NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 413

TOXICOLOGY AND CARCINOGENESIS STUDIES OF ETHYLENE GLYCOL

(CAS NO. 107-21-1)

IN B6C3F$_1$ MICE

(FEED STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF ETHYLENE GLYCOL

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IN B6C3F$_1$ MICE

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NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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CONTENTS

ABSTRACT

$HOCH_2CH_2OH$

Ethylene Glycol

CAS No. