (20%)
Infiltration cellular, histiocyte	1 (10%)					
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Cortex, mineralization	1 (10%)			2 (20%)	4 (40%)	1 (10%)
Renal tubule, regeneration	6 (60%)	6 (60%)	5 (50%)	7 (70%)	9 (90%)	5 (50%)
Urinary bladder	(10)	(3)	(1)	(1)	(1)	(10)
Calculus gross observation	1 (10%)	3 (100%)	1 (100%)		1 (100%)	
Calculus micro observation only	1 (10%)	3 (100%)	1 (100%)		1 (100%)	

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A6 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Cytoplasmic alteration		5 (50%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)
Degeneration				10 (100%)	10 (100%)	10 (100%)
Hepatodiaphragmatic nodule	2 (20%)			1 (10%)	1 (10%)	
Pigmentation			2 (20%)	10 (100%)	10 (100%)	10 (100%)
Cardiovascular System						
Heart	(10)					(9)
Cardiomyopathy	1 (10%)					
Endocrine System						
Pituitary gland	(10)					(10)
Pars distalis, cyst						1 (10%)
General Body System						
None						
Genital System						
Ovary	(10)	(10)	(9)	(10)	(10)	(10)
Cyst			4 (44%)			1 (10%)
Uterus	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy				1 (10%)	9 (90%)	8 (80%)
Dilatation		2 (20%)				

TABLE A6 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia					4 (40%)	3 (30%)
Lymph node	(10)		(1)			(10)
Pancreatic, infiltration cellular, histiocyte			1 (100%)			1 (10%)
Lymph node, mandibular	(10)					(10)
Angiectasis						1 (10%)
Infiltration cellular, histiocyte						1 (10%)
Lymph node, mesenteric	(10)					(8)
Infiltration cellular, histiocyte						1 (13%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Congestion					1 (10%)	
Hematopoietic cell proliferation					6 (60%)	10 (100%)
Pigmentation			1 (10%)	9 (90%)	10 (100%)	9 (90%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(9)					(10)
Hemorrhage	1 (11%)					
Infiltration cellular, histiocyte						3 (30%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Cortex, mineralization	10 (100%)	8 (80%)	8 (80%)	4 (40%)	7 (70%)	8 (80%)
Renal tubule, regeneration	3 (30%)	4 (40%)	8 (80%)	5 (50%)	8 (80%)	5 (50%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

APPENDIX B

Summary of Nonneoplastic Lesions in Mice

TABLE B1 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	0 ppm	2000 ppm	4000 ppm	6000 ppm	8000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(2)	(1)	(1)	(3)	(10)
Developmental malformation, focal						1 (10%)
Hematopoietic cell proliferation, focal	3 (30%)	1 (50%)				
Centrilobular, fatty change	1 (10%)		1 (100%)	1 (100%)	1 (33%)	
Tongue					(1)	
Mucosa, epithelium, hyperkeratosis, focal					1 (100%)	
Cardiovascular System						
None						
Endocrine System						
Parathyroid gland	(9)					(7)
Unilateral, cyst	1 (11%)					
General Body System						
None						
Genital System						
Preputial gland	(10)		(1)			(10)
Cyst			1 (100%)			
Testes	(10)	(9)	(10)	(10)	(10)	(10)
Seminiferous tubule, atrophy			3 (30%)	10 (100%)	10 (100%)	10 (100%)
Hematopoietic System						
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Red pulp, hematopoietic cell proliferation			10 (100%)	9 (90%)	9 (90%)	10 (100%)
Thymus	(9)			(10)	(10)	(10)
Depletion lymphoid					6 (60%)	9 (90%)
Integumentary System						
None						

TABLE B1 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	2000 ppm	4000 ppm	6000 ppm	8000 ppm	10,000 ppm
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(1)	(2)	(2)		(10)
Congestion, focal			1 (50%)			
Hemorrhage, focal	4 (40%)	1 (100%)	1 (50%)	1 (50%)		2 (20%)
Special Senses System						
None						
Urinary System						
Kidney	(10)		(1)			(10)
Mineralization, focal	1 (10%)					
Interstitium, inflammation, focal, subacute						1 (10%)
Urinary bladder	(10)			(3)		(10)
Calculus gross observation				3 (100%)		
Lumen, calculus micro observation only				3 (100%)		

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE B2 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	0 ppm	2000 ppm	4000 ppm	6000 ppm	8000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)				(1)	(10)
Hematopoietic cell proliferation, focal	4 (40%)					
Centrilobular, fatty change	1 (10%)					
Parenchyma, ectopic tissue					1 (100%)	
Salivary glands	(10)					(10)
Inflammation, focal, subacute	2 (20%)					
Cardiovascular System						
None						
Endocrine System						
Adrenal gland, cortex	(10)	(10)	(9)	(10)	(10)	(10)
Zona reticularis, hypertrophy		10 (100%)	9 (100%)	10 (100%)	10 (100%)	10 (100%)
Parathyroid gland	(6)					(2)
Unilateral, cyst	1 (17%)					
General Body System						
None						
Genital System						
Ovary	(10)		(1)		(10)	(8)
Atrophy						5 (63%)
Periovarian tissue, inflammation, chronic, focal			1 (100%)			
Uterus	(10)	(10)	(10)	(10)	(10)	(8)
Endometrium, atrophy						1 (13%)
Lumen, dilatation	1 (10%)					
Hematopoietic System						
Lymph node, mandibular	(10)					(9)
Hyperplasia, lymphoid	1 (10%)					
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Red pulp, hematopoietic cell proliferation		5 (50%)	10 (100%)	8 (80%)	9 (90%)	10 (100%)
Thymus	(9)				(10)	(10)
Depletion lymphoid						4 (40%)

TABLE B2 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	2000 ppm	4000 ppm	6000 ppm	8000 ppm	10,000 ppm
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(1)	(1)	(1)	(2)	(10)
Congestion				1 (100%)		
Hemorrhage, focal	2 (20%)				2 (100%)	3 (30%)
Special Senses System						
None						
Urinary System						
None						

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE B3 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	0 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, acute, focal	1 (10%)					
Mesentery				(1)		
Hemorrhage, focal				1 (100%)		
Fat, necrosis, focal				1 (100%)		
Pancreas	(10)			(1)		(10)
Duct, cyst, focal				1 (100%)		
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
Epididymis	(10)	(10)	(10)	(10)	(10)	(10)
Aspermia						10 (100%)
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Seminiferous tubule, atrophy						10 (100%)
Hematopoietic System						
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Red pulp, hematopoietic cell proliferation						10 (100%)
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE B3 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
Nervous System						
None						
Respiratory System						
Lung	(10)				(3)	(10)
Bronchiole, inflammation, acute						1 (10%)
Interstitium, inflammation, acute, *multifocal*					3 (100%)	
Nose	(10)					(10)
Exudate						1 (10%)
Special Senses System						
None						
Urinary System						
Urinary bladder	(10)				(1)	(10)
Calculus gross observation	1 (10%)				1 (100%)	
Calculus micro observation only	1 (10%)				1 (100%)	

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE B4 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	0 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, acute, focal	2 (20%)					
Centrilobular, hypertrophy		1 (10%)				
Cardiovascular System						
None						
Endocrine System						
Adrenal gland, cortex	(10)	(10)	(10)	(10)	(10)	(10)
Zona reticularis, hypertrophy			1 (10%)	8 (80%)	10 (100%)	9 (90%)
General Body System						
None						
Genital System						
Ovary	(10)		(10)	(10)	(10)	(10)
Bilateral, interstitium, atrophy						2 (20%)
Hematopoietic System						
Lymph node, mandibular	(10)		(1)			(10)
Hyperplasia, lymphoid			1 (100%)			
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia, lymphoid					2 (20%)	
Red pulp, hematopoietic cell proliferation				1 (10%)	9 (90%)	10 (100%)
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE B4 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol *(continued)*

	0 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
Nervous System						
None						
Respiratory System						
Lung	(10)		(1)		(1)	(10)
Hemorrhage, focal	2 (20%)		1 (100%)		1 (100%)	2 (20%)
Special Senses System						
None						
Urinary System						
None						

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE B5 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	1		3	7	10
Alimentary System						
None						
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
Preputial gland	(10)					(10)
Hyperplasia	1 (10%)					
Hematopoietic System						
Lymph node, mandibular	(10)				(1)	(10)
Congestion	1 (10%)					1 (10%)
Lymph node, mesenteric	(9)					(10)
Hyperplasia						1 (10%)
Spleen	(10)				(1)	(10)
Developmental malformation					1 (100%)	
Integumentary System						
Skin	(10)					(10)
Sebaceous gland, hyperplasia	1 (10%)					
Musculoskeletal System						
None						
Nervous System						
None						

TABLE B5 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Respiratory System						
Lung	(10)			(1)		(10)
Hemorrhage, focal	1 (10%)			1 (100%)		
Special Senses System						
None						
Urinary System						
Urinary bladder	(10)					(10)
Calculus micro observation only	1 (10%)					2 (20%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE B6 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	2		10	10	10
Alimentary System						
Liver	(10)	(1)		(1)		(10)
Inflammation, focal, subacute	2 (20%)					2 (20%)
Salivary glands	(10)					(10)
Parotid gland, inflammation, focal, subacute						1 (10%)
Stomach, forestomach	(10)					(10)
Hyperplasia, focal						1 (10%)
Cardiovascular System						
None						
Endocrine System						
Parathyroid gland	(8)					(8)
Unilateral, cyst	1 (13%)					
General Body System						
None						
Genital System						
Uterus	(10)			(2)	(1)	(10)
Endometrium, hyperplasia				1 (50%)	1 (100%)	
Vagina	(10)			(10)	(10)	(10)
Developmental malformation						1 (10%)
Hematopoietic System						
Spleen	(10)	(1)				(10)
Hyperplasia, lymphoid		1 (100%)				
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE B6 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
Nervous System						
None						
Respiratory System						
Lung	(10)				(1)	(10)
Hemorrhage, focal					1 (100%)	3 (30%)
Special Senses System						
None						
Urinary System						
None						

[1] Number of animals examined microscopically at site and number of animals with lesion.

APPENDIX C

Organ Weights and Organ-Weight-to-Body-Weight Ratios

TABLE C1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
n	10	10	10	10	10	10
Necropsy body wt						
2-Methoxyethanol	316 ± 7	295 ± 7	260 ± 5**	214 ± 5**	136 ± 20**[3]	—[4]
2-Ethoxyethanol	315 ± 5	309 ± 4	296 ± 4**	295 ± 8*	236 ± 5**	—
2-Butoxyethanol	308 ± 6	315 ± 4	309 ± 4	298 ± 3	280 ± 5**	264 ± 5**
Heart						
2-Methoxyethanol						
Absolute	1.084 ± 0.075	1.299 ± 0.105	1.120 ± 0.077	0.925 ± 0.045	0.705 ± 0.127[3]	—
Relative	3.42 ± 0.19	4.40 ± 0.32*	4.29 ± 0.23*	4.33 ± 0.21**	5.15 ± 0.18*[3]	—
2-Ethoxyethanol						
Absolute	1.240 ± 0.080	1.323 ± 0.053	1.235 ± 0.050	1.138 ± 0.054	1.071 ± 0.037	—
Relative	3.93 ± 0.22	4.30 ± 0.20	4.18 ± 0.16	3.86 ± 0.15	4.55 ± 0.16	—
2-Butoxyethanol						
Absolute	1.125 ± 0.050	1.162 ± 0.035	1.070 ± 0.026	1.148 ± 0.033	1.100 ± 0.033	1.061 ± 0.042
Relative	3.66 ± 0.19	3.69 ± 0.10	3.47 ± 0.06	3.85 ± 0.11	3.94 ± 0.13	4.03 ± 0.15
Right kidney						
2-Methoxyethanol						
Absolute	1.105 ± 0.034	1.051 ± 0.040	0.959 ± 0.023**	0.780 ± 0.027**	0.630 ± 0.060**[3]	—
Relative	3.50 ± 0.05	3.56 ± 0.07	3.70 ± 0.08	3.66 ± 0.13*	4.66 ± 0.24**[3]	—
2-Ethoxyethanol						
Absolute	1.079 ± 0.032	1.084 ± 0.028	1.043 ± 0.021	1.031 ± 0.028	0.901 ± 0.023**	—
Relative	3.42 ± 0.08	3.51 ± 0.06	3.53 ± 0.06	3.50 ± 0.04	3.83 ± 0.07**	—
2-Butoxyethanol						
Absolute	1.101 ± 0.028	1.255 ± 0.031	1.210 ± 0.031	1.093 ± 0.023	1.076 ± 0.016	1.074 ± 0.034
Relative	3.57 ± 0.04	3.98 ± 0.06**	3.91 ± 0.06**	3.66 ± 0.06*	3.85 ± 0.04**	4.07 ± 0.09**
Liver						
2-Methoxyethanol						
Absolute	10.16 ± 0.38	8.94 ± 0.41	7.93 ± 0.19**	6.87 ± 0.18**	5.04 ± 0.80**[3]	—
Relative	32.10 ± 0.70	30.30 ± 0.92	30.60 ± 0.57	32.20 ± 0.64	36.90 ± 0.43[3]	—
2-Ethoxyethanol						
Absolute	10.15 ± 0.31	9.95 ± 0.32	9.27 ± 0.19*	9.39 ± 0.30	6.51 ± 0.13**	—
Relative	32.20 ± 0.72	32.20 ± 0.93	31.40 ± 0.57	31.90 ± 0.59	27.60 ± 0.32**	—
2-Butoxyethanol						
Absolute	10.37 ± 0.35	10.93 ± 0.26	10.68 ± 0.23	10.35 ± 0.16	10.02 ± 0.25	9.71 ± 0.31
Relative	33.60 ± 0.56	34.70 ± 0.41	34.60 ± 0.69	34.70 ± 0.44	35.80 ± 0.45**	36.80 ± 0.86**
Lung						
2-Methoxyethanol						
Absolute	1.728 ± 0.024	1.852 ± 0.064	1.511 ± 0.066*	1.404 ± 0.076**	1.582 ± 0.194[3]	—
Relative	5.49 ± 0.14	6.28 ± 0.11**	5.81 ± 0.20*	6.53 ± 0.23**	11.66 ± 0.28**[3]	—
2-Ethoxyethanol						
Absolute	1.626 ± 0.094	1.679 ± 0.077	1.746 ± 0.100	1.543 ± 0.055[5]	1.374 ± 0.041*	—
Relative	5.15 ± 0.25	5.45 ± 0.26	5.91 ± 0.31	5.28 ± 0.13[5]	5.85 ± 0.19*	—
2-Butoxyethanol						
Absolute	1.710 ± 0.109	1.819 ± 0.125	1.585 ± 0.064	1.925 ± 0.195	1.507 ± 0.057	1.395 ± 0.022**
Relative	5.52 ± 0.28	5.78 ± 0.39	5.14 ± 0.21	6.47 ± 0.67	5.39 ± 0.16	5.31 ± 0.12

TABLE C1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
Right testis						
2-Methoxyethanol						
Absolute	1.398 ± 0.048	1.411 ± 0.019	0.603 ± 0.044**	0.442 ± 0.032**	0.254 ± 0.010**[3]	—
Relative	4.44 ± 0.15	4.81 ± 0.09	2.31 ± 0.14**	2.07 ± 0.15**	1.89 ± 0.20*[3]	—
2-Ethoxyethanol						
Absolute	1.394 ± 0.022	1.431 ± 0.023	1.443 ± 0.016	1.342 ± 0.025	0.618 ± 0.042**	—
Relative	4.43 ± 0.05	4.64 ± 0.05	4.89 ± 0.06	4.56 ± 0.09	2.62 ± 0.18*	—
2-Butoxyethanol						
Absolute	1.399 ± 0.036	1.424 ± 0.020	1.407 ± 0.023	1.425 ± 0.021	1.352 ± 0.010	1.396 ± 0.013
Relative	4.54 ± 0.07	4.52 ± 0.04	4.56 ± 0.06	4.78 ± 0.08*	4.85 ± 0.08**	5.31 ± 0.10**
Thymus						
2-Methoxyethanol						
Absolute	0.268 ± 0.026	0.198 ± 0.017*	0.160 ± 0.016**	0.095 ± 0.016**	0.072 ± 0.005**[3]	—
Relative	0.85 ± 0.080	0.67 ± 0.05	0.61 ± 0.06	0.45 ± 0.07**	0.53 ± 0.04[3]	—
2-Ethoxyethanol						
Absolute	0.299 ± 0.019	0.270 ± 0.021	0.213 ± 0.005**	0.258 ± 0.010**	0.154 ± 0.011**	—
Relative	0.95 ± 0.05	0.87 ± 0.06	0.72 ± 0.02**	0.87 ± 0.02*	0.65 ± 0.05**	—
2-Butoxyethanol						
Absolute	0.309 ± 0.012	0.294 ± 0.017	0.291 ± 0.013	0.327 ± 0.022	0.256 ± 0.013**	0.262 ± 0.017*
Relative	1.01 ± 0.04	0.93 ± 0.05	0.94 ± 0.04	1.10 ± 0.08	0.92 ± 0.04	0.99 ± 0.06

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] Doses administered to rats given 2-methoxyethanol or 2-butoxyethanol were: 0, 750, 1500, 3000, 4500, or 6000 ppm. Doses administered to rats given 2-ethoxyethanol were: 0, 1250, 2500, 5000, 10,000, or 20,000 ppm.

[3] n=2.

[4] n=0.

[5] n=9.

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.

** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female F344/N Rats in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
n	10	10	10	10	10	10
Necropsy body wt						
2-Methoxyethanol	189 ± 4	189 ± 2	170 ± 3**	145 ± 3**	151 ± 2**[3]	–[4]
2-Ethoxyethanol	185 ± 3	183 ± 3	177 ± 1	173 ± 3**	149 ± 1**	–
2-Butoxyethanol	186 ± 4	186 ± 3	181 ± 2	172 ± 2**	160 ± 2**	145 ± 2**
Heart						
2-Methoxyethanol						
Absolute	0.740 ± 0.028	0.702 ± 0.013	0.703 ± 0.033	0.653 ± 0.023*	0.649 ± 0.028[3]	–
Relative	3.94 ± 0.19	3.71 ± 0.09	4.17 ± 0.24	4.50 ± 0.14*	4.31 ± 0.18[3]	–
2-Ethoxyethanol						
Absolute	0.688 ± 0.033	0.717 ± 0.036	0.671 ± 0.012	0.674 ± 0.029	0.616 ± 0.019	–
Relative	3.73 ± 0.17	3.90 ± 0.15	3.80 ± 0.06	3.88 ± 0.13	4.14 ± 0.12**	–
2-Butoxyethanol						
Absolute	0.704 ± 0.028	0.697 ± 0.022	0.716 ± 0.027	0.686 ± 0.022	0.613 ± 0.019*	0.573 ± 0.016**
Relative	3.81 ± 0.20	3.75 ± 0.11	3.95 ± 0.16	3.99 ± 0.12	3.84 ± 0.11	3.94 ± 0.08
Right kidney						
2-Methoxyethanol						
Absolute	0.644 ± 0.015	0.656 ± 0.027	0.595 ± 0.016	0.544 ± 0.023**	0.577 ± 0.017*[3]	–
Relative	3.41 ± 0.07	3.46 ± 0.12	3.51 ± 0.08	3.76 ± 0.18**	3.83 ± 0.09**[3]	–
2-Ethoxyethanol						
Absolute	0.678 ± 0.008	0.683 ± 0.020	0.669 ± 0.010	0.663 ± 0.005	0.636 ± 0.010**	–
Relative	3.68 ± 0.07	3.74 ± 0.16	3.79 ± 0.07	3.83 ± 0.05	4.28 ± 0.06**	–
2-Butoxyethanol						
Absolute	0.668 ± 0.015	0.762 ± 0.010	0.759 ± 0.026	0.662 ± 0.011	0.668 ± 0.012	0.645 ± 0.011
Relative	3.59 ± 0.07	4.10 ± 0.06**	4.18 ± 0.12**	3.84 ± 0.04**	4.19 ± 0.09**	4.45 ± 0.09**
Liver						
2-Methoxyethanol						
Absolute	5.70 ± 0.13	5.66 ± 0.14	4.79 ± 0.17**	4.44 ± 0.21**	4.79 ± 0.33**[3]	–
Relative	30.10 ± 0.59	29.90 ± 0.67	28.20 ± 0.88	30.70 ± 1.70	31.80 ± 2.18[3]	–
2-Ethoxyethanol						
Absolute	5.38 ± 0.09	5.24 ± 0.17	5.08 ± 0.10	5.00 ± 0.10*	5.05 ± 0.11*	–
Relative	29.20 ± 0.65	28.50 ± 0.65	28.80 ± 0.45	28.90 ± 0.45	33.90 ± 0.72**	–
2-Butoxyethanol						
Absolute	5.56 ± 0.15	6.04 ± 0.16	6.00 ± 0.12	5.36 ± 0.10	5.13 ± 0.10	4.99 ± 0.11*
Relative	29.90 ± 0.54	32.50 ± 0.81*	33.00 ± 0.48**	31.10 ± 0.61*	32.20 ± 0.66*	34.40 ± 0.71**
Lung						
2-Methoxyethanol						
Absolute	1.133 ± 0.028	1.281 ± 0.049	1.167 ± 0.023	1.060 ± 0.039	1.061 ± 0.106[3]	–
Relative	5.99 ± 0.16	6.76 ± 0.20**	6.90 ± 0.20**	7.30 ± 0.24**	7.04 ± 0.66**[3]	–
2-Ethoxyethanol						
Absolute	1.109 ± 0.038	1.059 ± 0.028	1.102 ± 0.028	1.109 ± 0.048	0.991 ± 0.029*	–
Relative	6.01 ± 0.20	5.78 ± 0.13	6.24 ± 0.14	6.42 ± 0.30	6.66 ± 0.18**	–
2-Butoxyethanol						
Absolute	1.134 ± 0.035	1.056 ± 0.036	1.173 ± 0.037	1.134 ± 0.047	1.113 ± 0.095	1.025 ± 0.053
Relative	6.13 ± 0.25	5.68 ± 0.20	6.46 ± 0.19	6.59 ± 0.26	6.98 ± 0.59	7.05 ± 0.31*

TABLE C2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female F344/N Rats in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
Thymus						
2-Methoxyethanol						
Absolute	0.224 ± 0.010	0.180 ± 0.012*	0.125 ± 0.010**	0.084 ± 0.008**	0.099 ± 0.011**[3]	—
Relative	1.19 ± 0.06	0.95 ± 0.06**	0.74 ± 0.06**	0.57 ± 0.06**	0.66 ± 0.07**[3]	—
2-Ethoxyethanol						
Absolute	0.214 ± 0.013	0.210 ± 0.007	0.221 ± 0.013	0.186 ± 0.009	0.069 ± 0.006**	—
Relative	1.16 ± 0.07	1.15 ± 0.04	1.25 ± 0.08	1.07 ± 0.05	0.47 ± 0.04**	—
2-Butoxyethanol						
Absolute	0.233 ± 0.015	0.232 ± 0.013	0.237 ± 0.013	0.213 ± 0.005	0.242 ± 0.015	0.173 ± 0.012*
Relative	1.26 ± 0.08	1.25 ± 0.08	1.31 ± 0.08	1.24 ± 0.03	1.51 ± 0.09	1.19 ± 0.09

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] Doses administered to rats given 2-methoxyethanol or 2-butoxyethanol were: 0, 750, 1500, 3000, 4500, or 6000 ppm. Doses administered to rats given 2-ethoxyethanol were: 0, 1250, 2500, 5000, 10,000, or 20,000 ppm.

[3] n=5.

[4] n=0.

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

TABLE C3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats in the Stop-Exposure Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]			
	Dose 1	Dose 2	Dose 3	Dose 4
60-Day Treatment Period				
n	10	10	10	10
Necropsy body wt				
2-Methoxyethanol	294 ± 4	248 ± 6**	228 ± 7**	–[3]
2-Ethoxyethanol	306 ± 7	285 ± 6*	259 ± 5**	138 ± 21**[4]
2-Butoxyethanol	299 ± 5	282 ± 6	275 ± 4**	255 ± 4**
Right testis				
2-Methoxyethanol				
Absolute	1.412 ± 0.016	0.644 ± 0.028**	0.433 ± 0.015**	–
Relative	4.81 ± 0.07	2.59 ± 0.09**	1.90 ± 0.06**	–
2-Ethoxyethanol				
Absolute	1.368 ± 0.019	1.400 ± 0.016	0.609 ± 0.044**	0.361 ± 0.096**[4]
Relative	4.48 ± 0.09	4.93 ± 0.10	2.37 ± 0.19**	2.51 ± 0.27*[4]
2-Butoxyethanol				
Absolute	1.47 ± 0.02	1.38 ± 0.02**	1.35 ± 0.02**	1.34 ± 0.01**
Relative	4.91 ± 0.05	4.90 ± 0.08	4.91 ± 0.07	5.25 ± 0.07**
Epididymis				
2-Methoxyethanol				
Absolute	0.485 ± 0.017	0.281 ± 0.019**	0.237 ± 0.015**	–
Relative	1.65 ± 0.06	1.13 ± 0.07**	1.04 ± 0.05**	–
2-Ethoxyethanol				
Absolute	0.441 ± 0.012	0.420 ± 0.014	0.228 ± 0.012**	0.114 ± 0.018**[4]
Relative	1.44 ± 0.03	1.48 ± 0.06	0.88 ± 0.04**	0.83 ± 0.06**[4]
2-Butoxyethanol				
Absolute	0.472 ± 0.012	0.465 ± 0.016	0.450 ± 0.011	0.446 ± 0.014
Relative	1.58 ± 0.03	1.66 ± 0.07	1.64 ± 0.04	1.75 ± 0.04**
30-Day Recovery Period				
n	10	10	10	10
Necropsy body wt				
2-Methoxyethanol	339 ± 7	311 ± 7*	278 ± 5**	–
2-Ethoxyethanol	339 ± 8	339 ± 6	303 ± 3**	237 ± 37**[5]
2-Butoxyethanol	332 ± 6	331 ± 5	321 ± 11	329 ± 7
Right testis				
2-Methoxyethanol				
Absolute	1.432 ± 0.022	0.846 ± 0.051**	0.442 ± 0.015**	–
Relative	4.23 ± 0.07	2.74 ± 0.18**	1.59 ± 0.05**	–
2-Ethoxyethanol				
Absolute	1.460 ± 0.030	1.415 ± 0.021	0.652 ± 0.029**	0.395 ± 0.038**[5]
Relative	4.32 ± 0.05	4.19 ± 0.10	2.15 ± 0.10**	1.72 ± 0.10**[5]
2-Butoxyethanol				
Absolute	1.43 ± 0.02	1.48 ± 0.01	1.46 ± 0.03	1.40 ± 0.03
Relative	4.31 ± 0.07	4.48 ± 0.06	4.61 ± 0.20	4.26 ± 0.15

TABLE C3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats in the Stop-Exposure Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose			
	Dose 1	Dose 2	Dose 3	Dose 4
30-Day Recovery Period (continued)				
Epididymis				
2-Methoxyethanol				
Absolute	0.480 ± 0.015	0.308 ± 0.012**	0.261 ± 0.011**	—
Relative	1.41 ± 0.04	1.00 ± 0.05**	0.94 ± 0.04**	—
2-Ethoxyethanol				
Absolute	0.507 ± 0.018	0.497 ± 0.017	0.311 ± 0.015**	0.204 ± 0.014**[5]
Relative	1.49 ± 0.04	1.47 ± 0.05	1.03 ± 0.05**	0.91 ± 0.11**[5]
2-Butoxyethanol				
Absolute	0.520 ± 0.034	0.445 ± 0.020	0.475 ± 0.019	0.464 ± 0.017
Relative	1.57 ± 0.10	1.34 ± 0.05	1.50 ± 0.08	1.41 ± 0.06
56-Day Recovery Period				
n	10	10	10	5
Necropsy body wt				
2-Methoxyethanol	381 ± 8	343 ± 6**	324 ± 7**	—
2-Ethoxyethanol	384 ± 6	362 ± 8*	352 ± 6**[6]	272 ± 29**
Right testis				
2-Methoxyethanol				
Absolute	1.534 ± 0.024	0.914 ± 0.057**	0.478 ± 0.044**	—
Relative	4.04 ± 0.08	2.66 ± 0.14**	1.47 ± 0.12**	—
2-Ethoxyethanol				
Absolute	1.486 ± 0.022	1.362 ± 0.026**	0.678 ± 0.044**[6]	0.444 ± 0.023**
Relative	3.88 ± 0.07	3.77 ± 0.06	1.92 ± 0.12**[6]	1.72 ± 0.23**
Epididymis				
2-Methoxyethanol				
Absolute	0.544 ± 0.016	0.366 ± 0.025**	0.277 ± 0.016**	—
Relative	1.43 ± 0.05	1.06 ± 0.06**	0.86 ± 0.05**	—
2-Ethoxyethanol				
Absolute	0.533 ± 0.015	0.544 ± 0.021	0.319 ± 0.019**[6]	0.255 ± 0.024**
Relative	1.39 ± 0.04	1.51 ± 0.06	0.91 ± 0.05**[6]	0.95 ± 0.04**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).
[2] Doses administered to rats given 2-methoxyethanol or 2-butoxyethanol were: 0, 1500, 3000, or 6000 ppm. Doses administered to rats given 2-ethoxyethanol were: 0, 5000, 10,000, or 20,000 ppm.
[3] Data not available due to 100% mortality in the 6000 ppm 2-methoxyethanol group.
[4] n=4.
[5] n=5.
[6] n=9.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male B6C3F$_1$ Mice in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
n	10	10	10	10	10	10
Necropsy body wt						
2-Methoxyethanol	39.2 ± 0.8	39.6 ± 0.8	40.8 ± 0.8	37.8 ± 0.9	37.1 ± 0.8[3]	30.1 ± 1.0**
2-Ethoxyethanol	38.9 ± 0.8	40.9 ± 0.8	43.0 ± 1.1	40.5 ± 0.9	33.6 ± 0.9*	31.9 ± 0.7**
2-Butoxyethanol	40.2 ± 1.6	40.1 ± 0.7	40.2 ± 0.5	38.4 ± 0.9	39.1 ± 0.8	38.3 ± 0.8
Heart						
2-Methoxyethanol						
Absolute	0.165 ± 0.006	0.173 ± 0.004	0.168 ± 0.003	0.172 ± 0.005	0.172 ± 0.004[3]	0.165 ± 0.004
Relative	4.24 ± 0.21	4.40 ± 0.15	4.13 ± 0.12	4.55 ± 0.11	4.64 ± 0.08*[3]	5.52 ± 0.19**
2-Ethoxyethanol						
Absolute	0.183 ± 0.007	0.192 ± 0.007	0.198 ± 0.006	0.196 ± 0.008	0.171 ± 0.007	0.171 ± 0.007
Relative	4.70 ± 0.15	4.70 ± 0.19	4.63 ± 0.16	4.84 ± 0.19	5.15 ± 0.28	5.36 ± 0.16*
2-Butoxyethanol						
Absolute	0.173 ± 0.005	0.190 ± 0.006	0.186 ± 0.007[3]	0.179 ± 0.011	0.182 ± 0.009	0.175 ± 0.008
Relative	4.36 ± 0.20	4.74 ± 0.12	4.66 ± 0.18[3]	4.64 ± 0.23	4.64 ± 0.18	4.56 ± 0.16
Right kidney						
2-Methoxyethanol						
Absolute	0.288 ± 0.012	0.288 ± 0.006	0.293 ± 0.011	0.290 ± 0.007	0.340 ± 0.012**[3]	0.310 ± 0.013*
Relative	7.36 ± 0.30	7.30 ± 0.12	7.20 ± 0.29	7.66 ± 0.09	9.17 ± 0.23**[3]	10.32 ± 0.30**
2-Ethoxyethanol						
Absolute	0.341 ± 0.010	0.379 ± 0.010	0.367 ± 0.014	0.332 ± 0.012	0.331 ± 0.011	0.343 ± 0.010
Relative	8.78 ± 0.27	9.27 ± 0.20	8.54 ± 0.23	8.22 ± 0.32	9.88 ± 0.34*	10.75 ± 0.22**
2-Butoxyethanol						
Absolute	0.319 ± 0.009	0.385 ± 0.012	0.377 ± 0.007[3]	0.306 ± 0.007	0.319 ± 0.010	0.307 ± 0.008
Relative	7.98 ± 0.16	9.59 ± 0.20	9.41 ± 0.13[3]	7.99 ± 0.17	8.15 ± 0.18	8.02 ± 0.12
Liver						
2-Methoxyethanol						
Absolute	1.46 ± 0.09	1.50 ± 0.05	1.72 ± 0.05*	1.72 ± 0.07*	1.81 ± 0.07**[3]	1.58 ± 0.08*
Relative	37.30 ± 2.00	37.80 ± 0.71	42.20 ± 1.29*	45.20 ± 0.95**	48.80 ± 1.19**[3]	52.20 ± 1.50**
2-Ethoxyethanol						
Absolute	1.85 ± 0.06	2.03 ± 0.06	2.24 ± 0.10	2.00 ± 0.08	1.56 ± 0.07	1.75 ± 0.06
Relative	47.70 ± 1.42	49.50 ± 0.69	51.90 ± 1.38*	49.40 ± 1.59	46.30 ± 1.53	54.90 ± 1.23**
2-Butoxyethanol						
Absolute	1.56 ± 0.11	1.77 ± 0.04*	1.79 ± 0.03*[3]	1.48 ± 0.05	1.70 ± 0.07	1.58 ± 0.06
Relative	38.50 ± 1.45	44.20 ± 0.72**	44.70 ± 0.80**[3]	38.50 ± 0.85	43.30 ± 1.39	41.20 ± 1.11
Lung						
2-Methoxyethanol						
Absolute	0.246 ± 0.009	0.271 ± 0.022[3]	0.267 ± 0.013	0.267 ± 0.016	0.263 ± 0.013[3]	0.235 ± 0.006
Relative	6.26 ± 0.15	6.93 ± 0.68[3]	6.56 ± 0.33	7.07 ± 0.45	7.06 ± 0.22*[3]	7.90 ± 0.38**
2-Ethoxyethanol						
Absolute	0.256 ± 0.007	0.289 ± 0.015	0.277 ± 0.012	0.269 ± 0.027	0.244 ± 0.013	0.251 ± 0.009
Relative	6.61 ± 0.23	7.08 ± 0.39	6.46 ± 0.29	6.63 ± 0.62	7.28 ± 0.35	7.90 ± 0.29**
2-Butoxyethanol						
Absolute	0.264 ± 0.016	0.315 ± 0.014	0.257 ± 0.011[3]	0.259 ± 0.018	0.235 ± 0.013	0.251 ± 0.018
Relative	6.62 ± 0.40	7.85 ± 0.28	6.39 ± 0.20[3]	6.76 ± 0.49	6.03 ± 0.36	6.54 ± 0.41

TABLE C4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male B6C3F$_1$ Mice in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
Right testis						
2-Methoxyethanol						
Absolute	0.121 ± 0.002	0.120 ± 0.003	0.102 ± 0.003**	0.029 ± 0.002**	0.026 ± 0.001**[3]	0.023 ± 0.001**
Relative	3.11 ± 0.08	3.04 ± 0.06	2.51 ± 0.07**	0.77 ± 0.05**	0.69 ± 0.02**[3]	0.78 ± 0.06**
2-Ethoxyethanol						
Absolute	0.119 ± 0.002	0.124 ± 0.002	0.123 ± 0.005	0.119 ± 0.003	0.097 ± 0.004**	0.019 ± 0.002**
Relative	3.08 ± 0.08	3.05 ± 0.06	2.86 ± 0.11	2.95 ± 0.08	2.88 ± 0.11	0.59 ± 0.07**
2-Butoxyethanol						
Absolute	0.127 ± 0.002	0.126 ± 0.003	0.127 ± 0.004[3]	0.124 ± 0.002	0.122 ± 0.002	0.120 ± 0.002
Relative	3.19 ± 0.12	3.17 ± 0.11	3.17 ± 0.12[3]	3.23 ± 0.08	3.12 ± 0.07	3.15 ± 0.06
Thymus						
2-Methoxyethanol						
Absolute	0.046 ± 0.004	0.047 ± 0.004	0.047 ± 0.006	0.039 ± 0.002	0.036 ± 0.005*[3]	0.023 ± 0.003**
Relative	1.17 ± 0.08	1.18 ± 0.09	1.15 ± 0.14	1.04 ± 0.07	0.98 ± 0.12*[3]	0.76 ± 0.09**
2-Ethoxyethanol						
Absolute	0.055 ± 0.004	0.058 ± 0.005	0.057 ± 0.005	0.060 ± 0.004	0.041 ± 0.006	0.043 ± 0.004*
Relative	1.42 ± 0.11	1.40 ± 0.11	1.31 ± 0.09	1.47 ± 0.09	1.21 ± 0.16	1.33 ± 0.11
2-Butoxyethanol						
Absolute	0.052 ± 0.005	0.054 ± 0.004	0.050 ± 0.004[3]	0.050 ± 0.007	0.045 ± 0.004	0.041 ± 0.004
Relative	1.28 ± 0.09	1.35 ± 0.08	1.25 ± 0.10[3]	1.27 ± 0.16	1.16 ± 0.10	1.06 ± 0.08

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] Doses administered to mice given 2-methoxyethanol were: 0, 2000, 4000, 6000, 8000 or 10,000 ppm. Doses given to mice administered 2-ethoxyethanol were: 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm. Doses administered to mice given 2-butoxyethanol were: 0, 750, 1500, 3000, 4500, or 6000 ppm.

[3] n=9.

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.

** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female B6C3F$_1$ Mice in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
n	10	10	10	10	10	10
Necropsy body wt						
2-Methoxyethanol	29.7 ± 0.7	29.3 ± 0.7	29.6 ± 1.0	27.2 ± 1.2	26.0 ± 0.9**	23.9 ± 0.9**
2-Ethoxyethanol	31.3 ± 0.8	31.8 ± 1.2	33.2 ± 1.0	29.9 ± 1.5	27.8 ± 0.8*	24.8 ± 0.5**
2-Butoxyethanol	31.1 ± 0.7	31.8 ± 0.8	30.9 ± 1.5	28.0 ± 0.7*	28.4 ± 0.5*	27.8 ± 0.9**
Heart						
2-Methoxyethanol						
Absolute	0.123 ± 0.003	0.130 ± 0.006	0.144 ± 0.006*	0.127 ± 0.005	0.130 ± 0.004	0.127 ± 0.004
Relative	4.16 ± 0.18	4.44 ± 0.19	4.88 ± 0.11**	4.81 ± 0.35*	5.04 ± 0.19**	5.38 ± 0.15**
2-Ethoxyethanol						
Absolute	0.136 ± 0.007	0.138 ± 0.004	0.135 ± 0.004	0.129 ± 0.004	0.139 ± 0.003	0.134 ± 0.004
Relative	4.33 ± 0.13	4.43 ± 0.25	4.11 ± 0.21	4.38 ± 0.21	5.02 ± 0.13*	5.41 ± 0.14**
2-Butoxyethanol						
Absolute	0.132 ± 0.005	0.143 ± 0.004	0.141 ± 0.006	0.130 ± 0.004	0.130 ± 0.004	0.129 ± 0.006
Relative	4.27 ± 0.17	4.50 ± 0.18	4.67 ± 0.31	4.66 ± 0.12	4.60 ± 0.17	4.68 ± 0.22
Right kidney						
2-Methoxyethanol						
Absolute	0.185 ± 0.004	0.227 ± 0.005**	0.212 ± 0.010*	0.206 ± 0.010	0.201 ± 0.005	0.209 ± 0.006
Relative	6.26 ± 0.18	7.80 ± 0.27**	7.18 ± 0.33**	7.73 ± 0.57*	7.82 ± 0.34**	8.88 ± 0.39**
2-Ethoxyethanol						
Absolute	0.208 ± 0.005	0.236 ± 0.006*	0.207 ± 0.006	0.204 ± 0.010	0.206 ± 0.005	0.241 ± 0.004**
Relative	6.71 ± 0.27	7.49 ± 0.23*	6.25 ± 0.18	6.87 ± 0.20	7.45 ± 0.18*	9.73 ± 0.09**
2-Butoxyethanol						
Absolute	0.196 ± 0.004	0.244 ± 0.004**	0.245 ± 0.006**	0.209 ± 0.008	0.214 ± 0.004	0.227 ± 0.007*
Relative	6.33 ± 0.10	7.69 ± 0.14**	8.06 ± 0.29**	7.47 ± 0.19**	7.55 ± 0.18**	8.21 ± 0.26**
Liver						
2-Methoxyethanol						
Absolute	1.24 ± 0.05	1.38 ± 0.05	1.36 ± 0.06	1.26 ± 0.04	1.19 ± 0.05	1.18 ± 0.03
Relative	42.10 ± 2.17	47.00 ± 1.57	46.00 ± 1.46	47.60 ± 3.27	46.10 ± 2.59	49.90 ± 1.82**
2-Ethoxyethanol						
Absolute	1.22 ± 0.03	1.41 ± 0.05*	1.20 ± 0.05	1.23 ± 0.08	1.25 ± 0.04	1.22 ± 0.03
Relative	39.30 ± 1.25	44.60 ± 1.33**	36.30 ± 1.37	40.90 ± 0.85	45.00 ± 1.21**	49.10 ± 0.79**
2-Butoxyethanol						
Absolute	1.18 ± 0.04	1.36 ± 0.05	1.37 ± 0.05	1.16 ± 0.04	1.16 ± 0.04	1.16 ± 0.05
Relative	38.20 ± 1.25	42.70 ± 1.40	44.60 ± 1.12**	41.60 ± 0.92	41.00 ± 1.15	41.70 ± 1.15
Lung						
2-Methoxyethanol						
Absolute	0.241 ± 0.014	0.239 ± 0.006	0.242 ± 0.022	0.274 ± 0.015	0.251 ± 0.016	0.253 ± 0.021
Relative	8.11 ± 0.45	8.20 ± 0.32	8.18 ± 0.67	10.22 ± 0.61*	9.71 ± 0.59*	10.54 ± 0.70*
2-Ethoxyethanol						
Absolute	0.232 ± 0.008	0.240 ± 0.013	0.245 ± 0.015	0.248 ± 0.016	0.265 ± 0.020	0.209 ± 0.006
Relative	7.46 ± 0.31	7.65 ± 0.49	7.46 ± 0.53	8.44 ± 0.62	9.56 ± 0.67*	8.43 ± 0.16*
2-Butoxyethanol						
Absolute	0.263 ± 0.017	0.227 ± 0.009	0.235 ± 0.010	0.254 ± 0.017	0.259 ± 0.018	0.240 ± 0.016
Relative	8.51 ± 0.60	7.13 ± 0.22	7.68 ± 0.26	9.04 ± 0.56	9.16 ± 0.65	8.64 ± 0.50

TABLE C5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female B6C3F$_1$ Mice in the 13-Week Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
Thymus						
2-Methoxyethanol						
Absolute	0.048 ± 0.003	0.055 ± 0.005	0.049 ± 0.003	0.042 ± 0.002	0.037 ± 0.003*	0.026 ± 0.003**
Relative	1.63 ± 0.09	1.89 ± 0.19	1.67 ± 0.09	1.57 ± 0.10	1.46 ± 0.13	1.09 ± 0.14*
2-Ethoxyethanol						
Absolute	0.057 ± 0.003	0.055 ± 0.004	0.056 ± 0.004	0.056 ± 0.005	0.056 ± 0.003	0.043 ± 0.003**
Relative	1.84 ± 0.12	1.71 ± 0.12	1.69 ± 0.13	1.88 ± 0.12	2.04 ± 0.11	1.71 ± 0.12
2-Butoxyethanol						
Absolute	0.063 ± 0.005	0.062 ± 0.005	0.055 ± 0.003	0.051 ± 0.002*	0.051 ± 0.004*	0.054 ± 0.004
Relative	2.02 ± 0.14	1.94 ± 0.13	1.80 ± 0.08	1.82 ± 0.07	1.80 ± 0.12	1.91 ± 0.09

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] Doses administered to mice given 2-methoxyethanol were: 0, 2000, 4000, 6000, 8000 or 10,000 ppm. Doses given to mice administered 2-ethoxyethanol were: 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm. Doses administered to mice given 2-butoxyethanol were: 0, 750, 1500, 3000, 4500, or 6000 ppm.

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.

** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

APPENDIX D

Hematology, Clinical Chemistry, and Urinalysis Results

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
MALE						
Hematology						
n						
Week 1	10	10	10	10	9	9
Week 3	10	8	10	9	0	0
Week 13	8	9	10	10	2	0
Hematocrit (%)						
Week 1	46.1 ± 0.6	45.8 ± 0.5	46.2 ± 0.6	44.3 ± 0.4*	45.4 ± 0.3	43.6 ± 0.5**
Week 3	49.3 ± 0.6	45.6 ± 0.6**	46.2 ± 0.6**	41.6 ± 0.5**	—	—
Week 13	48.1 ± 0.4	46.9 ± 0.6	45.4 ± 0.7**	46.0 ± 0.8*	31.6 ± 7.0**	—
Hemoglobin (g/dL)						
Week 1	15.0 ± 0.1	14.7 ± 0.1	14.8 ± 0.2	14.3 ± 0.1**	14.4 ± 0.2**	13.9 ± 0.2**
Week 3	16.0 ± 0.2	14.9 ± 0.2**	14.9 ± 0.2**	13.8 ± 0.1**	—	—
Week 13	16.0 ± 0.2	15.5 ± 0.2	15.2 ± 0.2**	14.9 ± 0.2**	10.1 ± 1.9**	—
Erythrocytes (10^6/μL)						
Week 1	7.88 ± 0.12	7.88 ± 0.10	7.96 ± 0.12	7.60 ± 0.07	7.70 ± 0.11	7.44 ± 0.11*
Week 3	8.80 ± 0.10	8.32 ± 0.14*	8.47 ± 0.14*	7.61 ± 0.10**	—	—
Week 13	9.44 ± 0.11	9.40 ± 0.13	9.20 ± 0.13	9.08 ± 0.16	5.94 ± 1.24*	—
Reticulocytes (10^6/μL)						
Week 1	0.22 ± 0.03	0.27 ± 0.02[2]	0.21 ± 0.02	0.12 ± 0.02*	0.07 ± 0.01**	0.05 ± 0.01**
Week 3	0.18 ± 0.01	0.17 ± 0.02	0.15 ± 0.01	0.17 ± 0.01	—	—
Week 13	0.12 ± 0.01	0.17 ± 0.02	0.13 ± 0.02	0.13 ± 0.01	0.09 ± 0.03	—
Nucleated erythrocytes (10^3/μL)						
Week 1	0.02 ± 0.01[2]	0.07 ± 0.03[2]	0.02 ± 0.01[2]	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Week 3	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00[3]	—	—
Week 13	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.00 ± 0.00	—
Mean cell volume (fL)						
Week 1	58.6 ± 0.3	58.0 ± 0.3	58.0 ± 0.4	58.3 ± 0.4	59.1 ± 0.4	58.6 ± 0.2
Week 3	56.1 ± 0.3	54.9 ± 0.4*	54.6 ± 0.2**	54.8 ± 0.2**	—	—
Week 13	50.9 ± 0.3	49.8 ± 0.5	49.1 ± 0.2**	50.8 ± 0.2	53.0 ± 1.0	—
Mean cell hemoglobin (pg)						
Week 1	19.0 ± 0.2	18.6 ± 0.1	18.6 ± 0.1	18.7 ± 0.1	18.7 ± 0.1	18.8 ± 0.1
Week 3	18.2 ± 0.1	18.0 ± 0.2	17.6 ± 0.1*	18.1 ± 0.1	—	—
Week 13	16.9 ± 0.1	16.5 ± 0.1	16.5 ± 0.1	16.5 ± 0.2	17.0 ± 0.4	—
Mean cell hemoglobin concentration (g/dL)						
Week 1	32.4 ± 0.3	32.1 ± 0.2	32.0 ± 0.2	32.2 ± 0.2	31.7 ± 0.2*	32.0 ± 0.1
Week 3	32.5 ± 0.2	32.8 ± 0.4	32.3 ± 0.1	33.1 ± 0.2*	—	—
Week 13	33.2 ± 0.3	33.1 ± 0.4	33.5 ± 0.3	32.4 ± 0.4	32.1 ± 1.2	—
Platelets (10^3/μL)						
Week 1	937.5 ± 31.3	864.8 ± 12.1*	791.8 ± 13.0**	492.5 ± 18.6**	338.1 ± 21.0**	276.2 ± 20.8**
Week 3	797.7 ± 13.3	730.1 ± 16.5**	568.7 ± 11.8**	267.7 ± 7.9**	—	—
Week 13	582.4 ± 12.1	612.8 ± 18.0	490.9 ± 13.5**	401.9 ± 33.8**	265.5 ± 53.5**	—
Leukocytes (10^3/μL)						
Week 1	7.87 ± 0.51	7.45 ± 0.45	7.05 ± 0.37	4.94 ± 0.29**	3.37 ± 0.34**	2.92 ± 0.22**
Week 3	8.49 ± 0.40	7.68 ± 0.35	6.81 ± 0.46**	4.81 ± 0.19**	—	—
Week 13	7.49 ± 0.63	8.51 ± 0.73	6.47 ± 0.61	6.18 ± 0.54	1.80 ± 0.30*	—

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
MALE (continued)						
Hematology (continued)						
Segmented neutrophils (10^3/μL)						
Week 1	0.96 ± 0.12[2]	0.75 ± 0.08[2]	1.04 ± 0.13[2]	0.77 ± 0.12	0.51 ± 0.13*	0.39 ± 0.05**
Week 3	1.02 ± 0.06	1.07 ± 0.08	0.68 ± 0.08**	0.63 ± 0.11**	—	—
Week 13	1.26 ± 0.20	1.19 ± 0.14	1.06 ± 0.16	0.79 ± 0.07*	0.25 ± 0.06**	—
Lymphocytes (10^3/μL)						
Week 1	6.97 ± 0.56[2]	6.80 ± 0.38[2]	5.71 ± 0.32[2]	4.09 ± 0.18**	2.78 ± 0.24**	2.50 ± 0.21**
Week 3	7.36 ± 0.43	6.47 ± 0.31	6.03 ± 0.41*	4.14 ± 0.20**	—	—
Week 13	6.09 ± 0.45	7.17 ± 0.61	5.32 ± 0.51	5.19 ± 0.45	1.51 ± 0.25*	—
Monocytes (10^3/μL)						
Week 1	0.03 ± 0.02[2]	0.11 ± 0.03[2]	0.09 ± 0.03[2]	0.05 ± 0.02	0.06 ± 0.02	0.02 ± 0.01
Week 3	0.08 ± 0.03	0.11 ± 0.03	0.09 ± 0.02	0.04 ± 0.01	—	—
Week 13	0.08 ± 0.03	0.10 ± 0.03	0.05 ± 0.03	0.15 ± 0.04	0.02 ± 0.02	—
Eosinophils (10^3/μL)						
Week 1	0.02 ± 0.01[2]	0.01 ± 0.01[2]	0.05 ± 0.03[2]	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00
Week 3	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	—	—
Week 13	0.05 ± 0.03	0.06 ± 0.02	0.03 ± 0.01	0.06 ± 0.02	0.01 ± 0.01	—
Methemoglobin (g/dL)						
Week 1	0.09 ± 0.02	0.10 ± 0.02	0.11 ± 0.01	0.13 ± 0.02	0.07 ± 0.02	0.10 ± 0.02
Week 3	0.11 ± 0.02	0.09 ± 0.02	0.07 ± 0.01	0.10 ± 0.02	—	—
Week 13	0.11 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.13 ± 0.08	—
Total bone marrow cellularity (10^6/femur)						
Week 1	70.6 ± 2.7	—[4]	66.6 ± 3.2	53.5 ± 2.9**	32.7 ± 2.0**[3]	25.5 ± 1.2**[3]
Week 3	66.1 ± 2.9	82.2 ± 3.6[3]	75.1 ± 3.9	53.2 ± 2.4	—	—
Week 13	66.0 ± 2.9[3]	71.1 ± 3.0[3]	58.4 ± 2.1	57.0 ± 2.2*	31.4 ± 12.2**	—
Clinical Chemistry						
n						
Week 1	10	10	10	10	10	10
Week 3	10	10	10	9	0	0
Week 13	10	10	10	10	2	0
Urea nitrogen (mg/dL)						
Week 1	18.6 ± 0.6	19.7 ± 1.0	16.9 ± 0.6	16.0 ± 0.6	18.2 ± 0.7	17.4 ± 0.9
Week 3	20.4 ± 0.4	18.4 ± 0.6	20.5 ± 0.8	21.1 ± 0.9	—	—
Week 13	16.7 ± 1.1	17.9 ± 0.9	20.0 ± 1.6	16.9 ± 0.7	34.5 ± 14.5	—
Creatinine (mg/dL)						
Week 1	0.45 ± 0.02	0.41 ± 0.01	0.41 ± 0.01*	0.38 ± 0.01**	0.39 ± 0.02**	0.36 ± 0.02**
Week 3	0.59 ± 0.02	0.60 ± 0.02	0.56 ± 0.02	0.50 ± 0.00**	—	—
Week 13	0.55 ± 0.03	0.50 ± 0.02	0.49 ± 0.01	0.49 ± 0.02	0.35 ± 0.05**	—
Total protein (g/dL)						
Week 1	6.1 ± 0.0	5.9 ± 0.1*	5.8 ± 0.1**	5.5 ± 0.0**	5.6 ± 0.1**	5.4 ± 0.1**
Week 3	6.3 ± 0.1	6.3 ± 0.1	6.1 ± 0.1	5.6 ± 0.0**	—	—
Week 13	6.6 ± 0.1	6.4 ± 0.1	6.2 ± 0.1**	6.0 ± 0.1**	5.0 ± 0.1**	—

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
MALE (continued)						
Clinical Chemistry (continued)						
Albumin (g/dL)						
Week 1	3.4 ± 0.0	3.4 ± 0.1	3.3 ± 0.1	3.2 ± 0.1*	3.2 ± 0.1*	3.0 ± 0.1**
Week 3	3.7 ± 0.0	3.5 ± 0.1*	3.6 ± 0.0*	3.3 ± 0.1**	—	—
Week 13	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.0	3.5 ± 0.0	2.8 ± 0.3**	—
Alkaline phosphatase (IU/L)						
Week 1	442 ± 8	401 ± 10**	364 ± 10**	321 ± 14**	317 ± 18**	308 ± 8**
Week 3	271 ± 5	281 ± 10	238 ± 8*	137 ± 5**	—	—
Week 13	138 ± 2	131 ± 2	135 ± 5	152 ± 8	89 ± 10	—
Alanine aminotransferase (IU/L)						
Week 1	34 ± 2	36 ± 2	32 ± 1	34 ± 1	37 ± 2	31 ± 1
Week 3	35 ± 1	32 ± 1	33 ± 1	27 ± 1**	—	—
Week 13	34 ± 1	33 ± 1	33 ± 1	36 ± 1	31 ± 0	—
Creatine kinase (IU/L)						
Week 1	412 ± 39	415 ± 44	436 ± 52	425 ± 60[2]	395 ± 36	304 ± 29
Week 3	153 ± 25[2]	212 ± 27	187 ± 23	93 ± 12	—	—
Week 13	79 ± 10	143 ± 27	133 ± 18	87 ± 10	89 ± 24	—
Bile acids (μmol/L)						
Week 1	10.10 ± 0.85	13.70 ± 1.12*	15.30 ± 3.84	14.50 ± 1.93*	25.30 ± 5.14**	16.80 ± 0.76**
Week 3	9.30 ± 1.56	11.44 ± 1.36[2]	23.50 ± 4.66**	33.78 ± 7.85**	—	—
Week 13	17.20 ± 4.57	19.40 ± 3.34	11.70 ± 1.41	18.50 ± 3.79	16.00 ± 3.00	—
Urinalysis						
n	10	10	10	10	2	0
Volume (mL/16 hr)						
Week 13	5.6 ± 0.3	4.8 ± 0.4[2]	3.9 ± 0.2**	3.8 ± 0.3**	6.8 ± 2.8	—
Specific gravity						
Week 13	1.042 ± 0.002	1.045 ± 0.004	1.064 ± 0.003**	1.063 ± 0.003**	1.046 ± 0.004	—
pH						
Week 13	7.40 ± 0.22	6.75 ± 0.08	6.65 ± 0.08*	6.95 ± 0.19	7.00 ± 0.00	—

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
FEMALE						
Hematology						
n						
Week 1	10	10	10	10	10	10
Week 3	7	9	8	5	0	0
Week 13	9	9	8	10	5	0
Hematocrit (%)						
Week 1	46.8 ± 0.4	45.6 ± 0.7	44.8 ± 0.6*	43.2 ± 0.5**	43.7 ± 0.6**	43.4 ± 0.7**
Week 3	48.6 ± 0.6	48.4 ± 0.5	47.4 ± 0.6	43.1 ± 1.2**	—	—
Week 13	44.5 ± 0.4	43.8 ± 0.4	42.2 ± 0.8*	41.5 ± 0.5**	40.7 ± 0.9**	—
Hemoglobin (g/dL)						
Week 1	15.8 ± 0.1	15.4 ± 0.2	15.1 ± 0.2*	14.5 ± 0.1**	14.8 ± 0.2**	14.9 ± 0.2**
Week 3	16.0 ± 0.1	15.8 ± 0.1	15.8 ± 0.2	14.3 ± 0.2**	—	—
Week 13	15.2 ± 0.1	14.8 ± 0.1*	14.5 ± 0.2**	13.7 ± 0.1**	13.6 ± 0.3**	—
Erythrocytes (10^6/μL)						
Week 1	8.14 ± 0.09	7.94 ± 0.12	7.86 ± 0.12	7.43 ± 0.10**	7.66 ± 0.15**	7.57 ± 0.12**
Week 3	8.73 ± 0.11	8.80 ± 0.12	8.85 ± 0.14	8.09 ± 0.22	—	—
Week 13	8.34 ± 0.09	8.30 ± 0.09	8.24 ± 0.13	8.13 ± 0.12	7.91 ± 0.18	—
Reticulocytes (10^6/μL)						
Week 1	0.22 ± 0.02	0.15 ± 0.02*	0.09 ± 0.00**	0.05 ± 0.01**	0.03 ± 0.00**	0.03 ± 0.00**
Week 3	0.16 ± 0.01	0.13 ± 0.01	0.11 ± 0.02	0.18 ± 0.02	—	—
Week 13	0.09 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.11 ± 0.01	0.09 ± 0.01	—
Nucleated erythrocytes (10^3/μL)						
Week 1	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 3	0.00 ± 0.00	0.00 ± 0.00[5]	0.01 ± 0.01	0.00 ± 0.00	—	—
Week 13	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.03 ± 0.01	0.00 ± 0.00	—
Mean cell volume (fL)						
Week 1	57.5 ± 0.2	57.5 ± 0.3	57.2 ± 0.4	58.2 ± 0.3	57.1 ± 0.4	57.4 ± 0.3
Week 3	55.7 ± 0.3	55.1 ± 0.2	53.6 ± 0.3**	53.2 ± 0.2**	—	—
Week 13	53.3 ± 0.3	52.8 ± 0.2	51.3 ± 0.3**	50.9 ± 0.3**	51.6 ± 0.2**	—
Mean cell hemoglobin (pg)						
Week 1	19.5 ± 0.2	19.4 ± 0.2	19.3 ± 0.1	19.5 ± 0.2	19.3 ± 0.2	19.7 ± 0.1
Week 3	18.3 ± 0.2	18.0 ± 0.2	18.0 ± 0.2	17.8 ± 0.2	—	—
Week 13	18.3 ± 0.2	17.9 ± 0.1	17.6 ± 0.1**	16.9 ± 0.2**	17.3 ± 0.1**	—
Mean cell hemoglobin concentration (g/dL)						
Week 1	33.9 ± 0.3	33.8 ± 0.3	33.8 ± 0.3	33.5 ± 0.3	33.9 ± 0.3	34.3 ± 0.2
Week 3	32.9 ± 0.3	32.7 ± 0.3	33.2 ± 0.2	33.3 ± 0.4	—	—
Week 13	34.2 ± 0.3	33.8 ± 0.3	34.3 ± 0.3	33.1 ± 0.3*	33.5 ± 0.2	—
Platelets (10^3/μL)						
Week 1	852.8 ± 19.7	775.3 ± 14.6*	539.0 ± 12.9**	261.6 ± 10.6**	180.1 ± 22.3**	159.9 ± 21.7**
Week 3	861.4 ± 20.1	658.0 ± 11.3**	531.1 ± 13.7**	349.6 ± 20.7**	—	—
Week 13	658.9 ± 24.3	650.6 ± 12.0	534.9 ± 25.4**	400.7 ± 27.2**	376.0 ± 32.0**	—
Leukocytes (10^3/μL)						
Week 1	9.24 ± 0.36	7.35 ± 0.35**	5.80 ± 0.39**	4.49 ± 0.23**	3.51 ± 0.37**	3.45 ± 0.30**
Week 3	7.87 ± 0.56	7.48 ± 0.39	8.24 ± 0.61	5.36 ± 0.52*	—	—
Week 13	7.14 ± 0.23	6.76 ± 0.18	5.74 ± 0.26**	4.16 ± 0.45**	4.62 ± 0.50**	—

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
FEMALE (continued)						
Hematology (continued)						
Segmented neutrophils (10^3/μL)						
Week 1	1.07 ± 0.19	0.69 ± 0.07	0.75 ± 0.08	0.54 ± 0.06**	0.42 ± 0.08**	0.43 ± 0.10**
Week 3	0.97 ± 0.10	0.85 ± 0.14	0.91 ± 0.11	0.79 ± 0.12	—	—
Week 13	0.94 ± 0.14	0.97 ± 0.12	0.75 ± 0.06	0.48 ± 0.08**	0.53 ± 0.15*	—
Lymphocytes (10^3/μL)						
Week 1	8.02 ± 0.31	6.55 ± 0.34**	4.96 ± 0.36**	3.91 ± 0.19**	3.03 ± 0.33**	2.95 ± 0.24**
Week 3	6.79 ± 0.47	6.53 ± 0.40	7.22 ± 0.58	4.44 ± 0.49	—	—
Week 13	6.08 ± 0.34	5.63 ± 0.12	4.80 ± 0.24**	3.56 ± 0.43**	4.00 ± 0.46**	—
Monocytes (10^3/μL)						
Week 1	0.12 ± 0.03	0.09 ± 0.03	0.07 ± 0.02	0.05 ± 0.01	0.05 ± 0.02	0.06 ± 0.02
Week 3	0.08 ± 0.03	0.09 ± 0.03	0.08 ± 0.03	0.10 ± 0.06	—	—
Week 13	0.06 ± 0.03	0.10 ± 0.03	0.08 ± 0.03	0.07 ± 0.02	0.02 ± 0.01	—
Eosinophils (10^3/μL)						
Week 1	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
Week 3	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	—	—
Week 13	0.06 ± 0.02	0.06 ± 0.02	0.11 ± 0.03	0.05 ± 0.02	0.06 ± 0.03	—
Methemoglobin (g/dL)						
Week 1	0.12 ± 0.02	0.14 ± 0.02	0.13 ± 0.02	0.15 ± 0.02	0.14 ± 0.02	0.14 ± 0.03
Week 3	0.15 ± 0.02	0.12 ± 0.02	0.12 ± 0.01	0.12 ± 0.02	—	—
Week 13	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.12 ± 0.02	0.11 ± 0.01	—
Total bone marrow cellularity (10^6/femur)						
Week 1	55.2 ± 2.4	—[4]	43.6 ± 2.0**	25.9 ± 1.1**	21.5 ± 1.4**	19.9 ± 1.3**
Week 3	46.2 ± 1.4[3]	40.6 ± 1.8*[3]	34.7 ± 1.5**[3]	30.2 ± 2.7**	—	—
Week 13	38.9 ± 1.7[3]	45.5 ± 1.3[3]	42.6 ± 1.8[3]	33.0 ± 2.7	39.1 ± 2.2	—
Clinical Chemistry						
n						
Week 1	10	10	10	10	10	10
Week 3	10	10	10	5	0	0
Week 13	10	10	10	10	5	0
Urea nitrogen (mg/dL)						
Week 1	19.0 ± 0.4	16.7 ± 0.9	18.0 ± 0.7	19.7 ± 1.0	22.3 ± 2.7	19.5 ± 1.3
Week 3	16.8 ± 0.4	17.4 ± 0.7	20.3 ± 0.7**	23.2 ± 0.7**	—	—
Week 13	22.3 ± 1.4	19.2 ± 0.6**	19.0 ± 1.1**	18.8 ± 1.1*	18.4 ± 1.9*	—
Creatinine (mg/dL)						
Week 1	0.48 ± 0.01	0.51 ± 0.02	0.48 ± 0.01	0.45 ± 0.02	0.52 ± 0.04	0.51 ± 0.02
Week 3	0.59 ± 0.02	0.57 ± 0.02	0.54 ± 0.02	0.52 ± 0.02*	—	—
Week 13	0.55 ± 0.02	0.51 ± 0.02	0.47 ± 0.02**	0.48 ± 0.04**	0.52 ± 0.04	—
Total protein (g/dL)						
Week 1	6.1 ± 0.1	5.7 ± 0.1**	5.5 ± 0.1**	5.2 ± 0.1**	5.1 ± 0.1**	5.3 ± 0.1**
Week 3	6.0 ± 0.1	5.7 ± 0.1*	5.6 ± 0.1*	5.4 ± 0.1**	—	—
Week 13	6.6 ± 0.1	6.4 ± 0.1	6.1 ± 0.1**	5.9 ± 0.1**	5.8 ± 0.1**	—

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
FEMALE (continued)						
Clinical Chemistry (continued)						
Albumin (g/dL)						
Week 1	3.4 ± 0.0	3.4 ± 0.1	3.2 ± 0.0**	3.1 ± 0.1**	3.0 ± 0.1**	3.1 ± 0.1**
Week 3	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.2 ± 0.1**	—	—
Week 13	3.79 ± 0.07	3.62 ± 0.07	3.62 ± 0.03	3.57 ± 0.08*	3.46 ± 0.09**	—
Alkaline phosphatase (IU/L)						
Week 1	333 ± 7	285 ± 7**	257 ± 7**	251 ± 6**	227 ± 12**	242 ± 8**
Week 3	188 ± 5	175 ± 11	120 ± 5**	85 ± 4**	—	—
Week 13	192 ± 10	171 ± 10	157 ± 12*	155 ± 13*	137 ± 9**	—
Alanine aminotransferase (IU/L)						
Week 1	26 ± 1	23 ± 1	23 ± 1	23 ± 1	29 ± 1	26 ± 2
Week 3	31 ± 2	31 ± 2	31 ± 1	29 ± 2	—	—
Week 13	36 ± 2	36 ± 3	34 ± 2	51 ± 7	35 ± 2	—
Creatine kinase (IU/L)						
Week 1	261 ± 25	309 ± 33	352 ± 60	203 ± 19	199 ± 25	199 ± 30[2]
Week 3	300 ± 36	418 ± 113	220 ± 20	170 ± 27*	—	—
Week 13	88 ± 11[2]	116 ± 26	125 ± 15	93 ± 21	114 ± 28	—
Bile acids (µmol/L)						
Week 1	6.20 ± 0.49[6]	5.57 ± 0.57[7]	8.88 ± 1.95[5]	22.70 ± 4.16**	13.33 ± 2.46*[2]	21.22 ± 3.84**[2]
Week 3	11.75 ± 2.46[5]	23.00 ± 5.21*	18.80 ± 2.93	31.80 ± 7.12**	—	—
Week 13	21.40 ± 4.08	19.30 ± 3.39	19.80 ± 3.33	21.90 ± 3.26	30.00 ± 8.91	—
Urinalysis						
n	10	10	10	10	5	0
Volume (mL/16 hr)						
Week 13	5.05 ± 0.34	5.55 ± 0.44	3.75 ± 0.19*	4.15 ± 0.55*	3.50 ± 0.95	—
Specific gravity						
Week 13	1.052 ± 0.002	1.047 ± 0.004	1.057 ± 0.003	1.058 ± 0.002	1.078 ± 0.010*	—
pH						
Week 13	7.10 ± 0.10	7.15 ± 0.17	7.10 ± 0.10	7.15 ± 0.20	7.70 ± 0.12*	—

[1] Mean ± standard error.
[2] n=9.
[3] n=10.
[4] Not measured at this exposure level.
[5] n=8.
[6] n=5.
[7] n=7.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
MALE						
Hematology						
n						
Week 1	10	10	9	9	10	9
Week 3	10	[illegible]	8	9	10	10
Week 13	9	10	10	10	8	0
Hematocrit (%)						
Week 1	43.6 ± 0.3	42.7 ± 0.5	43.8 ± 0.3	44.7 ± 0.7	45.9 ± 0.5**	45.0 ± 0.5*
Week 3	47.1 ± 0.7	46.7 ± 0.8	46.1 ± 0.8	43.0 ± 0.5**	42.7 ± 0.6**	38.0 ± 0.3**
Week 13	46.6 ± 0.8	47.1 ± 0.7	45.8 ± 0.7	42.9 ± 1.1*	25.3 ± 1.3**	—
Hemoglobin (g/dL)						
Week 1	14.6 ± 0.1	14.3 ± 0.1	14.4 ± 0.1	14.6 ± 0.2	13.9 ± 0.2**	13.5 ± 0.1**
Week 3	15.6 ± 0.2	15.4 ± 0.2	15.2 ± 0.1	14.2 ± 0.1**	13.9 ± 0.2**	12.2 ± 0.1**
Week 13	15.5 ± 0.4	15.5 ± 0.2	15.2 ± 0.2	14.2 ± 0.3**	8.4 ± 0.4**	—
Erythrocytes (10^6/μL)						
Week 1	7.39 ± 0.05	7.09 ± 0.10*	7.31 ± 0.06	7.43 ± 0.13	7.13 ± 0.09*	7.02 ± 0.09**
Week 3	8.49 ± 0.16	8.32 ± 0.15	8.27 ± 0.16	7.64 ± 0.08**	7.47 ± 0.11**	6.29 ± 0.05**
Week 13	8.98 ± 0.15	9.27 ± 0.16	8.97 ± 0.13	8.27 ± 0.23*	3.87 ± 0.19**	—
Reticulocytes (10^6/μL)						
Week 1	0.13 ± 0.02	0.09 ± 0.01*	0.10 ± 0.02	0.06 ± 0.01**	0.01 ± 0.00**	0.02 ± 0.01**
Week 3	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.07 ± 0.02	0.27 ± 0.07
Week 13	0.09 ± 0.02[2]	0.05 ± 0.02[3]	0.05 ± 0.01	0.06 ± 0.02	0.68 ± 0.07**	—
Nucleated erythrocytes (10^3/μL)						
Week 1	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 3	0.03 ± 0.02	0.03 ± 0.02	0.04 ± 0.03	0.04 ± 0.02	0.00 ± 0.00	0.08 ± 0.04[3]
Week 13	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	4.04 ± 0.92**	—
Mean cell volume (fL)						
Week 1	59.1 ± 0.3	60.4 ± 0.4*	60.1 ± 0.3*	60.1 ± 0.4*	64.3 ± 0.4**	64.0 ± 0.4**
Week 3	55.5 ± 0.5	56.2 ± 0.2	55.8 ± 0.3	56.2 ± 0.2	57.1 ± 0.2**	60.3 ± 0.3**
Week 13	51.9 ± 0.2	50.8 ± 0.2	51.1 ± 0.3	52.0 ± 0.3	65.4 ± 1.4**	—
Mean cell hemoglobin (pg)						
Week 1	19.8 ± 0.2	20.2 ± 0.2	19.7 ± 0.2	19.6 ± 0.1	19.4 ± 0.1	19.3 ± 0.2
Week 3	18.4 ± 0.2	18.5 ± 0.2	18.5 ± 0.3	18.6 ± 0.2	18.6 ± 0.2	19.4 ± 0.2**
Week 13	17.3 ± 0.4	16.7 ± 0.2	16.9 ± 0.2	17.2 ± 0.3	21.7 ± 0.5**	—
Mean cell hemoglobin concentration (g/dL)						
Week 1	33.6 ± 0.3	33.4 ± 0.3	32.9 ± 0.3	32.6 ± 0.3*	30.3 ± 0.3**	30.1 ± 0.3**
Week 3	33.2 ± 0.3	33.0 ± 0.3	33.1 ± 0.4	33.1 ± 0.3	32.5 ± 0.2	32.2 ± 0.4*
Week 13	33.4 ± 0.7	32.9 ± 0.4	33.1 ± 0.3	33.2 ± 0.4	33.2 ± 0.4	—
Platelets (10^3/μL)						
Week 1	895.4 ± 21.7	905.0 ± 11.2	843.8 ± 20.6	809.4 ± 15.5*	556.8 ± 24.8**	554.2 ± 17.2**
Week 3	793.5 ± 18.4	812.0 ± 16.3	643.0 ± 65.9*	580.6 ± 46.7**	604.9 ± 36.2**[3]	539.4 ± 50.2**[3]
Week 13	557.6 ± 8.0	570.2 ± 16.1	503.8 ± 9.7*	518.5 ± 9.3	581.5 ± 36.5	—
Leukocytes (10^3/μL)						
Week 1	6.35 ± 0.30	6.53 ± 0.34	6.43 ± 0.19	5.86 ± 0.20	3.45 ± 0.24**	4.40 ± 0.29**
Week 3	7.30 ± 0.41	9.19 ± 0.50	9.55 ± 0.46	7.77 ± 0.46	6.04 ± 0.53	4.18 ± 0.22**
Week 13	6.07 ± 0.35	6.08 ± 0.40	6.51 ± 0.39	6.84 ± 0.30	27.71 ± 3.95**	—

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
MALE (continued)						
Hematology (continued)						
Segmented neutrophils ($10^3/\mu L$)						
Week 1	0.91 ± 0.11	1.09 ± 0.17	1.03 ± 0.10	1.07 ± 0.12	0.71 ± 0.12	1.55 ± 0.14*
Week 3	1.28 ± 0.14	1.10 ± 0.12	1.08 ± 0.18	1.26 ± 0.08	0.68 ± 0.08**	0.77 ± 0.07**[3]
Week 13	1.45 ± 0.17	1.53 ± 0.12	1.52 ± 0.17	1.35 ± 0.12	5.44 ± 1.08**	—
Lymphocytes ($10^3/\mu L$)						
Week 1	5.39 ± 0.26	5.34 ± 0.20	5.35 ± 0.22	4.66 ± 0.18*	2.71 ± 0.25**	2.79 ± 0.18**
Week 3	5.93 ± 0.36	8.01 ± 0.42	8.30 ± 0.43	6.37 ± 0.48	5.27 ± 0.47	3.43 ± 0.26**[3]
Week 13	4.47 ± 0.30	4.41 ± 0.34	4.84 ± 0.30	5.41 ± 0.31	21.96 ± 3.09**	—
Monocytes ($10^3/\mu L$)						
Week 1	0.04 ± 0.02	0.09 ± 0.04	0.02 ± 0.01	0.10 ± 0.04	0.01 ± 0.01	0.04 ± 0.03
Week 3	0.05 ± 0.02	0.06 ± 0.03	0.11 ± 0.03	0.07 ± 0.05	0.03 ± 0.02	0.03 ± 0.01[3]
Week 13	0.08 ± 0.03	0.07 ± 0.02	0.11 ± 0.05	0.05 ± 0.02	0.13 ± 0.06	—
Eosinophils ($10^3/\mu L$)						
Week 1	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Week 3	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.07 ± 0.03	0.05 ± 0.02	0.04 ± 0.01[3]
Week 13	0.05 ± 0.02	0.04 ± 0.02	0.03 ± 0.01	0.03 ± 0.02	0.27 ± 0.17	—
Methemoglobin (g/dL)						
Week 3	0.20 ± 0.03	0.19 ± 0.02	0.34 ± 0.10[3]	0.25 ± 0.03[2]	0.17 ± 0.01	0.14 ± 0.02
Week 13	0.15 ± 0.05	0.15 ± 0.03	0.12 ± 0.03	0.13 ± 0.04	0.16 ± 0.06	—
Total bone marrow cellularity (10^6/femur)						
Week 1	61.8 ± 2.9	—[4]	55.8 ± 2.8[5]	73.5 ± 11.2[5]	33.5 ± 2.1**[3]	—[4]
Week 3	65.0 ± 5.7	—[4]	72.5 ± 4.9[5]	72.9 ± 5.2[5]	59.6 ± 3.9	—[4]
Week 13	46.8 ± 1.9[5]	—[4]	42.7 ± 2.8	43.7 ± 2.3	79.3 ± 2.9**[5]	—
Clinical Chemistry						
n						
Week 1	10	10	10	10	10	10
Week 3	10	10	10	10	10	10
Week 13	10	10	10	10	10	0
Urea nitrogen (mg/dL)						
Week 1	19.2 ± 0.6	16.7 ± 1.0	18.5 ± 0.6	17.1 ± 0.5	18.6 ± 0.8	18.4 ± 0.5
Week 3	18.7 ± 0.7	18.0 ± 0.6	18.6 ± 0.9	14.7 ± 0.6**	14.5 ± 0.4**	17.7 ± 1.5*
Week 13	22.0 ± 1.5	23.4 ± 0.8	19.3 ± 1.5	19.4 ± 0.7	21.9 ± 1.6	—
Creatinine (mg/dL)						
Week 1	0.48 ± 0.02	0.47 ± 0.02	0.53 ± 0.02	0.51 ± 0.02	0.49 ± 0.01	0.41 ± 0.01**
Week 3	0.61 ± 0.01	0.65 ± 0.02	0.62 ± 0.04	0.60 ± 0.03	0.59 ± 0.02	0.54 ± 0.02*
Week 13	0.67 ± 0.05	0.70 ± 0.03	0.67 ± 0.05	0.62 ± 0.03	0.65 ± 0.04	—
Total protein (g/dL)						
Week 1	6.11 ± 0.09	5.93 ± 0.05	5.96 ± 0.06	5.92 ± 0.06	5.86 ± 0.25**	5.31 ± 0.06**
Week 3	6.3 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	5.7 ± 0.1**	5.6 ± 0.1**	5.1 ± 0.1**
Week 13	6.7 ± 0.1	6.8 ± 0.1	6.5 ± 0.1	6.2 ± 0.1**	5.9 ± 0.1**	—
Albumin (g/dL)						
Week 1	3.3 ± 0.1	3.2 ± 0.1	3.3 ± 0.0	3.2 ± 0.1	3.2 ± 0.1	3.0 ± 0.0**
Week 3	3.5 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.3 ± 0.1	3.2 ± 0.0**	3.1 ± 0.1**
Week 13	3.6 ± 0.1	3.6 ± 0.1	3.7 ± 0.1	3.5 ± 0.1	3.4 ± 0.1*	—

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
MALE (continued)						
Clinical Chemistry (continued)						
Alkaline phosphatase (IU/L)						
Week 1	664 ± 7	564 ± 12**	511 ± 15**	436 ± 10**	291 ± 10**	283 ± 7**
Week 3	316 ± 10	286 ± 4*	253 ± 5**	198 ± 4**	154 ± 6**	76 ± 4**
Week 13	132 ± 7	123 ± 7	104 ± 6**	108 ± 7**	69 ± 3**	—
Alanine aminotransferase (IU/L)						
Week 1	40 ± 2	36 ± 2	39 ± 1	38 ± 1	44 ± 2	43 ± 3
Week 3	36 ± 2	35 ± 1	37 ± 1	41 ± 2	35 ± 2	39 ± 2
Week 13	41 ± 2	41 ± 2	40 ± 2	36 ± 2	42 ± 2	—
Creatine kinase (IU/L)						
Week 1	378 ± 28	393 ± 57	478 ± 39	622 ± 98*	550 ± 50*	628 ± 155
Week 3	392 ± 44	492 ± 47	494 ± 85	563 ± 140	591 ± 67	407 ± 47
Week 13	303 ± 48	215 ± 34[3]	239 ± 24	255 ± 33	315 ± 52	—
Bile acids (μmol/L)						
Week 1	11.80 ± 1.51	13.80 ± 1.56	13.40 ± 1.70	16.10 ± 2.85	16.00 ± 2.62	18.20 ± 2.92
Week 3	11.30 ± 1.40	8.60 ± 0.60	11.50 ± 1.52	15.40 ± 4.55	27.00 ± 7.05*	44.30 ± 8.01**
Week 13	15.70 ± 2.25	14.70 ± 1.38	12.44 ± 1.51[3]	16.80 ± 2.52	24.80 ± 4.25	—
Urinalysis						
n	10	9	10	10	10	0
Volume (mL/16 hr)						
Week 13	3.5 ± 0.3	2.9 ± 0.2	3.4 ± 0.2	3.4 ± 0.3	2.3 ± 0.1**	—
Specific gravity						
Week 13	1.079 ± 0.002	1.088 ± 0.001**	1.084 ± 0.002	1.075 ± 0.003	1.083 ± 0.002	—
pH						
Week 13	6.35 ± 0.08	6.17 ± 0.08[5]	6.35 ± 0.08	6.30 ± 0.13	5.70 ± 0.08**	—
FEMALE						
n						
Week 1	9	7	10	8	9	9
Week 3	10	10	8	11	10	10
Week 13	9	10	10	8	10	0
Hematology						
Hematocrit (%)						
Week 1	45.0 ± 1.1	44.2 ± 1.1	44.3 ± 0.5	43.5 ± 0.7	44.1 ± 0.6	43.1 ± 0.9
Week 3	45.8 ± 0.4	45.0 ± 0.5	44.8 ± 0.6	43.6 ± 0.6*	44.2 ± 0.5*	38.5 ± 0.5**
Week 13	45.8 ± 0.8	45.9 ± 0.7	45.3 ± 0.4	44.0 ± 0.5*	41.1 ± 0.9**	—
Hemoglobin (g/dL)						
Week 1	15.3 ± 0.2	15.2 ± 0.3	14.7 ± 0.1*	14.4 ± 0.2*	13.8 ± 0.1**	13.2 ± 0.3**
Week 3	15.3 ± 0.1	15.1 ± 0.1	14.9 ± 0.1**	14.4 ± 0.2**	14.3 ± 0.1**	12.0 ± 0.2**
Week 13	15.4 ± 0.2	15.2 ± 0.2	15.1 ± 0.1	14.6 ± 0.2**	13.2 ± 0.3**	—

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
FEMALE (continued)						
Hematology (continued)						
Erythrocytes (10^6/μL)						
Week 1	7.68 ± 0.21	7.47 ± 0.22	7.32 ± 0.11	7.12 ± 0.15*	7.02 ± 0.09**	6.67 ± 0.17**
Week 3	8.14 ± 0.07	7.92 ± 0.07	7.90 ± 0.10	7.52 ± 0.10**	7.59 ± 0.10**	6.21 ± 0.12**
Week 13	8.38 ± 0.14	8.41 ± 0.15	8.35 ± 0.09	8.04 ± 0.10*	5.76 ± 0.19**	—
Reticulocytes (10^6/μL)						
Week 1	0.06 ± 0.02	0.03 ± 0.01	0.05 ± 0.01	0.02 ± 0.01*	0.01 ± 0.01**	0.01 ± 0.00**
Week 3	0.07 ± 0.02	0.01 ± 0.01	0.04 ± 0.02	0.05 ± 0.02	0.07 ± 0.03	0.31 ± 0.05**
Week 13	0.06 ± 0.02	0.07 ± 0.02	0.08 ± 0.03	0.05 ± 0.01	1.40 ± 0.11**	—
Nucleated erythrocytes (10^3/μL)						
Week 1	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.10 ± 0.10
Week 3	0.04 ± 0.02	0.01 ± 0.01	0.05 ± 0.03	0.03 ± 0.01	0.03 ± 0.03	0.12 ± 0.05
Week 13	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	1.46 ± 0.35**	—
Mean cell volume (fL)						
Week 1	58.7 ± 0.4	59.1 ± 0.4	60.7 ± 0.3**	61.0 ± 0.6**	62.8 ± 0.5**	64.7 ± 0.6**
Week 3	56.2 ± 0.3	56.7 ± 0.3	56.5 ± 0.4	57.8 ± 0.2**	58.2 ± 0.3**	62.0 ± 0.5**
Week 13	54.8 ± 0.3	54.5 ± 0.3	54.3 ± 0.2	54.6 ± 0.4	71.7 ± 1.2**	—
Mean cell hemoglobin (pg)						
Week 1	19.9 ± 0.3	20.3 ± 0.3	20.0 ± 0.3	20.2 ± 0.3	19.7 ± 0.2	19.8 ± 0.3
Week 3	18.8 ± 0.1	19.1 ± 0.2	18.9 ± 0.2	19.1 ± 0.2	18.9 ± 0.2	19.4 ± 0.2**
Week 13	18.4 ± 0.1	18.1 ± 0.2	18.1 ± 0.1	18.2 ± 0.2	23.1 ± 0.4**	—
Mean cell hemoglobin concentration (g/dL)						
Week 1	34.1 ± 0.5	34.4 ± 0.4	33.1 ± 0.3	33.0 ± 0.5	31.4 ± 0.4**	30.6 ± 0.5**
Week 3	33.4 ± 0.3	33.7 ± 0.4	33.3 ± 0.4	33.0 ± 0.3	32.3 ± 0.3*	31.3 ± 0.3**
Week 13	33.6 ± 0.3	33.1 ± 0.3	33.4 ± 0.2	33.2 ± 0.2	32.2 ± 0.3**	—
Platelets (10^3/μL)						
Week 1	810.6 ± 28.0	747.1 ± 18.3	633.0 ± 18.4**	528.6 ± 13.2**	377.8 ± 34.3**	257.8 ± 24.6**
Week 3	816.8 ± 19.0	723.9 ± 19.3**	666.4 ± 16.0**	612.8 ± 12.0**	562.3 ± 20.8**	248.4 ± 16.9**
Week 13	671.3 ± 17.4	622.4 ± 9.2*	611.5 ± 16.9*	542.6 ± 12.1**	360.2 ± 27.5**	—
Leukocytes (10^3/μL)						
Week 1	7.69 ± 0.42	7.26 ± 0.36	5.86 ± 0.47**	5.48 ± 0.34**	4.82 ± 0.14**	4.22 ± 0.37**
Week 3	7.18 ± 0.39	7.73 ± 0.35	8.13 ± 0.17	7.02 ± 0.52	5.92 ± 0.38	3.59 ± 0.21**
Week 13	5.57 ± 0.26	4.52 ± 0.37	5.73 ± 0.32	6.40 ± 0.40	26.20 ± 4.96**	—
Segmented neutrophils (10^3/μL)						
Week 1	1.07 ± 0.18	1.17 ± 0.18	0.89 ± 0.14	1.04 ± 0.12	1.03 ± 0.16	1.09 ± 0.19
Week 3	0.94 ± 0.05	0.86 ± 0.16	1.29 ± 0.07	0.77 ± 0.14	0.71 ± 0.10	0.53 ± 0.07**
Week 13	1.50 ± 0.13	1.09 ± 0.12	1.39 ± 0.12	1.24 ± 0.16	3.87 ± 1.09	—
Lymphocytes (10^3/μL)						
Week 1	6.49 ± 0.38	5.97 ± 0.30	4.65 ± 0.35**	4.39 ± 0.35**	3.76 ± 0.12**	3.09 ± 0.24**
Week 3	6.22 ± 0.37	6.79 ± 0.28	6.79 ± 0.21	6.25 ± 0.47	5.18 ± 0.35	3.14 ± 0.17**
Week 13	3.95 ± 0.23	3.34 ± 0.31	4.18 ± 0.25	5.00 ± 0.33*	21.55 ± 3.74**	—
Monocytes (10^3/μL)						
Week 1	0.06 ± 0.03	0.08 ± 0.04	0.04 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01
Week 3	0.02 ± 0.01	0.06 ± 0.03	0.03 ± 0.02	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Week 13	0.02 ± 0.01	0.02 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.33 ± 0.20	—
Eosinophils (10^3/μL)						
Week 1	0.07 ± 0.03	0.04 ± 0.04	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
Week 3	0.02 ± 0.02	0.02 ± 0.01	0.05 ± 0.03	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Week 13	0.09 ± 0.02	0.06 ± 0.02	0.08 ± 0.03	0.14 ± 0.03	0.43 ± 0.19	—

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
FEMALE (continued)						
Hematology (continued)						
Methemoglobin (g/dL)						
Week 3	0.12 ± 0.02	0.16 ± 0.02	0.15 ± 0.02	0.13 ± 0.01	0.14 ± 0.02	0.11 ± 0.02
Week 13	0.17 ± 0.01	0.14 ± 0.03	0.13 ± 0.02	0.12 ± 0.02	0.13 ± 0.02	—
Total bone marrow cellularity (10^6/femur)						
Week 1	45.2 ± 3.0	—[4]	46.5 ± 1.8	39.9 ± 2.0	35.6 ± 3.5	—[4]
Week 3	51.6 ± 2.5	—[4]	46.8 ± 3.0[3]	48.5 ± 1.1	46.8 ± 2.1	—[4]
Week 13	32.6 ± 0.9[5]	—[4]	35.2 ± 1.4*	36.3 ± 1.2*[5]	60.2 ± 3.0**	—
Clinical Chemistry						
n						
Week 1	10	10	11	9	10	10
Week 3	10	10	9	11	10	10
Week 13	10	10	10	10	10	0
Urea nitrogen (mg/dL)						
Week 1	19.2 ± 0.7	20.4 ± 0.7	20.5 ± 1.3	17.3 ± 0.6	15.8 ± 1.1	20.2 ± 1.1
Week 3	24.2 ± 0.8	23.5 ± 1.0	22.9 ± 0.8	23.6 ± 1.0	22.9 ± 0.8	28.4 ± 0.9*
Week 13	24.6 ± 0.8	23.3 ± 1.5	22.3 ± 1.3	18.8 ± 1.3*	23.8 ± 2.4	—
Creatinine (mg/dL)						
Week 1	0.50 ± 0.03	0.45 ± 0.03	0.53 ± 0.03	0.51 ± 0.03	0.41 ± 0.02	0.46 ± 0.02
Week 3	0.57 ± 0.02	0.54 ± 0.02	0.56 ± 0.02	0.55 ± 0.02	0.50 ± 0.02*	0.50 ± 0.00**
Week 13	0.69 ± 0.03	0.69 ± 0.06	0.63 ± 0.03	0.60 ± 0.03	0.60 ± 0.04	—
Total protein (g/dL)						
Week 1	5.8 ± 0.2	5.7 ± 0.2	5.9 ± 0.4	5.7 ± 0.2	4.7 ± 0.2**	4.9 ± 0.2**
Week 3	6.5 ± 0.1	6.2 ± 0.1*	6.1 ± 0.1**	5.9 ± 0.1**	5.9 ± 0.1**	5.2 ± 0.1**
Week 13	7.0 ± 0.3	6.9 ± 0.3	6.5 ± 0.3	5.6 ± 0.3**	5.3 ± 0.3**	—
Albumin (g/dL)						
Week 1	3.3 ± 0.1	3.2 ± 0.1	3.3 ± 0.2	3.2 ± 0.1	2.8 ± 0.1*	2.9 ± 0.1*
Week 3	3.8 ± 0.1	3.7 ± 0.1	3.6 ± 0.1*	3.4 ± 0.0**	3.5 ± 0.1**	3.3 ± 0.0**
Week 13	4.0 ± 0.1	4.0 ± 0.2	3.8 ± 0.2	3.3 ± 0.2*	3.2 ± 0.2**	—
Alkaline phosphatase (IU/L)						
Week 1	389 ± 28	302 ± 22*	347 ± 28	294 ± 14*	225 ± 12**	198 ± 7**
Week 3	326 ± 14	269 ± 6**	246 ± 9**	223 ± 7**	194 ± 7**	94 ± 7**
Week 13	114 ± 12	123 ± 12	108 ± 8	115 ± 5	69 ± 5**	—
Alanine aminotransferase (IU/L)						
Week 1	36 ± 1	35 ± 1	36 ± 3	40 ± 2	35 ± 2	42 ± 3*
Week 3	32 ± 1	32 ± 1	35 ± 1	38 ± 1**	37 ± 2*	47 ± 2**
Week 13	39 ± 2	40 ± 5	37 ± 2	33 ± 2	48 ± 7	—
Creatine kinase (IU/L)						
Week 1	784 ± 172	1039 ± 223	815 ± 81	954 ± 141	612 ± 45	672 ± 76
Week 3	287 ± 45	412 ± 54	549 ± 111**	407 ± 51*	575 ± 74**	353 ± 35*
Week 13	312 ± 51	522 ± 88	362 ± 99	417 ± 80	307 ± 55	—
Bile acids (µmol/L)						
Week 1	25.60 ± 5.25	19.40 ± 1.89	25.55 ± 3.54	29.00 ± 4.59	19.60 ± 3.06	27.70 ± 2.90
Week 3	10.89 ± 2.34[3]	22.11 ± 6.10*[3]	13.00 ± 1.90	37.55 ± 6.66**	35.50 ± 4.19**	50.10 ± 5.31**
Week 13	27.30 ± 6.90	24.20 ± 8.78	16.67 ± 3.54[3]	15.30 ± 3.40	17.90 ± 2.61	—

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol (continued)

	0 ppm	1250 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
FEMALE (continued)						
Urinalysis						
n	10	10	10	10	9	0
Volume (mL/16 hr)						
Week 13	2.7 ± 0.3	3.3 ± 0.4	3.2 ± 0.3	3.2 ± 0.3	2.3 ± 0.3	—
Specific gravity						
Week 13	1.065 ± 0.003	1.060 ± 0.004	1.063 ± 0.003	1.062 ± 0.004	1.075 ± 0.003	—
pH						
Week 13	6.65 ± 0.18	6.70 ± 0.13	7.05 ± 0.12	7.10 ± 0.27	6.28 ± 0.12	—

[1] *Mean ± standard error.*
[2] n=8.
[3] n=9.
[4] Not measured at this exposure level.
[5] n=10.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE D3 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
MALE						
Hematology						
n						
Week 1	10	9	10	10	10	10
Week 3	8	9	10	10	10	10
Week 13	8	10	10	10	10	9
Hematocrit (%)						
Week 1	43.1 ± 0.8	44.6 ± 0.5	41.8 ± 1.3	42.1 ± 1.0	45.8 ± 0.8	46.4 ± 0.8*
Week 3	46.6 ± 0.6	46.2 ± 0.5	46.1 ± 0.6	47.0 ± 0.6	45.9 ± 0.3	48.0 ± 1.0
Week 13	44.8 ± 0.8	45.0 ± 0.6	44.7 ± 0.4	44.1 ± 0.7	42.3 ± 0.6*	43.4 ± 0.4
Hemoglobin (g/dL)						
Week 1	14.5 ± 0.1	14.5 ± 0.1	13.5 ± 0.3*	13.0 ± 0.2**	14.0 ± 0.2*	13.9 ± 0.2*
Week 3	15.4 ± 0.2	15.4 ± 0.2	15.2 ± 0.2	15.3 ± 0.2	14.8 ± 0.1*	15.1 ± 0.2
Week 13	15.0 ± 0.2	15.2 ± 0.1	14.9 ± 0.1	14.6 ± 0.1	14.0 ± 0.1**	13.7 ± 0.2**
Erythrocytes (10^6/μL)						
Week 1	7.26 ± 0.13	7.40 ± 0.09	6.63 ± 0.17*	6.19 ± 0.16**	6.64 ± 0.12**	6.17 ± 0.11**
Week 3	8.23 ± 0.14	8.07 ± 0.10	7.89 ± 0.13*	7.81 ± 0.10**	7.39 ± 0.08**	7.45 ± 0.15**
Week 13	8.64 ± 0.15	8.74 ± 0.10	8.54 ± 0.09	8.11 ± 0.12*	7.48 ± 0.12**	7.18 ± 0.12**
Reticulocytes (10^6/μL)						
Week 1	0.24 ± 0.02[2]	0.35 ± 0.11	0.27 ± 0.03	0.41 ± 0.05*	0.87 ± 0.11**	1.08 ± 0.13**
Week 3	0.28 ± 0.02	0.27 ± 0.02	0.23 ± 0.02	0.21 ± 0.02[2]	0.27 ± 0.02	0.26 ± 0.02
Week 13	0.14 ± 0.03	0.24 ± 0.06[2]	0.15 ± 0.02	0.18 ± 0.02	0.22 ± 0.05	0.46 ± 0.07**
Nucleated erythrocytes (10^3/μL)						
Week 1	0.05 ± 0.01	0.06 ± 0.03	0.13 ± 0.04	0.75 ± 0.17**	0.69 ± 0.26**	1.58 ± 0.42**
Week 3	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.07 ± 0.03
Week 13	0.00 ± 0.00	0.00 ± 0.00[2]	0.01 ± 0.01[2]	0.01 ± 0.01	0.00 ± 0.00	0.04 ± 0.02**
Mean cell volume (fL)						
Week 1	59.5 ± 0.3	60.3 ± 0.4	63.0 ± 0.7**	68.2 ± 0.7**	69.0 ± 0.6**	75.3 ± 1.5**
Week 3	56.5 ± 0.5	57.2 ± 0.2	58.4 ± 0.4**	60.1 ± 0.6**	62.3 ± 0.5**	64.4 ± 0.4**
Week 13	52.0 ± 0.4	51.5 ± 0.3	52.3 ± 0.4	54.4 ± 0.3**	56.7 ± 0.5**	60.6 ± 1.1**
Mean cell hemoglobin (pg)						
Week 1	19.9 ± 0.3	19.7 ± 0.2	20.3 ± 0.2	21.1 ± 0.3*	21.1 ± 0.2**	22.5 ± 0.3**
Week 3	18.7 ± 0.3	19.1 ± 0.1	19.2 ± 0.2	19.5 ± 0.2**	20.1 ± 0.1**	20.3 ± 0.2**
Week 13	17.4 ± 0.2	17.4 ± 0.1	17.5 ± 0.2	18.0 ± 0.2*	18.7 ± 0.3**	19.1 ± 0.3**
Mean cell hemoglobin concentration (g/dL)						
Week 1	33.6 ± 0.4	32.6 ± 0.3	32.3 ± 0.5	31.0 ± 0.5**	30.7 ± 0.3**	30.0 ± 0.5**
Week 3	33.1 ± 0.3	33.4 ± 0.2	32.9 ± 0.2	32.5 ± 0.2	32.3 ± 0.2	31.6 ± 0.4**
Week 13	33.4 ± 0.4	33.8 ± 0.3	33.4 ± 0.3	33.1 ± 0.3	33.0 ± 0.3	31.5 ± 0.4**
Platelets (10^3/μL)						
Week 3	831.6 ± 16.5	831.2 ± 16.7	775.6 ± 21.7	803.6 ± 14.0[2]	765.3 ± 15.9*	778.0 ± 6.9*
Week 13	648.1 ± 6.7	634.3 ± 12.2	616.3 ± 11.7	623.4 ± 10.7	595.2 ± 17.9**	602.8 ± 19.0*
Leukocytes (10^3/μL)						
Week 1	8.43 ± 0.31	8.54 ± 0.49	7.93 ± 0.41	10.36 ± 0.26**	15.95 ± 2.87**	25.59 ± 3.33**
Week 3	9.21 ± 0.67	8.86 ± 0.42	9.31 ± 0.49	7.97 ± 0.52	8.80 ± 0.76	8.75 ± 0.62
Week 13	5.64 ± 0.32	6.40 ± 0.28	5.97 ± 0.36	6.28 ± 0.39	6.74 ± 0.31	5.64 ± 0.32

TABLE D3 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
MALE (continued)						
Hematology (continued)						
Segmented neutrophils (10^3/μL)						
Week 1	1.32 ± 0.17	0.99 ± 0.15	1.36 ± 0.16	2.44 ± 0.14**	2.09 ± 0.48*	3.44 ± 0.76**
Week 3	0.87 ± 0.10	0.83 ± 0.12	1.08 ± 0.15	1.00 ± 0.13	1.01 ± 0.11	1.12 ± 0.14
Week 13	1.06 ± 0.12	1.38 ± 0.09[2]	1.12 ± 0.12	1.27 ± 0.11	1.58 ± 0.20	0.98 ± 0.08
Lymphocytes (10^3/μL)						
Week 1	6.92 ± 0.41	7.39 ± 0.41	6.39 ± 0.33	7.66 ± 0.34	13.26 ± 2.33**	21.66 ± 3.04**
Week 3	8.19 ± 0.64	7.84 ± 0.48	8.07 ± 0.40	6.84 ± 0.51	7.66 ± 0.74	7.54 ± 0.55
Week 13	4.53 ± 0.36	5.18 ± 0.20[2]	4.77 ± 0.37	4.93 ± 0.46	5.11 ± 0.30	4.57 ± 0.29
Monocytes (10^3/μL)						
Week 1	0.13 ± 0.04	0.12 ± 0.04	0.09 ± 0.03	0.15 ± 0.04	0.46 ± 0.19	0.29 ± 0.08
Week 3	0.12 ± 0.04	0.14 ± 0.03	0.13 ± 0.04	0.10 ± 0.02	0.09 ± 0.04	0.07 ± 0.03
Week 13	0.04 ± 0.02	0.02 ± 0.01[2]	0.05 ± 0.02	0.05 ± 0.03	0.04 ± 0.02	0.05 ± 0.03
Eosinophils (10^3/μL)						
Week 1	0.01 ± 0.01	0.02 ± 0.01	0.05 ± 0.02	0.06 ± 0.02*	0.03 ± 0.02	0.13 ± 0.05*
Week 3	0.02 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.01
Week 13	0.01 ± 0.01	0.03 ± 0.01[2]	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02
Methemoglobin (g/dL)						
Week 1	0.15 ± 0.03	0.15 ± 0.02	0.17 ± 0.02	0.15 ± 0.02[2]	0.17 ± 0.02	0.16 ± 0.02
Week 3	0.15 ± 0.03	0.17 ± 0.02	0.19 ± 0.03	0.22 ± 0.04	0.12 ± 0.03	0.17 ± 0.03
Week 13	0.12 ± 0.02	0.12 ± 0.02	0.14 ± 0.02	0.09 ± 0.03	0.13 ± 0.04	0.10 ± 0.02
Total bone marrow cellularity (10^6/femur)						
Week 1	56.5 ± 2.5	—[3]	—	62.7 ± 2.5	71.0 ± 1.9**	71.8 ± 2.2**
Week 3	73.7 ± 3.1[4]	—	—	71.9 ± 5.5	75.3 ± 4.0	74.8 ± 3.8
Week 13	61.5 ± 2.0[4]	—	—	64.3 ± 2.0	72.3 ± 3.2**	68.1 ± 1.8*[4]
Clinical Chemistry						
n						
Week 1	10	10	10	10	10	10
Week 3	10	10	10	10	10	10
Week 13	9	10	10	10	10	10
Urea nitrogen (mg/dL)						
Week 1	15.9 ± 0.4	16.4 ± 0.9	21.9 ± 0.8**	25.7 ± 1.0**	24.0 ± 1.0**	22.6 ± 1.0**
Week 3	20.5 ± 1.1	24.3 ± 1.0*	23.5 ± 0.7*	23.2 ± 0.9	25.8 ± 0.7**	26.9 ± 0.9**
Week 13	17.3 ± 0.7	19.0 ± 0.4	18.9 ± 0.8	20.2 ± 0.5**	20.1 ± 0.9**	22.6 ± 0.8**
Creatinine (mg/dL)						
Week 1	0.45 ± 0.02	0.48 ± 0.02	0.51 ± 0.03	0.46 ± 0.02	0.48 ± 0.02	0.53 ± 0.03*
Week 3	0.55 ± 0.03	0.51 ± 0.02	0.50 ± 0.04	0.51 ± 0.02	0.55 ± 0.02	0.60 ± 0.03
Week 13	0.61 ± 0.02	0.60 ± 0.02	0.61 ± 0.02	0.62 ± 0.02	0.62 ± 0.03	0.60 ± 0.02
Total protein (g/dL)						
Week 1	5.6 ± 0.1	5.7 ± 0.1	6.3 ± 0.1**	6.3 ± 0.1**	6.1 ± 0.1**	6.0 ± 0.1**
Week 3	6.3 ± 0.1	6.5 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.3 ± 0.1
Week 13	6.8 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.5 ± 0.1**	6.3 ± 0.1**	6.0 ± 0.1**

TABLE D3 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
MALE (continued)						
Clinical Chemistry (continued)						
Albumin (g/dL)						
Week 1	3.19 ± 0.06	3.22 ± 0.05	3.59 ± 0.10*	3.65 ± 0.05**	3.51 ± 0.06**	3.48 ± 0.05**
Week 3	3.5 ± 0.1	3.5 ± 0.0	3.4 ± 0.1	3.4 ± 0.0	3.3 ± 0.0	3.5 ± 0.1
Week 13	3.5 ± 0.0	3.5 ± 0.1	3.5 ± 0.0	3.4 ± 0.0**	3.4 ± 0.0**	3.3 ± 0.1**
Alkaline phosphatase (IU/L)						
Week 1	377 ± 12	412 ± 10	450 ± 14**	476 ± 15**	432 ± 18**	422 ± 13**
Week 3	319 ± 11	343 ± 11	304 ± 7	335 ± 11	341 ± 7	357 ± 11*
Week 13	145 ± 8	147 ± 6	144 ± 4	152 ± 7	145 ± 7	156 ± 5
Alanine aminotransferase (IU/L)						
Week 1	48 ± 2	49 ± 3	58 ± 3	58 ± 4	41 ± 3	39 ± 3*
Week 3	30 ± 1	31 ± 1	31 ± 2	31 ± 1	31 ± 2	32 ± 1
Week 13	30 ± 1	33 ± 2	32 ± 1	34 ± 2	33 ± 1	36 ± 2*
Creatine kinase (IU/L)						
Week 1	782 ± 96	544 ± 72	712 ± 101	914 ± 122	747 ± 80	767 ± 112
Week 3	416 ± 62	597 ± 114	300 ± 28	353 ± 62	440 ± 83	437 ± 65
Week 13	280 ± 50	226 ± 37	294 ± 43	226 ± 36	214 ± 20	270 ± 38
Bile acids (µmol/L)						
Week 1	11.40 ± 2.05	8.80 ± 1.04	11.70 ± 1.17	11.60 ± 0.85	9.50 ± 1.01	9.11 ± 1.06[2]
Week 3	11.44 ± 1.43[2]	18.40 ± 2.15	11.67 ± 1.72[2]	12.90 ± 2.40	10.20 ± 1.07	17.80 ± 3.15
Week 13	12.00 ± 2.35[5]	21.33 ± 6.38[6]	12.75 ± 1.85[7]	16.50 ± 4.38[7]	11.17 ± 2.43[6]	16.75 ± 4.36[7]
Urinalysis						
n	10	10	10	10	10	10
Volume (mL/16 hr)						
Week 13	4.8 ± 0.6	2.8 ± 0.3	2.3 ± 0.2**	2.8 ± 0.4	3.4 ± 0.5	4.4 ± 0.4
Specific gravity						
Week 13	1.046 ± 0.003	1.064 ± 0.002**	1.066 ± 0.002**	1.064 ± 0.003**	1.061 ± 0.003*	1.055 ± 0.002
pH						
Week 13	6.85 ± 0.11	6.55 ± 0.12	6.85 ± 0.13	6.60 ± 0.07	6.50 ± 0.13	6.50 ± 0.11*

TABLE D3 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
FEMALE						
Hematology						
n						
Week 1	10	10	10	9	10	10
Week 3	8	8	9	10	10	10
Week 13	10	10	10	10	10	9
Hematocrit (%)						
Week 1	46.7 ± 0.4	44.8 ± 0.5**	43.8 ± 0.5**	39.3 ± 0.7**	41.1 ± 0.6**	40.5 ± 0.8**
Week 3	47.5 ± 0.9	46.7 ± 0.5	46.8 ± 0.7	46.9 ± 0.5	46.5 ± 0.7	47.4 ± 0.6
Week 13	44.8 ± 0.6	43.2 ± 0.8	42.8 ± 0.7	43.6 ± 0.7	44.4 ± 0.7	46.1 ± 0.7
Hemoglobin (g/dL)						
Week 1	15.5 ± 0.1	15.3 ± 0.1	14.6 ± 0.1**	13.1 ± 0.3**	13.4 ± 0.1**	13.3 ± 0.3**
Week 3	16.0 ± 0.3	15.6 ± 0.2	15.6 ± 0.2	15.3 ± 0.1	15.0 ± 0.2*	14.4 ± 0.1**
Week 13	14.9 ± 0.2	14.4 ± 0.2*	13.9 ± 0.2**	14.2 ± 0.2**	14.0 ± 0.2**	13.4 ± 0.2**
Erythrocytes (10^6/μL)						
Week 1	7.98 ± 0.10	7.51 ± 0.07**	7.18 ± 0.09**	6.06 ± 0.18**	5.63 ± 0.11**	5.50 ± 0.14**
Week 3	8.48 ± 0.20	8.15 ± 0.08	7.72 ± 0.15**	7.45 ± 0.11**	7.15 ± 0.12**	6.74 ± 0.10**
Week 13	8.15 ± 0.09	7.59 ± 0.15**	7.09 ± 0.14**	7.00 ± 0.12**	6.80 ± 0.11**	6.58 ± 0.14**
Reticulocytes (10^6/μL)						
Week 1	0.25 ± 0.03	0.25 ± 0.03	0.22 ± 0.04	0.55 ± 0.09*	1.11 ± 0.09**	1.15 ± 0.12**
Week 3	0.15 ± 0.02	0.17 ± 0.03	0.14 ± 0.02	0.15 ± 0.02	0.20 ± 0.03	0.24 ± 0.08
Week 13	0.12 ± 0.02	0.17 ± 0.03	0.19 ± 0.03	0.28 ± 0.03**	0.28 ± 0.05**	0.27 ± 0.05**
Nucleated erythrocytes (10^3/μL)						
Week 1	0.05 ± 0.02	0.10 ± 0.03	0.11 ± 0.03	0.81 ± 0.19**	6.13 ± 1.19**	4.97 ± 0.99**
Week 3	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.05 ± 0.03	0.13 ± 0.05*
Week 13	0.01 ± 0.01[2]	0.03 ± 0.02	0.02 ± 0.01	0.05 ± 0.02	0.10 ± 0.03*	0.16 ± 0.04**
Mean cell volume (fL)						
Week 1	58.6 ± 0.5	59.5 ± 0.3	60.8 ± 0.3**	65.1 ± 1.0**	73.3 ± 1.3**	73.7 ± 1.4**
Week 3	56.1 ± 0.3	57.3 ± 0.5	60.7 ± 0.5**	63.1 ± 0.4**	65.1 ± 0.6**	70.5 ± 1.0**
Week 13	54.8 ± 0.3	57.0 ± 0.4**	60.5 ± 0.4**	62.4 ± 0.6**	65.3 ± 0.6**	70.1 ± 0.9**
Mean cell hemoglobin (pg)						
Week 1	19.4 ± 0.2	20.4 ± 0.1**	20.4 ± 0.2**	21.7 ± 0.3**	23.9 ± 0.4**	24.3 ± 0.5**
Week 3	18.9 ± 0.2	19.2 ± 0.3	20.3 ± 0.2**	20.6 ± 0.2**	21.0 ± 0.2**	21.3 ± 0.2**
Week 13	18.3 ± 0.2	18.9 ± 0.2	19.7 ± 0.2**	20.2 ± 0.3**	20.6 ± 0.2**	20.4 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Week 1	33.1 ± 0.3	34.2 ± 0.3	33.4 ± 0.3	33.4 ± 0.5	32.8 ± 0.4	32.9 ± 0.4
Week 3	33.6 ± 0.3	33.5 ± 0.4	33.4 ± 0.3	32.6 ± 0.2*	32.4 ± 0.4*	30.3 ± 0.4**
Week 13	33.3 ± 0.3	33.3 ± 0.3	32.6 ± 0.3	32.6 ± 0.4	31.5 ± 0.3**	29.1 ± 0.3**
Platelets (10^3/μL)						
Week 1	934.8 ± 33.7	959.0 ± 35.1	921.2 ± 49.2	1045.8 ± 45.4	1118.9 ± 29.5**	1097.7 ± 45.8*
Week 3	819.0 ± 20.8	805.4 ± 27.4	799.1 ± 28.1	754.6 ± 22.1[2]	739.4 ± 13.3**	705.8 ± 15.5**
Week 13	691.3 ± 17.0	653.9 ± 13.6	688.4 ± 25.1	578.1 ± 21.8**	495.6 ± 9.7**	495.7 ± 11.4**
Leukocytes (10^3/μL)						
Week 1	7.95 ± 0.20	7.95 ± 0.36	7.93 ± 0.54	12.07 ± 1.62	35.71 ± 3.98**	31.31 ± 3.92**
Week 3	8.56 ± 0.70	7.89 ± 0.42	7.77 ± 0.69	8.48 ± 0.66	9.96 ± 0.82	8.54 ± 0.56
Week 13	6.42 ± 0.43[2]	5.98 ± 0.35	5.99 ± 0.32	6.10 ± 0.27	6.05 ± 0.28	6.97 ± 0.55

TABLE D3 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
FEMALE (continued)						
Hematology (continued)						
Segmented neutrophils ($10^3/\mu L$)						
Week 1	1.10 ± 0.13	0.86 ± 0.11	0.74 ± 0.11	1.48 ± 0.38	4.71 ± 0.94**	4.00 ± 0.93**
Week 3	0.97 ± 0.23	1.15 ± 0.22	0.81 ± 0.16	0.90 ± 0.18	1.21 ± 0.18	0.90 ± 0.15
Week 13	0.94 ± 0.13[2]	1.20 ± 0.24	1.08 ± 0.22	1.19 ± 0.19	1.02 ± 0.15	1.29 ± 0.14
Lymphocytes ($10^3/\mu L$)						
Week 1	6.62 ± 0.26	6.92 ± 0.36	6.92 ± 0.46	10.20 ± 1.30*	30.06 ± 3.32**	26.80 ± 3.21**
Week 3	7.41 ± 0.61	6.61 ± 0.37	6.77 ± 0.57	7.38 ± 0.49	8.56 ± 0.76	7.40 ± 0.45
Week 13	5.52 ± 0.41	4.63 ± 0.20	4.81 ± 0.16	4.86 ± 0.31	4.93 ± 0.33	5.51 ± 0.47
Monocytes ($10^3/\mu L$)						
Week 1	0.14 ± 0.03	0.12 ± 0.04	0.15 ± 0.04	0.32 ± 0.08	0.66 ± 0.30	0.30 ± 0.12
Week 3	0.15 ± 0.05	0.11 ± 0.03	0.10 ± 0.04	0.16 ± 0.05	0.13 ± 0.04	0.19 ± 0.02
Week 13	0.06 ± 0.02	0.09 ± 0.03	0.05 ± 0.02	0.03 ± 0.01	0.05 ± 0.03	0.08 ± 0.02
Eosinophils ($10^3/\mu L$)						
Week 1	0.04 ± 0.02	0.02 ± 0.02	0.05 ± 0.02	0.01 ± 0.01	0.08 ± 0.05	0.05 ± 0.05
Week 3	0.03 ± 0.01	0.02 ± 0.02	0.07 ± 0.02	0.03 ± 0.02	0.06 ± 0.04	0.05 ± 0.02
Week 13	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.04 ± 0.02	0.05 ± 0.02
Methemoglobin (g/dL)						
Week 1	0.15 ± 0.02	0.18 ± 0.02	0.15 ± 0.02	0.16 ± 0.03	0.20 ± 0.02	0.20 ± 0.02
Week 3	0.12 ± 0.02	0.12 ± 0.02	0.11 ± 0.02	0.13 ± 0.02	0.10 ± 0.01	0.09 ± 0.01
Week 13	0.11 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	0.11 ± 0.01
Total bone marrow cellularity (10^6/femur)						
Week 1	50.6 ± 2.0	—	—	55.1 ± 1.1[4]	59.2 ± 1.6*	53.8 ± 2.6
Week 3	47.6 ± 2.0[4]	—	—	51.7 ± 1.7	49.9 ± 3.5	49.3 ± 2.6
Week 13	40.7 ± 3.8	—	—	47.3 ± 1.9*	54.5 ± 1.2**	54.5 ± 2.4**[4]
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Week 1	19.6 ± 0.5	19.0 ± 1.4	20.3 ± 0.7	20.1 ± 0.9	22.2 ± 1.0	21.7 ± 0.8
Week 3	23.3 ± 0.9	21.5 ± 0.6	22.6 ± 0.9	26.1 ± 0.8*	29.1 ± 1.5**	30.9 ± 1.1**
Week 13	18.4 ± 0.5	18.7 ± 0.9	20.6 ± 0.4**	21.1 ± 1.2*	26.2 ± 0.8**	31.2 ± 1.3**
Creatinine (mg/dL)						
Week 1	0.35 ± 0.02	0.39 ± 0.01	0.39 ± 0.03	0.38 ± 0.01	0.39 ± 0.01	0.43 ± 0.02**
Week 3	0.50 ± 0.02	0.52 ± 0.01	0.53 ± 0.05	0.48 ± 0.02	0.51 ± 0.02	0.53 ± 0.02
Week 13	0.56 ± 0.02	0.56 ± 0.02	0.60 ± 0.03	0.65 ± 0.03*	0.66 ± 0.02**	0.66 ± 0.03**
Total protein (g/dL)						
Week 1	5.7 ± 0.1	5.8 ± 0.1	5.6 ± 0.0	5.5 ± 0.1	5.3 ± 0.1**	5.6 ± 0.1
Week 3	6.6 ± 0.1	6.6 ± 0.1	6.4 ± 0.1*	6.3 ± 0.1**	6.0 ± 0.1**	5.9 ± 0.0**
Week 13	6.9 ± 0.1	6.8 ± 0.1	6.4 ± 0.1**	6.3 ± 0.1**	5.8 ± 0.1**	5.7 ± 0.1**
Albumin (g/dL)						
Week 1	3.4 ± 0.1	3.5 ± 0.1	3.3 ± 0.1	3.3 ± 0.1	3.1 ± 0.1*	3.3 ± 0.1
Week 3	3.7 ± 0.1	3.8 ± 0.1	3.6 ± 0.0	3.6 ± 0.0	3.5 ± 0.1*	3.5 ± 0.0**
Week 13	3.8 ± 0.1	3.8 ± 0.1	3.6 ± 0.1	3.5 ± 0.0**	3.4 ± 0.0**	3.4 ± 0.1**

TABLE D3 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol (continued)

	0 ppm	750 ppm	1500 ppm	3000 ppm	4500 ppm	6000 ppm
FEMALE (continued)						
Clinical Chemistry (continued)						
Alkaline phosphatase (IU/L)						
Week 1	298 ± 8	307 ± 11	323 ± 12	325 ± 13	313 ± 5	335 ± 13*
Week 3	281 ± 25	235 ± 7	249 ± 6	242 ± 7	230 ± 4	242 ± 7
Week 13	140 ± 11	142 ± 9	157 ± 10	175 ± 14	235 ± 15**	254 ± 13**
Alanine aminotransferase (IU/L)						
Week 1	24 ± 1	23 ± 0	24 ± 1	25 ± 1	28 ± 1*	31 ± 1**
Week 3	35 ± 3	29 ± 1	28 ± 2	31 ± 2	32 ± 1	32 ± 1
Week 13	27 ± 1	28 ± 1	29 ± 1	29 ± 1	31 ± 1**	33 ± 2**
Creatine kinase (IU/L)						
Week 1	542 ± 36	590 ± 57	524 ± 37	651 ± 58	644 ± 72	647 ± 55
Week 3	429 ± 102	670 ± 175	470 ± 157	431 ± 111	284 ± 51	412 ± 94
Week 13	152 ± 21	154 ± 17	166 ± 18	234 ± 30*	264 ± 22**	210 ± 27*
Bile acids (μmol/L)						
Week 1	15.22 ± 1.61[2]	23.13 ± 9.88[7]	13.89 ± 3.21[2]	15.50 ± 2.60[7]	21.20 ± 5.10	17.00 ± 3.89[2]
Week 3	12.40 ± 1.60	23.10 ± 5.84	23.00 ± 3.67*	22.70 ± 5.48	20.50 ± 3.82	22.80 ± 3.23*
Week 13	8.75 ± 1.54[7]	19.14 ± 5.26[8]	7.50 ± 1.23[6]	11.25 ± 1.84[7]	11.00 ± 1.45	13.30 ± 2.86
Urinalysis						
n	10	10	10	10	10	10
Volume (mL/16 hr)						
Week 13	3.8 ± 0.4	2.5 ± 0.1*	2.1 ± 0.1**	2.1 ± 0.1**	2.3 ± 0.2**	2.3 ± 0.1**
Specific gravity						
Week 13	1.055 ± 0.005	1.060 ± 0.003	1.067 ± 0.002*	1.075 ± 0.002**	1.074 ± 0.002**	1.082 ± 0.003**
pH						
Week 13	6.60 ± 0.10	6.55 ± 0.12	6.65 ± 0.11	6.55 ± 0.05	6.90 ± 0.07	6.60 ± 0.07

[1] Mean ± standard error.
[2] n=9.
[3] Not measured at this exposure level.
[4] n=10.
[5] n=5.
[6] n=6.
[7] n=8.
[8] n=7.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

APPENDIX E

Reproductive Tissue Evaluations and Estrous Cycle Characterization

TABLE E1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

Study Parameters	0 ppm	750 ppm	1500 ppm	3000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	316 ± 7	295 ± 7	260 ± 5**	214 ± 5**
Left epididymis	0.431 ± 0.012	0.427 ± 0.009	0.206 ± 0.007**	0.162 ± 0.005**
Left cauda epididymis	0.194 ± 0.006	0.189 ± 0.005	0.082 ± 0.004**	0.068 ± 0.002**
Left testis	1.494 ± 0.032	1.488 ± 0.020	0.673 ± 0.046**	0.500 ± 0.025**
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.140 ± 0.317	8.630 ± 0.331	1.790 ± 0.520**	0.000 ± 0.000**
Spermatid heads (10^7/testis)	13.69 ± 0.63	12.84 ± 0.48	1.41 ± 0.50**	0.00 ± 0.00**
Spermatid count (mean/10^{-4} mL suspension)	68.43 ± 3.17	64.20 ± 2.42	7.03 ± 2.51**	0.00 ± 0.00**
Spermatozoal measurements				
Motility (%)	98.43 ± 0.15	97.49 ± 0.39	0.00 ± 0.00**	0.00 ± 0.00**
Concentration (10^6/g caudal epididymal tissue)	755.4 ± 25.6	655.8 ± 14.1*	13.0 ± 3.4**	7.2 ± 2.2**

[1] Data presented as mean ± standard error.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

Study Parameters	0 ppm	1500 ppm	3000 ppm	4500 ppm
n	9	6	3	2
Necropsy body weight (g)	189 ± 4[2]	170 ± 3**[2]	145 ± 3**[2]	151 ± 2**[3]
Estrous cycle length (days)	6.72 ± 0.49[4]	7.67 ± 0.49[5]	5.17 ± 0.17[6]	7.00 ± 1.00[7]
Estrous stages (% of cycle)				
Diestrus	41.7	52.4	70.0	70.0
Proestrus	13.3	10.5	7.5	6.7
Estrus	32.5	26.7	16.7	13.3
Metestrus	12.5	10.5	5.8	10.0

[1] Data presented as mean ± standard error. Differences from the control group for estrous cycle length were not significant by Dunn's test. There is evidence to suggest that animals in the 1500 ppm (P≤0.01) and 3000 ppm (P≤0.05) dose groups differed significantly from the controls in the relative frequency of time spent in estrous stages (Wilk's Criterion). Although the 4500 ppm group also appeared different, Wilk's Criterion gave a P-value of 0.09. The lack of significance at this dose level may have been due to increased variability and/or the small sample size (n=5).
[2] n=10.
[3] n=5.
[4] Estrous cycle longer than 12 days or unclear in 1 of 10 animals.
[5] Estrous cycle longer than 12 days or unclear in 4 of 10 animals.
[6] Estrous cycle longer than 12 days or unclear in 7 of 10 animals.
[7] Estrous cycle longer than 12 days or unclear in three of five animals.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E3 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

Study Parameters	0 ppm	2500 ppm	5000 ppm	10,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	315 ± 5	296 ± 4**	295 ± 8*	236 ± 5**
Left epididymis	0.444 ± 0.007	0.447 ± 0.007	0.417 ± 0.008*	0.199 ± 0.008**
Left cauda epididymis	0.185 ± 0.003	0.190 ± 0.003	0.173 ± 0.004*	0.081 ± 0.003**
Left testis	1.459 ± 0.024	1.519 ± 0.020	1.410 ± 0.025	0.727 ± 0.042**
Spermatid measurements				
Spermatid heads (10^7/g testis)	8.980 ± 0.352	9.630 ± 0.273	9.410 ± 0.376	1.610 ± 0.399**
Spermatid heads (10^7/testis)	13.12 ± 0.58	14.63 ± 0.50	13.27 ± 0.60	1.17 ± 0.31**
Spermatid count (mean/10^{-4} mL suspension)	65.58 ± 2.90	73.15 ± 2.49	66.35 ± 3.00	5.83 ± 1.57**
Spermatozoal measurements				
Motility (%)	96.55 ± 1.02	97.88 ± 0.67	97.07 ± 0.93	0.56 ± 0.44**
Concentration (10^6/g caudal epididymal tissue)	763.9 ± 23.1	658.3 ± 14.8**	669.0 ± 25.2**	27.2 ± 5.2**

[1] Data presented as mean ± standard error.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E4 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

Study Parameters	0 ppm	2500 ppm	5000 ppm	10,000 ppm
n	10	6	9	6
Necropsy body weight (g)	185 ± 3	177 ± 1[2]	173 ± 3**[2]	149 ± 1**[2]
Estrous cycle length (days)	5.40 ± 0.15	5.83 ± 0.40[3]	5.83 ± 0.26[4]	6.50 ± 0.43*[3]
Estrous stages (% of cycle)				
Diestrus	36.7	37.3	42.5	55.0
Proestrus	15.0	11.0	15.8	10.0
Estrus	39.2	44.1	30.0	25.8
Metestrus	9.2	7.6	11.7	9.2

[1] Data presented as mean ± standard error. There is evidence that animals in the 10,000 ppm group differed significantly (P≤0.05, Wilk's Criterion) from the controls in the relative frequency of time spent in estrous stages. Females in this group spent more time in diestrus and less time in proestrus and estrus than did controls.
[2] n=10.
[3] Estrous cycle longer than 12 days or unclear in 4 of 10 animals.
[4] Estrous cycle longer than 12 days or unclear in 1 of 10 animals.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E5 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

Study Parameters	0 ppm	3000 ppm	4500 ppm	6000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	308 ± 6	298 ± 3	280 ± 5**	264 ± 5**
Left epididymis	0.426 ± 0.010	0.429 ± 0.004	0.405 ± 0.007*	0.405 ± 0.008*
Left cauda epididymis	0.179 ± 0.003	0.183 ± 0.003	0.176 ± 0.003	0.173 ± 0.005
Left testis	1.480 ± 0.031	1.480 ± 0.018	1.420 ± 0.018	1.420 ± 0.021
Spermatid measurements				
Spermatid heads (10^7/g testis)	8.540 ± 0.291	9.210 ± 0.423	8.970 ± 0.374	9.290 ± 0.217
Spermatid heads (10^7/testis)	12.64 ± 0.39	13.60 ± 0.54	12.72 ± 0.52	13.26 ± 0.44
Spermatid count (mean/10^{-4} mL suspension)	63.20 ± 1.94	67.98 ± 2.69	63.60 ± 2.62	66.28 ± 2.20
Spermatozoal measurements				
Motility (%)	98.57 ± 0.08	98.31 ± 0.23	98.48 ± 0.12	98.49 ± 0.16
Concentration (10^6/g caudal epididymal tissue)	713.9 ± 16.2	633.0 ± 13.1**	656.3 ± 13.3**	617.2 ± 22.9**

[1] Data presented as mean ± standard error. Differences from the control group for cauda epididymal weights, spermatid measurements, and sperm motility are not significant by Dunn's test; differences from the control group for left testis weights are not significant by Shirley's test.

* Significantly different (P≤0.05) from the control group by Shirley's test.

** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E6 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

Study Parameters	0 ppm	3000 ppm	4500 ppm	6000 ppm
n	7	6	7	6
Necropsy body weight (g)	186 ± 4[2]	172 ± 2**[2]	160 ± 2**[2]	145 ± 2**[2]
Estrous cycle length (days)	6.50 ± 0.70[3]	6.83 ± 0.95[4]	7.57 ± 0.53[3]	5.83 ± 0.70[4]
Estrous stages (% of cycle)				
Diestrus	28.9	45.6	52.8	67.5
Proestrus	8.8	11.4	13.9	7.0
Estrus	57.9	38.6	20.4	17.5
Metestrus	4.4	4.4	13.0	7.9

[1] Data presented as mean ± standard error. Differences from the control group for estrous cycle lengths are not significant by Dunn's test. There is evidence that animals in the 4500 and 6000 ppm groups differed significantly (P≤0.01, Wilk's Criterion) from the controls in the relative frequency of time spent in estrous stages. Females in these groups spent more time in diestrus and less time in proestrus, metestrus, and estrus than did controls.

[2] n=10.

[3] Estrous cycle longer than 12 days or unclear in 3 of 10 animals.

[4] Estrous cycle longer than 12 days or unclear in 4 of 10 animals.

** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E7 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

Study Parameters	0 ppm	2000 ppm	4000 ppm	6000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	39.2 ± 0.8	39.6 ± 0.8	40.8 ± 0.8	37.8 ± 0.9
Left epididymis	0.045 ± 0.001	0.046 ± 0.002	0.042 ± 0.001	0.031 ± 0.001**
Left cauda epididymis	0.016 ± 0.001	0.017 ± 0.001	0.016 ± 0.001	0.013 ± 0.001*
Left testis	0.114 ± 0.001	0.113 ± 0.003	0.097 ± 0.003**	0.025 ± 0.001**
Spermatid measurements				
Spermatid heads (10^7/g testis)	19.440 ± 0.630	19.490 ± 0.693	16.790 ± 0.950*	1.490 ± 0.582**
Spermatid heads (10^7/testis)	2.22 ± 0.08	2.21 ± 0.11	1.63 ± 0.11**	0.04 ± 0.01**
Spermatid count (mean/10^{-4} mL suspension)	69.43 ± 2.67	69.18 ± 3.32	50.78 ± 3.29**	1.20 ± 0.46**
Spermatozoal measurements				
Motility (%)	99.29 ± 0.07	99.06 ± 0.08*	98.93 ± 0.24	0.00 ± 0.00**
Concentration (10^6/g caudal epididymal tissue)	1587.8 ± 69.03	1181.0 ± 56.29**	1077.4 ± 38.70**	335.9 ± 40.13**

[1] Data presented as mean ± standard error.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E8 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Methoxyethanol[1]

Study Parameters	0 ppm	6000 ppm	8000 ppm	10,000 ppm
n	10	3	4	2
Necropsy body weight (g)	29.7 ± 0.7	27.2 ± 1.2[2]	26.0 ± 0.9**[2]	23.9 ± 0.9**[2]
Estrous cycle length (days)	4.60 ± 0.22	7.17 ± 0.83*[3]	5.63 ± 0.47*[4]	8.50 ± 1.50*[5]
Estrous stages (% of cycle)				
Diestrus	30.0	18.3	10.0	39.2
Proestrus	19.2	4.2	8.3	2.5
Estrus	34.2	70.8	62.5	50.0
Metestrus	16.7	6.7	19.2	8.3

[1] Data presented as mean ± standard error. All dose groups differed significantly from controls in the relative frequency of time spent in estrous stages (Wilk's Criterion, P≤0.01).
[2] n=10.
[3] Estrous cycle longer than 12 days or unclear in 7 of 10 animals.
[4] Estrous cycle longer than 12 days or unclear in 6 of 10 animals.
[5] Estrous cycle longer than 12 days or unclear in 8 of 10 animals.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E9 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

Study Parameters	0 ppm	5000 ppm	10,000 ppm	20,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	38.9 ± 0.8	43.0 ± 1.1	40.5 ± 0.9	33.6 ± 0.9*
Left epididymis	0.046 ± 0.001	0.045 ± 0.001	0.047 ± 0.001	0.038 ± 0.001**
Left cauda epididymis	0.017 ± 0.001	0.018 ± 0.001	0.017 ± 0.001	0.014 ± 0.001*
Left testis	0.118 ± 0.002	0.116 ± 0.004	0.120 ± 0.002	0.091 ± 0.004**
Spermatid measurements				
Spermatid heads (10^7/g testis)	19.160 ± 0.745	19.340 ± 0.767	19.970 ± 0.961	18.710 ± 1.018
Spermatid heads (10^7/testis)	2.26 ± 0.10	2.27 ± 0.15	2.39 ± 0.10	1.72 ± 0.12*
Spermatid count (mean/10^{-4} mL suspension)	70.68 ± 3.16	70.85 ± 4.74	74.68 ± 3.18	53.68 ± 3.88*
Spermatozoal measurements				
Motility (%)	98.65 ± 0.24	98.40 ± 0.30	97.92 ± 0.25	97.35 ± 0.45*
Concentration (10^6/g caudal epididymal tissue)	1126.7 ± 55.7	1036.2 ± 94.5	1133.2 ± 63.4	1139.7 ± 91.0

[1] Data presented as mean ± standard error. Spermatozoal concentration and spermatid heads per gram of testis are not significant by Dunn's test.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E10 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Ethoxyethanol[1]

Study Parameters	0 ppm	5000 ppm	10,000 ppm	20,000 ppm
n	10	10	10	9
Necropsy body weight (g)	31.3 ± 0.8	33.2 ± 1.0	29.9 ± 1.5	27.8 ± 0.8*[2]
Estrous cycle length (days)	4.30 ± 0.11	4.85 ± 0.15*	5.25 ± 0.23**	5.50 ± 0.47**[3]
Estrous stages (% of cycle)				
Diestrus	31.7	27.5	32.5	40.8
Proestrus	23.3	20.8	18.3	19.2
Estrus	29.2	41.7	37.5	33.3
Metestrus	15.8	10.0	11.7	6.7

[1] Data presented as mean ± standard error. By multivariate analysis of variance, dosed groups do not differ significantly from controls in the relative length of time spent in the estrous stages.
[2] n=10.
[3] Estrous cycle longer than 12 days or unclear in 1 of 10 animals.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E11 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

Study Parameters	0 ppm	3000 ppm	4500 ppm	6000 ppm
n	9	9	10	8
Weights (g)				
Necropsy body weight	40.2 ± 1.6	38.4 ± 0.9	39.1 ± 0.8	38.3 ± 0.8
Left epididymis	0.048 ± 0.001	0.046 ± 0.001	0.044 ± 0.001	0.046 ± 0.001
Left cauda epididymis	0.018 ± 0.001	0.020 ± 0.001	0.017 ± 0.001	0.017 ± 0.001
Left testis	0.124 ± 0.002	0.113 ± 0.002**	0.117 ± 0.002**	0.116 ± 0.002**
Spermatid measurements				
Spermatid heads (10^7/g testis)	19.140 ± 0.642	17.810 ± 1.331	17.830 ± 0.395	18.510 ± 0.645
Spermatid heads (10^7/testis)	2.32 ± 0.09[2]	2.08 ± 0.16[2]	2.08 ± 0.03	2.16 ± 0.09[2]
Spermatid count (mean/10^{-4} mL suspension)	72.35 ± 2.80[2]	65.15 ± 4.86[2]	64.90 ± 0.95	67.48 ± 2.83[2]
Spermatozoal measurements				
Motility (%)	98.78 ± 0.11	97.97 ± 0.38**	98.27 ± 0.23*	92.67 ± 3.01**
Concentration (10^6/g caudal epididymal tissue)	1278.2 ± 99.7	1167.1 ± 46.0	1394.7 ± 61.5	1437.2 ± 77.9

[1] Data presented as mean ± standard error. Differences from the control group for epididymal and cauda epididymal weights and spermatid measurements are not significant by Dunn's test; spermatozoal concentrations are not significant by Shirley's test.
[2] n=10.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE E12 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Drinking Water Study of 2-Butoxyethanol[1]

Study Parameters	0 ppm	3000 ppm	4500 ppm	6000 ppm
n	10	10	9	10
Necropsy body weight (g)	31.1 ± 0.7	28.0 ± 0.7*	28.4 ± 0.5*[2]	27.8 ± 0.9**
Estrous cycle length (days)	4.40 ± 0.16	4.95 ± 0.46	4.44 ± 0.18[3]	4.60 ± 0.15
Estrous stages (% of cycle)				
Diestrus	30.0	34.2	36.1	42.1
Proestrus	19.2	19.3	19.4	16.7
Estrus	36.7	36.0	31.5	33.3
Metestrus	14.2	10.5	13.0	7.9

[1] Data presented as mean ± standard error; n=10. Estrous cycle lengths are not significant by Dunn's test. By multivariate analysis of variance, dosed groups do not differ significantly from controls in the relative length of time spent in the estrous stages.
[2] n=10.
[3] *Estrous cycle longer than 12 days or unclear in 1 of 10 animals.*
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

APPENDIX F

Leukemia Inhibition Studies in Male F344/N Rats

LEUKEMIA INHIBITION STUDIES

Materials and Methods

Twenty male rats per dose group per isomer were used to investigate the effect of ethylene glycol ethers on the growth of F344 rat leukemia cells. Ten rats per dose group received the test article only. The remaining 10 animals per dose group received the test article as well as a subcutaneous injection of 2.5×10^7 F344 rat leukemia cells on the first (2-ethoxyethanol and 2-butoxyethanol) or the second (2-methoxyethanol) day of dosing. Dose levels for 2-methoxyethanol and 2-butoxyethanol were 0, 3000, and 6000 ppm and dose levels for 2-ethoxyethanol were 0, 2500, and 5000 ppm. Test articles were administered in drinking water, which was available *ad libitum*, until clinical signs of leukemia appeared in rats that were administered F344 rat leukemia cells. After approximately 9 weeks, animals were killed then necropsied and the spleens and livers were weighed.

Hematology analyses were also performed on rats in the leukemia inhibition studies. At terminal sacrifice, rats were anesthetized with CO_2, and blood samples were collected from the inferior vena cava. Blood was placed in EDTA tubes, and an aliquot was used for hematologic analyses.

Results

Survival, weight gain, and water and compound consumption data, organ weights and organ-weight-to-body-weight ratios, and hematology data are presented in Tables F1 to F3.

TABLE F1 Survival, Weight Gain, Water Consumption, and Compound Consumption in Male F344/N Rats at 9 Weeks in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers

Dose (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change[2]			
2-Methoxyethanol							
Not injected with leukemia cells							
0	10/10	138	272	135		22.2	
3000	10/10	139	170	31	63	19.7	349
6000	0/10[4]	139	—	—	—	15.7	677
Injected with leukemia cells							
0	9/10[5]	140	273	131		21.1	
3000	10/10	142	185	42	68	19.0	328
6000	0/10[6]	137	—	—	—	14.2	626
2-Ethoxyethanol							
Not injected with leukemia cells							
0	10/10	133	296	163		22.9	
2500	10/10	137	278	141	94	22.2	255
5000	10/10	134	273	139	92	20.0	459
Injected with leukemia cells							
0	10/10	133	277	144		22.2	
2500	10/10	137	277	140	100	20.6	231
5000	10/10	136	270	135	98	18.9	438
2-Butoxyethanol							
Not injected with leukemia cells							
0	10/10	127	258	131		21.4	
3000	10/10	131	264	133	102	18.2	246
6000	10/10	128	246	118	95	14.1	407
Injected with leukemia cells							
0	9/10[5]	127	269	141		20.9	
3000	5/10[7]	131	241	106	90	16.8	237
6000	10/10	126	239	113	89	13.8	408

[1] Number surviving at 9 weeks/number of animals per dose group.
[2] Mean weight change.
[3] (Dosed group mean/control group mean) × 100.
[4] Week of death: 4 (four rats), 6 (two rats), 7 (four rats).
[5] Week of death: 9.
[6] Week of death: 4 (four rats), 5 (six rats).
[7] Week of death: 8 (one rat), 9 (four rats).

TABLE F2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]		
	Dose 1	Dose 2	Dose 3
Not injected with leukemia cells			
n	10	10	10
Necropsy body wt.			
2-Methoxyethanol	287 ± 4	178 ± 5**	—[3]
2-Ethoxyethanol	300 ± 6	277 ± 5*	273 ± 5**
2-Butoxyethanol	283 ± 10	281 ± 6	255 ± 4**
Liver			
2-Methoxyethanol			
Absolute	11.21 ± 0.27	6.75 ± 0.15**	—
Relative	39.11 ± 0.54	37.94 ± 0.62	—
2-Ethoxyethanol			
Absolute	11.58 ± 0.34	9.67 ± 0.18**	10.16 ± 0.33**
Relative	38.57 ± 0.63	34.94 ± 0.37**	37.32 ± 1.03
2-Butoxyethanol			
Absolute	10.68 ± 0.34	11.60 ± 0.33	10.67 ± 0.27
Relative	38.05 ± 1.55	41.36 ± 0.71*	41.87 ± 0.70**
Spleen			
2-Methoxyethanol			
Absolute	0.641 ± 0.010	0.492 ± 0.016**	—
Relative	2.24 ± 0.03	2.76 ± 0.08**	—
2-Ethoxyethanol			
Absolute	0.623 ± 0.034	0.552 ± 0.037	0.652 ± 0.008[4]
Relative	2.08 ± 0.11	1.99 ± 0.12	2.40 ± 0.04*[4]
2-Butoxyethanol			
Absolute	0.598 ± 0.016	0.674 ± 0.019*	0.910 ± 0.051**
Relative	2.13 ± 0.09	2.41 ± 0.05**	3.58 ± 0.21**

TABLE F2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose		
	Dose 1	Dose 2	Dose 3
Injected with leukemia cells			
n			
2-Methoxyethanol	8	9	0
2-Ethoxyethanol	9	10	10
2-Butoxethanol	6	5	10
Necropsy body wt.			
2-Methoxyethanol	264 ± 16	194 ± 4**	–
2-Ethoxyethanol	268 ± 10	272 ± 9	271 ± 6
2-Butoxyethanol	289 ± 19	228 ± 16*	232 ± 10*
Liver			
2-Methoxyethanol			
Absolute	13.64 ± 0.71	7.00 ± 0.18**	–
Relative	52.01 ± 1.44	36.10 ± 0.44**	–
2-Ethoxyethanol			
Absolute	13.85 ± 0.40	12.42 ± 0.48	10.92 ± 0.55**
Relative	51.82 ± 1.38	45.96 ± 1.99	40.48 ± 2.19**
2-Butoxyethanol			
Absolute	14.21 ± 0.80	12.65 ± 0.78	12.31 ± 0.50
Relative	49.69 ± 2.80	55.54 ± 1.46	53.22 ± 0.87
Spleen			
2-Methoxyethanol			
Absolute	10.10 ± 0.91	0.54 ± 0.03**	–
Relative	39.34 ± 4.14	2.80 ± 0.13**	–
2-Ethoxyethanol			
Absolute	9.28 ± 0.91	7.21 ± 1.25	4.13 ± 1.45**
Relative	35.67 ± 4.24	27.68 ± 5.58	15.65 ± 5.53*
2-Butoxyethanol			
Absolute	10.44 ± 0.75	8.27 ± 0.48	8.44 ± 0.26*
Relative	37.26 ± 4.35	36.91 ± 3.23	37.34 ± 1.58

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] Doses administered to rats given 2-methoxyethanol or 2-butoxyethanol were 0, 3000, or 6000 ppm. Doses administered to rats given 2-ethoxyethanol were 0, 2500, or 5000 ppm.

[3] Data not available due to total mortality in the 6000 ppm 2-methoxyethanol group.

[4] n=9.

* Significantly different (P≤0.05) from the control group by Shirley's test.

** Significantly different (P≤0.01) from the control group by Shirley's, Dunn's, or Wilcoxon's test.

TABLE F3 Hematology Data for Male F344/N Rats in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers[1]

	Dose[2]		
	Dose 1	Dose 2	Dose 3
Not injected with leukemia cells			
n			
2-Methoxyethanol	10	10	0
2-Ethoxyethanol	10	8	10
2-Butoxyethanol	9	10	8
Hematocrit (%)			
2-Methoxyethanol	44.5 ± 0.5	38.5 ± 0.9**	—
2-Ethoxyethanol	45.6 ± 0.6	45.0 ± 0.5	45.7 ± 0.6
2-Butoxyethanol	46.0 ± 0.6	44.5 ± 0.5*	43.0 ± 1.2*
Hemoglobin (g/dL)			
2-Methoxyethanol	15.1 ± 0.1	12.8 ± 0.2**	—
2-Ethoxyethanol	15.0 ± 0.1	14.3 ± 0.1**	13.9 ± 0.2**
2-Butoxyethanol	15.7 ± 0.2	14.5 ± 0.1**	13.6 ± 0.1**
Erythrocytes (10^6/μL)			
2-Methoxyethanol	8.60 ± 0.10	7.22 ± 0.20**	—
2-Ethoxyethanol	9.09 ± 0.09	8.76 ± 0.08*	8.61 ± 0.14**
2-Butoxyethanol	9.22 ± 0.19	8.10 ± 0.08**	7.18 ± 0.12**
Reticulocytes (10^6/μL)			
2-Methoxyethanol	0.14 ± 0.01	0.19 ± 0.04	—
2-Ethoxyethanol	0.13 ± 0.03	0.19 ± 0.04	0.13 ± 0.02
2-Butoxyethanol	0.05 ± 0.01	0.07 ± 0.01	0.16 ± 0.03**
Nucleated erythrocytes (10^3/μL)			
2-Methoxyethanol	0.05 ± 0.02	0.01 ± 0.01	—
2-Ethoxyethanol	0.07 ± 0.02	0.01 ± 0.01	0.04 ± 0.02
2-Butoxyethanol	0.00 ± 0.00	0.02 ± 0.01	0.23 ± 0.07**
Mean cell volume (fL)			
2-Methoxyethanol	51.8 ± 0.2	53.4 ± 0.3**	—
2-Ethoxyethanol	50.1 ± 0.4	51.3 ± 0.3*	53.0 ± 0.3**
2-Butoxyethanol	49.9 ± 0.5	54.9 ± 0.4**	59.9 ± 1.1**
Mean cell hemoglobin (pg)			
2-Methoxyethanol	17.5 ± 0.1	17.8 ± 0.3	—
2-Ethoxyethanol	16.5 ± 0.1	16.4 ± 0.1	16.2 ± 0.1*
2-Butoxyethanol	17.1 ± 0.2	17.9 ± 0.2**	19.0 ± 0.2**
Mean cell hemoglobin concentration (g/dL)			
2-Methoxyethanol	33.8 ± 0.2	33.4 ± 0.4	—
2-Ethoxyethanol	33.0 ± 0.3	31.9 ± 0.2*	30.5 ± 0.2**
2-Butoxyethanol	34.2 ± 0.2	32.6 ± 0.3**	31.8 ± 0.8**
Platelets (10^3/μL)			
2-Methoxyethanol	659.5 ± 7.1	325.7 ± 21.4**	—
2-Ethoxyethanol	605.1 ± 20.0	563.0 ± 8.8*	520.7 ± 15.5**
2-Butoxyethanol	568.9 ± 13.0	522.2 ± 15.0	575.8 ± 16.9

TABLE F3 Hematology Data for Male F344/N Rats in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose		
	Dose 1	Dose 2	Dose 3
Not injected with leukemia cells (continued)			
Leukocytes (10^3/μL)			
2-Methoxyethanol	6.07 ± 0.16	2.73 ± 0.28**	—
2-Ethoxyethanol	6.38 ± 0.22	5.76 ± 0.31	5.31 ± 0.22**
2-Butoxyethanol	7.38 ± 0.37	6.07 ± 0.17**	6.36 ± 0.33*
Segmented neutrophils (10^3/μL)			
2-Methoxyethanol	0.98 ± 0.09	0.47 ± 0.04**	—
2-Ethoxyethanol	1.18 ± 0.12	1.24 ± 0.15	0.85 ± 0.09*
2-Butoxyethanol	1.29 ± 0.16	1.03 ± 0.07	1.51 ± 0.17
Bands (10^3/μL)			
2-Methoxyethanol	0.00 ± 0.00	0.02 ± 0.01**	—
2-Ethoxyethanol	0.05 ± 0.03	0.03 ± 0.02	0.02 ± 0.01
2-Butoxyethanol	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01
Lymphocytes (10^3/μL)			
2-Methoxyethanol	4.99 ± 0.11	2.20 ± 0.26**	—
2-Ethoxyethanol	4.89 ± 0.18	4.41 ± 0.29	4.34 ± 0.23
2-Butoxyethanol	5.94 ± 0.31	4.89 ± 0.16*	4.75 ± 0.34*
Monocytes (10^3/μL)			
2-Methoxyethanol	0.04 ± 0.01	0.01 ± 0.01	—
2-Ethoxyethanol	0.08 ± 0.02	0.05 ± 0.02	0.03 ± 0.02*
2-Butoxyethanol	0.06 ± 0.02	0.04 ± 0.01	0.02 ± 0.01
Eosinophils (10^3/μL)			
2-Methoxyethanol	0.02 ± 0.01	0.03 ± 0.01	—
2-Ethoxyethanol	0.09 ± 0.02	0.02 ± 0.02	0.05 ± 0.01
2-Butoxyethanol	0.09 ± 0.02	0.09 ± 0.03	0.02 ± 0.01
Methemoglobin (g/dL)			
2-Methoxyethanol	0.11 ± 0.02	0.10 ± 0.02	—
2-Ethoxyethanol	0.12 ± 0.01	0.11 ± 0.02	0.12 ± 0.02
2-Butoxyethanol	0.13 ± 0.02	0.10 ± 0.01	0.13 ± 0.02
Undifferentiated mononuclear cells (10^3/mL)			
2-Methoxyethanol	0.05 ± 0.02	0.01 ± 0.00[3]	—
2-Ethoxyethanol	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01
2-Butoxyethanol	0.00 ± 0.00	0.01 ± 0.01	0.04 ± 0.02*

TABLE F3 Hematology Data for Male F344/N Rats in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose		
	Dose 1	Dose 2	Dose 3
Injected with leukemia cells			
n			
2-Methoxyethanol	8	9	0
2-Ethoxyethanol	9	9	9
2-Butoxyethanol	5	3	7
Hematocrit (%)			
2-Methoxyethanol	24.4 ± 2.2	40.4 ± 0.9**	—
2-Ethoxyethanol	25.2 ± 3.2	33.8 ± 3.5	42.4 ± 1.9**
2-Butoxyethanol	19.5 ± 1.3	20.9 ± 3.6	19.0 ± 1.2[4]
Hemoglobin (g/dL)			
2-Methoxyethanol	11.0 ± 0.7	13.1 ± 0.2	—
2-Ethoxyethanol	10.9 ± 0.7	12.3 ± 0.7	13.4 ± 0.3**
2-Butoxyethanol	10.8 ± 0.6	9.7 ± 1.7	8.3 ± 0.3**
Erythrocytes (10^6/μL)			
2-Methoxyethanol	4.34 ± 0.45	7.45 ± 0.15**	—
2-Ethoxyethanol	4.84 ± 0.68	6.47 ± 0.71	8.02 ± 0.36**
2-Butoxyethanol	3.91 ± 0.33	3.76 ± 0.81	2.88 ± 0.18*
Reticulocytes (10^6/μL)			
2-Methoxyethanol	0.08 ± 0.03	0.16 ± 0.01	—
2-Ethoxyethanol	0.04 ± 0.02	0.04 ± 0.01	0.10 ± 0.03
2-Butoxyethanol	0.02 ± 0.00	0.03 ± 0.02[5]	0.03 ± 0.00
Nucleated erythrocytes (10^3/μL)			
2-Methoxyethanol	1.16 ± 0.70	0.02 ± 0.01	—
2-Ethoxyethanol	3.00 ± 1.59	0.94 ± 0.82	0.08 ± 0.04
2-Butoxyethanol	0.33 ± 0.33	1.44 ± 1.44	0.00 ± 0.00
Mean cell volume (fL)			
2-Methoxyethanol	56.6 ± 0.9	54.3 ± 0.3*	—
2-Ethoxyethanol	52.7 ± 0.8	52.8 ± 0.9	52.8 ± 0.4
2-Butoxyethanol	50.0 ± 1.1	56.7 ± 2.6	63.0 ± 1.8**[4]
Mean cell hemoglobin (pg)			
2-Methoxyethanol	26.1 ± 1.4	17.5 ± 0.2**	—
2-Ethoxyethanol	24.2 ± 1.9	20.7 ± 2.2	16.9 ± 0.6**
2-Butoxyethanol	27.8 ± 0.9	26.1 ± 1.2	29.1 ± 1.2
Mean cell hemoglobin concentration (g/dL)			
2-Methoxyethanol	46.1 ± 2.5	32.4 ± 0.5**	—
2-Ethoxyethanol	45.7 ± 3.0	38.9 ± 3.4	32.0 ± 1.1**
2-Butoxyethanol	38.2 ± 5.1	32.8 ± 2.1	31.9 ± 0.6
Platelets (10^3/μL)			
2-Methoxyethanol	202.5 ± 36.6	364.0 ± 33.9**	—
2-Ethoxyethanol	157.0 ± 32.4	248.9 ± 48.9	382.1 ± 45.3**
2-Butoxyethanol	163.6 ± 41.2	160.0 ± 40.1	130.0 ± 7.9

TABLE F3 Hematology Data for Male F344/N Rats in the Leukemia Inhibition Drinking Water Studies of Ethylene Glycol Ethers (continued)

	Dose		
	Dose 1	Dose 2	Dose 3
Injected with leukemia cells (continued)			
Leukocytes ($10^3/\mu L$)			
2-Methoxyethanol	315.60 ± 71.09	3.11 ± 0.23**	—
2-Ethoxyethanol	315.64 ± 64.70	114.68 ± 39.79*	20.23 ± 9.39**
2-Butoxyethanol	325.6 ± 81.1	285.0 ± 96.4	243.4 ± 37.9
Segmented neutrophils ($10^3/\mu L$)			
2-Methoxyethanol	4.12 ± 1.83	0.51 ± 0.10	—
2-Ethoxyethanol	6.13 ± 1.31	4.09 ± 1.07	1.63 ± 0.42**
2-Butoxyethanol	7.04 ± 1.82	9.90 ± 3.36	6.28 ± 1.13
Bands ($10^3/\mu L$)			
2-Methoxyethanol	0.12 ± 0.12	0.01 ± 0.01	—
2-Ethoxyethanol	1.37 ± 0.99	1.13 ± 0.65	0.34 ± 0.22
2-Butoxyethanol	0.33 ± 0.33	1.44 ± 1.44	0.00 ± 0.00
Lymphocytes ($10^3/\mu L$)			
2-Methoxyethanol	5.98 ± 2.02	2.54 ± 0.18	—
2-Ethoxyethanol	21.59 ± 6.54	14.15 ± 4.53	7.65 ± 2.45*
2-Butoxyethanol	13.49 ± 6.00	56.24 ± 39.25	19.59 ± 6.86
Monocytes ($10^3/\mu L$)			
2-Methoxyethanol	0.00 ± 0.00	0.03 ± 0.02[6]	—
2-Ethoxyethanol	0.20 ± 0.13	0.36 ± 0.14	0.09 ± 0.06
2-Butoxyethanol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eosinophils ($10^3/\mu L$)			
2-Methoxyethanol	0.00 ± 0.00	0.01 ± 0.01	—
2-Ethoxyethanol	0.33 ± 0.33	0.03 ± 0.02	0.05 ± 0.03
2-Butoxyethanol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Methemoglobin (g/dL)			
2-Methoxyethanol	0.18 ± 0.03	0.12 ± 0.03[6]	—
2-Ethoxyethanol	0.20 ± 0.04[6]	0.27 ± 0.06	0.14 ± 0.03
2-Butoxyethanol	0.34 ± 0.13	0.22 ± 0.08	0.34 ± 0.02
Undifferentiated mononuclear cells (10^3/mL)			
2-Methoxyethanol	305.21 ± 71.29	0.01 ± 0.01**	—
2-Ethoxyethanol	280.80 ± 65.26	92.14 ± 34.32*	10.22 ± 6.72**
2-Butoxyethanol	304.7 ± 86.3	217.4 ± 76.3	217.6 ± 40.1

[1] Mean ± standard error.
[2] Doses administered to rats given 2-methoxyethanol or 2-butoxyethanol were 0, 3000, or 6000 ppm; doses administered to rats given 2-ethoxyethanol were 0, 2500, or 5000 ppm. All rats treated with 6000 ppm 2-methoxyethanol, with and without leukemia cells, died or were killed before hematology evaluations were conducted.
[3] n=9.
[4] n=6.
[5] n=2.
[6] n=8.
* Significantly different (P≤0.05) from the control group by Shirley's or Wilcoxon's test.
** Significantly different (P≤0.01) from the control group by Shirley's or Wilcoxon's test.

APPENDIX G

Genetic Toxicology

TABLE G1 Mutagenicity of 2-Methoxyethanol in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	+ hamster S9 10%	+ hamster S9 30%	+ rat S9 10%	+ rat S9 30%
TA100	0	131 ± 9.1	168 ± 12.4	148 ± 7.0	187 ± 2.3	131 ± 3.5
	100	122 ± 3.6	177 ± 7.3	141 ± 4.1	171 ± 5.4	132 ± 4.5
	333	131 ± 0.9	164 ± 5.2	128 ± 6.6	186 ± 3.8	132 ± 5.5
	1000	140 ± 9.9	179 ± 7.3	148 ± 6.4	193 ± 12.3	119 ± 10.9
	3333	131 ± 6.7	167 ± 5.9	131 ± 6.4	168 ± 5.5	123 ± 1.5
	10,000	129 ± 9.7	155 ± 6.1	127 ± 9.5	165 ± 1.2	130 ± 5.4
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control[3]		530 ± 29.1	1212 ± 79.3	520 ± 2.6	1599 ± 52.9	1582 ± 57.7
TA1535	0	25 ± 3.4	11 ± 1.9	12 ± 2.3	14 ± 3.2	17 ± 3.7
	100	18 ± 1.2	8 ± 1.3	12 ± 0.9	9 ± 1.2	11 ± 2.4
	333	21 ± 1.7	10 ± 1.3	12 ± 2.7	10 ± 0.5	16 ± 1.0
	1000	19 ± 4.9	12 ± 0.7	11 ± 2.4	11 ± 0.3	15 ± 1.2
	3333	22 ± 4.0	9 ± 1.9	10 ± 2.2	12 ± 0.9	13 ± 3.6
	10,000	18 ± 3.0	10 ± 1.7	11 ± 2.2	10 ± 1.2	14 ± 2.3
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		445 ± 7.8	62 ± 4.3	251 ± 11.3	243 ± 5.2	178 ± 11.5
TA97	0	128 ± 6.1	128 ± 4.7	135 ± 1.3	132 ± 4.8	128 ± 5.2
	100	140 ± 4.2	155 ± 13.5	142 ± 4.4	126 ± 4.1	138 ± 2.8
	333	140 ± 12.3	119 ± 4.9	127 ± 2.5	138 ± 6.8	129 ± 4.0
	1000	148 ± 4.2	137 ± 8.7	128 ± 0.9	134 ± 4.1	141 ± 4.3
	3333	128 ± 3.5	129 ± 2.6	134 ± 5.8	142 ± 5.5	137 ± 0.9
	10,000	128 ± 5.1	122 ± 7.8	128 ± 1.2	137 ± 4.3	121 ± 3.5
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		542 ± 14.7	1528 ± 9.5	2127 ± 86.6	2774 ± 68.6	1058 ± 75.8
TA98	0	30 ± 3.4	29 ± 2.1	42 ± 2.3	36 ± 3.5	41 ± 1.3
	100	31 ± 0.9	27 ± 1.5	40 ± 2.6	34 ± 3.8	46 ± 2.4
	333	33 ± 2.1	31 ± 3.7	35 ± 1.7	27 ± 4.2	47 ± 1.9
	1000	29 ± 2.4	32 ± 3.5	43 ± 1.2	29 ± 3.3	35 ± 6.5
	3333	30 ± 1.5	41 ± 2.3	32 ± 2.6	34 ± 2.3	45 ± 1.2
	10,000	29 ± 1.3	31 ± 0.9	38 ± 1.9	29 ± 2.7	46 ± 5.3
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		363 ± 28.2	385 ± 15.4	273 ± 9.7	559 ± 13.2	305 ± 17.3

[1] Study performed at Microbiological Associates, Inc. The detailed protocol and these data are presented in Zeiger *et al.* (1992). 0 µg/plate is the solvent control.

[2] Revertants are presented as mean ± the standard error from three plates.

[3] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537 and TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE G2 Mutagenicity of 2-Ethoxyethanol in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	+ 10% hamster S9 Trial 1	+ 10% hamster S9 Trial 2	+ 10% hamster S9 Trial 3	+ 10% rat S9 Trial 1	+ 10% rat S9 Trial 2
TA100	0	152 ± 6.2	145 ± 2.9	133 ± 9.8	132 ± 9.6	139 ± 7.4	138 ± 10.1	146 ± 9.9
	100	136 ± 5.7	156 ± 5.6	123 ± 10.4	117 ± 5.9	126 ± 5.4	140 ± 6.1	155 ± 1.3
	333	138 ± 2.9	145 ± 8.1	130 ± 11.6	134 ± 5.2	131 ± 6.3	138 ± 2.7	137 ± 4.1
	1000	142 ± 4.3	136 ± 4.9	119 ± 7.5	131 ± 7.1	114 ± 9.7	149 ± 9.7	157 ± 7.2
	3333	133 ± 4.4	134 ± 9.0	129 ± 6.9	118 ± 6.7	129 ± 4.6	140 ± 2.3	139 ± 6.5
	10,000	147 ± 3.8	139 ± 9.3	127 ± 6.2	114 ± 8.8	134 ± 7.5	138 ± 3.7	149 ± 5.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control[3]		2113 ± 13.0	1355 ± 13.4	125 ± 4.7	1339 ± 21.1	2408 ± 9.7	1429 ± 40.9	1119 ± 28.1
TA1535	0	29 ± 1.2	24 ± 4.0	11 ± 2.4	10 ± 1.7	16 ± 2.3	11 ± 2.4	16 ± 2.2
	100	31 ± 0.9	30 ± 3.6	11 ± 1.3	8 ± 1.3	16 ± 2.6	12 ± 1.9	13 ± 1.2
	333	23 ± 3.7	30 ± 3.1	13 ± 2.5	10 ± 1.9	14 ± 3.2	15 ± 3.6	17 ± 1.2
	1000	27 ± 2.2	29 ± 2.3	11 ± 0.9	10 ± 1.2	15 ± 1.2	11 ± 0.3	15 ± 2.3
	3333	28 ± 2.2	23 ± 4.1	13 ± 2.5	7 ± 0.9	15 ± 1.8	12 ± 1.2	15 ± 2.2
	10,000	22 ± 2.0	27 ± 0.6	10 ± 1.3	8 ± 1.2	15 ± 2.3	11 ± 2.1	15 ± 1.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1562 ± 70.0	1132 ± 19.2	10 ± 0.7	123 ± 0.6	156 ± 10.0	106 ± 12.6	45 ± 6.2
TA1537	0	12 ± 3.5	12 ± 2.7	11 ± 1.7	9 ± 0.9	13 ± 1.7	8 ± 0.7	11 ± 2.3
	100	10 ± 0.3	10 ± 2.1	9 ± 0.7	8 ± 1.5	10 ± 3.3	9 ± 1.5	15 ± 0.7
	333	13 ± 1.5	9 ± 2.9	12 ± 1.5	8 ± 1.7	9 ± 1.0	9 ± 3.1	16 ± 0.3
	1000	8 ± 2.9	7 ± 2.1	13 ± 3.8	10 ± 2.0	11 ± 3.2	11 ± 3.0	12 ± 0.6
	3333	6 ± 1.2	10 ± 2.2	10 ± 1.7	8 ± 1.2	10 ± 3.0	10 ± 1.2	11 ± 2.3
	10,000	10 ± 1.5	11 ± 1.5	12 ± 1.9	11 ± 1.5	13 ± 1.3	8 ± 1.5	16 ± 0.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control		390 ± 21.0	255 ± 37.8	10 ± 1.5	146 ± 7.1	221 ± 23.7	149 ± 7.0	78 ± 10.4
TA98	0	24 ± 3.1	21 ± 0.3	35 ± 2.2	35 ± 0.7	33 ± 1.7	32 ± 3.5	41 ± 2.2
	100	25 ± 2.1	21 ± 1.2	33 ± 6.8	36 ± 4.5	33 ± 2.3	30 ± 1.2	37 ± 1.7
	333	24 ± 4.0	19 ± 1.9	40 ± 4.0	30 ± 3.1	33 ± 3.8	30 ± 2.9	39 ± 3.6
	1000	20 ± 1.3	26 ± 4.4	32 ± 5.6	34 ± 4.8	37 ± 4.4	35 ± 4.3	28 ± 1.2
	3333	25 ± 0.7	19 ± 0.3	27 ± 3.5	31 ± 3.5	35 ± 1.2	32 ± 6.0	39 ± 6.4
	10,000	30 ± 4.7	25 ± 4.0	32 ± 4.6	31 ± 4.7	33 ± 5.7	32 ± 2.5	35 ± 4.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1869 ± 72.9	1323 ± 18.3	21 ± 2.3	1197 ± 34.9	2187 ± 67.0	1092 ± 26.1	912 ± 38.4

[1] Study performed at EG&G Mason Research Institute. The detailed protocol and these data are presented in Zeiger *et al.* (1985). 0 µg/plate is the solvent control.

[2] Revertants are presented as mean ± the standard error from three plates.

[3] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537 and TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE G3 Mutagenicity of 2-Butoxyethanol in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	+ hamster S9 10%	+ hamster S9 30%	+ rat S9 5%	+ rat S9 10%	+ rat S9 30%
TA100	0	164 ± 5.5	153 ± 9.5	161 ± 7.5		172 ± 6.1	111 ± 2.8
	100	156 ± 11.6	157 ± 3.2	166 ± 3.2		155 ± 8.3	127 ± 12.1
	333	157 ± 7.1	161 ± 12.7	157 ± 17.5		167 ± 8.5	99 ± 5.0
	1000	165 ± 17.0	156 ± 1.8	150 ± 1.5		169 ± 4.7	96 ± 3.7
	3333	166 ± 2.6	151 ± 4.8	151 ± 13.3		156 ± 3.3	150 ± 2.3
	10,000	112 ± 7.8	132 ± 15.5	149 ± 12.1		142 ± 9.2	119 ± 4.0
Trial summary		Negative	Negative	Negative		Negative	Negative
Positive control[3]		428 ± 30.9	930 ± 56.0	731 ± 52.2		471 ± 17.0	621 ± 9.8
TA1535	0	30 ± 4.9	14 ± 0.9	14 ± 2.6		12 ± 0.3	13 ± 0.3
	100	39 ± 0.3	13 ± 0.6	12 ± 1.5		10 ± 4.7	14 ± 1.5
	333	33 ± 4.3	12 ± 1.8	14 ± 3.4		11 ± 1.3	14 ± 0.3
	1000	25 ± 3.2	8 ± 1.8	12 ± 2.0		11 ± 0.7	12 ± 2.5
	3333	25 ± 3.2	13 ± 4.0	12 ± 0.6		8 ± 0.9	10 ± 0.3
	10,000	22 ± 2.5	7 ± 2.5	10 ± 1.0		11 ± 0.6	10 ± 2.0
Trial summary		Negative	Negative	Negative		Negative	Negative
Positive control		585 ± 26.0	203 ± 10.1	698 ± 29.8		195 ± 16.0	186 ± 4.5
TA1537	0	11 ± 3.2		13 ± 1.5			13 ± 3.4
	100	13 ± 2.6		14 ± 2.1			11 ± 1.8
	333	13 ± 1.9		7 ± 1.2			8 ± 1.2
	1000	10 ± 1.9		12 ± 1.5			9 ± 3.3
	3333	9 ± 1.3		10 ± 2.3			12 ± 4.1
	10,000	14 ± 2.4		11 ± 1.3			7 ± 0.6
Trial summary		Negative		Negative			Negative
Positive control		742 ± 61.5		64 ± 3.8			49 ± 2.9
TA97	0	180 ± 15.1	171 ± 10.4	180 ± 3.0	183 ± 11.9	178 ± 6.6	198 ± 11.3
	100	178 ± 4.9	170 ± 18.0	210 ± 8.2	177 ± 8.9	195 ± 8.5	215 ± 13.2
	333	190 ± 8.4	169 ± 3.0	197 ± 5.2	187 ± 2.0	195 ± 16.5	210 ± 5.0
	666				154 ± 9.5	195 ± 15.1	170 ± 15.2
	1000	214 ± 3.7	204 ± 6.9	193 ± 3.3	169 ± 10.3	184 ± 6.4	149 ± 11.4
	1666				161 ± 19.1	166 ± 22.1	178 ± 2.9
	3333	190 ± 2.7	172 ± 11.5	164 ± 0.7			
	10,000	181 ± 1.8	148 ± 10.3	130 ± 4.1			
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		799 ± 76.2	285 ± 14.7	456 ± 20.5	494 ± 22.3	355 ± 13.1	308 ± 8.8

TABLE G3 Mutagenicity of 2-Butoxyethanol in *Salmonella typhimurium* (continued)

		Revertants/plate[2]					
	Dose	-S9	+ hamster S9		+ rat S9		
Strain	(µg/plate)	Trial 1	10%	30%	5%	10%	30%
TA98	0	25 ± 2.3	19 ± 0.6	32 ± 1.9		34 ± 1.9	40 ± 0.6
	100	24 ± 3.0	26 ± 1.0	22 ± 3.4		33 ± 3.5	35 ± 4.7
	333	22 ± 2.5	20 ± 0.9	28 ± 2.0		22 ± 3.4	37 ± 5.7
	1000	25 ± 5.0	27 ± 0.6	28 ± 0.9		24 ± 3.2	34 ± 1.2
	3333	21 ± 2.8	26 ± 2.9	30 ± 1.2		27 ± 1.7	34 ± 2.3
	10,000	11 ± 1.5[4]	21 ± 4.3	27 ± 1.2		23 ± 2.8	42 ± 1.2
Trial summary		Negative	Negative	Negative		Negative	Negative
Positive control[3]		488 ± 48.6	933 ± 29.6	528 ± 35.3		355 ± 7.4	135 ± 6.9

[1] Study performed at SRI International. The detailed protocol and these data are presented in Zeiger *et al.* (1992). 0 µg/plate is the solvent control.

[2] Revertants are presented as mean ± the standard error from three plates.

[3] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537 and TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

[4] Slight toxicity.

TABLE G4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by 2-Ethoxyethanol[1]

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[2]	Average Mutant Fraction
-S9						
Trial 1						
Distilled water						
		72	86	150	69	
		86	106	163	63	
		92	94	130	47	
		101	114	127	42	55
Methyl methanesulfonate (μg/mL)						
	5	54	48	636	393	
		49	31	716	489	441*
2-Ethoxyethanol (μL/mL)						
	1	65	84	67	34	
		72	107	83	39	
		95	118	106	37	37
	1.5	70	106	73	35	
		64	88	72	38	
		59	85	68	38	37
	2	51	62	60	39	
		73	101	80	36	
		70	86	84	40	39
	3	73	109	105	48	
		57	71	123	72	
		74	103	105	47	56
	4	80	90	106	44	
		98	126	105	36	
		91	122	92	34	38
	5	63	110	80	42	
		65	78	106	55	
		59	113	94	53	50
Trial 2						
Distilled water						
		89	85	129	48	
		87	109	85	32	
		87	98	92	35	
		86	99	102	40	39
Methyl methanesulfonate (μg/mL)						
	5	31	23	468	503	
		43	33	466	363	433*

TABLE G4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by 2-Ethoxyethanol (continued)

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9 (continued)						
Trial 2 (continued)						
2-Ethoxyethanol (µL/mL)						
	1	60	73	40	22	
		64	62	49	25	24
	1.5	76	66	92	40	
		73	72	80	36	
		74	74	85	38	38
	2	53	78	63	40	
		65	74	58	30	
		54	54	72	45	38
	3	67	84	107	53	
		50	63	72	48	
		63	52	105	56	52
	4	70	77	82	39	
		81	62	96	40	
		70	81	106	50	43
	5	58	56	71	41	
		62	70	93	50	
		69	51	62	30	40
+S9						
Trial 1						
Distilled water						
		95	89	104	37	
		101	109	89	29	
		87	98	60	23	
		95	104	91	32	30
Methylcholanthrene (µg/mL)						
	2.5	47	16	654	462	
		50	15	690	462	
		51	15	727	472	465*
2-Ethoxyethanol (µL/mL)						
	0.5	73	68	103	47	
		96	87	87	30	
		90	92	76	28	35
	1	92	84	83	30	
		72	85	72	33	
		85	88	82	32	32
	2	87	74	105	40	
		101	79	118	39	
		96	78	111	38	39
	3	104	77	101	32	
		107	91	120	37	
		107	69	137	43	38
	4	77	84	121	52	
		72	78	103	48	
		88	71	117	44	48*
	5	113	66	173	51	

TABLE G4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by 2-Ethoxyethanol (continued)

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9 (continued)						
Trial 2						
Distilled water						
		103	149	116	38	
		66	73	77	39	
		82	82	90	37	
		103	96	78	25	35
Methylcholanthrene (μg/mL)						
	2.5	31	15	783	851	
		53	28	800	506	
		51	18	748	489	615*
2-Ethoxyethanol (μL/mL)						
	0.5	87	103	65	25	
		82	100	95	39	
		92	137	69	25	25
	1	104	122	76	24	
		82	89	65	26	
		92	137	69	25	25
	2	94	117	109	39	
		91	122	57	21	
		85	106	73	29	29
	3	75	89	99	44	
		114	169	125	37	
		87	125	105	40	40
	4	97	108	135	46	
		86	100	126	49	
		75	78	106	47	47
	5	71	83	128	60	
		96	81	131	46	
		107	104	125	39	48
Trial 3						
Ethanol		84	69	75	30	
		93	157	96	34	
		83	88	94	38	
		85	86	91	36	34
Methylcholanthrene (μg/mL)						
	2.5	40	11	723	603	
		27	7	473	577	
		45	8	858	633	604*

TABLE G4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by 2-Ethoxyethanol (continued)

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9 (continued)						
Trial 3 (continued)						
2-Ethoxyethanol (μL/mL)						
	0.5	63	77	89	47	
		84	101	100	40	
		64	54	96	50	46
	1	78	83	85	37	
		98	91	111	38	
		71	91	109	51	42
	2	114	90	122	36	
		65	74	94	49	42
	3	90	61	163	60	
		80	75	119	49	
		81	84	121	50	53*
	4	85	111	106	42	
		85	48	126	50	46
	5	96	101	115	40	
		92	111	138	50	
		55	30	110	67	52*

[1] Study performed at Litton Bionetics, Inc. The experimental protocol is presented in detail in Myhr *et al.* (1985). All doses were tested in triplicate; the average of the three tests is presented in the table.

[2] Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF/1 x 10^6 cells treated); MF=mutant fraction.

* Significant positive response ($P \leq 0.05$).

TABLE G5 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 2-Ethoxyethanol[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)[2]
-S9								
Trial 1								
Summary: Positive								
Medium		50	1048	444	0.42	8.9	25.5	
Mitomycin-C	0.005	50	1036	1583	1.52	31.7	25.5	260.66
2-Ethoxyethanol	951	50	1039	480	0.46	9.6	25.5	9.04
	3170	50	1041	676	0.64	13.5	25.5	53.28*
	9510	50	1017	1368	1.34	27.4	25.5	217.50*
								P<0.001[3]
+S9								
Trial 1								
Summary: Positive								
Medium		50	1041	415	0.39	8.3	25.5	
Cyclophosphamide	1.5	50	1040	1408	1.35	28.2	25.5	239.61
2-Ethoxyethanol	951	50	1042	454	0.43	9.1	25.5	9.29
	3170	50	1041	517	0.49	10.3	25.5	24.58*
	9510	50	1031	609	0.59	12.2	25.5	48.17*
								P<0.001

[1] Study performed at Litton Bionetics, Inc. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the protocol and these data are presented in Galloway *et al.* (1987).

[2] SCEs/chromosome of culture exposed to 2-ethoxyethanol relative to those of culture exposed to solvent.

[3] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose.

* Positive (>20% increase over solvent control).

TABLE G6 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 2-Butoxyethanol[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)[2]
-S9								
Trial 1								
Summary: Equivocal								
Medium		50	1016	418	0.41	8.4	26.0	
Mitomycin-C	0.001	50	1017	568	0.55	11.4	26.0	35.75
	0.010	5	103	150	1.45	30.0	26.0	253.98
2-Butoxyethanol	1510	50	1004	410	0.40	8.2	31.0[3]	–0.74
	2220	50	998	453	0.45	9.1	31.0[3]	10.33
	3000	50	1013	496	0.48	9.9	31.0[3]	19.01
								P=0.001[4]
Trial 2								
Summary: Negative								
Medium		50	1027	485	0.47	9.7	26.0	
Mitomycin-C	0.001	50	1015	626	0.61	12.5	26.0	30.60
	0.010	5	102	202	1.98	40.4	26.0	319.36
2-Butoxyethanol	2500	50	1007	531	0.52	10.6	36.0[3]	11.66
	3000	50	1009	541	0.53	10.8	36.0[3]	13.54
	3500	50	1007	551	0.54	11.0	36.0[3]	15.86
								P=0.010
+S9								
Trial 1								
Summary: Negative								
Medium		50	1006	491	0.48	9.8	26.0	
Cyclophosphamide	0.4	50	1038	705	0.67	14.1	26.0	39.16
	2.0	5	102	128	1.25	25.6	26.0	157.11
2-Butoxyethanol	500	50	1019	485	0.47	9.7	26.0	–2.48
	1670	50	1015	479	0.47	9.6	26.0	–3.31
	5000	50	1026	497	0.48	9.9	26.0	–0.75
								P=0.563

[1] Study performed at Litton Bionetics, Inc. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the protocol is presented by Galloway *et al.* (1987).

[2] SCEs/chromosome of culture exposed to 2-butoxyethanol relative to those of culture exposed to solvent.

[3] Because 2-butoxyethanol induced a delay in the cell division cycle, harvest time was extended to maximize the proportion of second-division cells available for analysis.

[4] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose.

TABLE G7 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 2-Ethoxyethanol[1]

-S9					+S9				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 — Harvest time: 10.5 hours Summary: Positive					**Trial 1** — Harvest time: 10.5 hours Summary: Negative				
Medium					Medium				
	100	2	0.02	2.0		100	1	0.01	1.0
Triethylenemelamine					Cyclophosphamide				
0.75	100	28	0.28	21.0	25.0	80	38	0.48	26.0
2-Ethoxyethanol					2-Ethoxyethanol				
4780	100	3	0.03	3.0	4780	100	1	0.01	1.0
6830	100	12	0.12	11.0[2]	6830	100	1	0.01	1.0
9510	100	15	0.15	13.0[2]	9510	100	1	0.01	1.0
				P<0.001					P=0.500

[1] Study performed at Litton Bionetics, Inc. Abs=aberrations. A detailed presentation of the protocol and these data are found in Galloway *et al.* (1987).
[2] Clear increase in complex aberrations.
[3] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose.

TABLE G8 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 2-Butoxyethanol[1]

-S9					+S9				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 — Harvest time: 10.5 hours Summary: Negative					**Trial 1** — Harvest time: 12.5 hours Summary: Negative				
Medium					Medium				
	200	7	0.04	3.5		200	6	0.03	3.0
Mitomycin-C					Cyclophosphamide				
0.25	200	22	0.11	10.5	7.5	200	20	0.10	8.0
0.75	25	14	0.56	36.0	37.5	25	10	0.40	36.0
2-Butoxyethanol					2-Butoxyethanol				
2513	200	3	0.02	1.5	2513	100	1	0.01	1.0
3750	200	2	0.01	1.0	3750	200	8	0.04	3.5
5000	100	0	0.00	0.0	5000	200	6	0.03	3.0
				P=0.991					P=0.368
Trial 2 — Harvest time: 20.5 hours[2] Summary: Weak positive									
Medium									
	100	0	0.00	0.0					
Mitomycin-C[3]									
0.05	25	22	0.88	36.0					
0.08	200	16	0.08	5.0					
2-Butoxyethanol									
2513	100	4	0.04	3.0					
3750	100	1	0.01	1.0					
5000	100	8	0.08	7.0*					
				P=0.007					

TABLE G8 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 2-Butoxyethanol (continued)

-S9					+S9				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 3 — Harvest time: 20.7 hours[2]									
Summary: Negative									
Medium									
	100	1	0.01	1.0					
Mitomycin-C									
0.05	100	27	0.27	22.0					
0.08	25	15	0.60	40.0					
2-Butoxyethanol									
4500	100	1	0.01	1.0					
4700	100	3	0.03	3.0					
5000	100	2	0.02	2.0					
				P=0.215					

[1] Study performed at Litton Bionetics, Inc. Abs=aberrations. A detailed presentation of the protocol is found in Galloway *et al.* (1987).
[2] Because of significant 2-butoxyethanol-induced cell cycle delay, incubation time prior to addition of Colcemid was lengthened to provide sufficient metaphases at harvest.
[3] Control doses were switched.
* Positive (P≤0.05).

TABLE G9 Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by 2-Ethoxyethanol[1]

Route of Exposure	Dose (ppm)	Incidence of Deaths (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested			
				Mating 1	Mating 2	Mating 3	Total[2]
Test 1							
Feeding	5110	4	14	2/2019	2/2004	3/1997	7/6020 (0.12%)
	0			1/2029	2/1959	0/1924	3/5912 (0.05%)
Injection	5170	10	0	0/2057	3/2055	1/1991	4/6103 (0.07%)
	0			0/2026	1/2004	1/2029	2/6059 (0.03%)
Test 2							
Feeding	20,000	2	0	2/1946	1/2451	3/1900	6/6297 (0.10%)
	0			2/2033	2/2259	1/2082	5/6374 (0.08%)
Injection	50,000	2	0	0/1969	2/1900	1/1929	3/5798 (0.05%)
	0			0/1950	0/2018	0/1897	0/5865 (0.00%)

[1] A detailed description of the protocol and the data from Test 1 are found in Valencia *et al.* (1985). Protocol and data from Test 2 are found in Mason *et al.* (1992). Results were not significant at the 5% level (Margolin *et al.*, 1983).
[2] Combined total number of lethal mutations/number of X chromosomes tested for three mating trials.

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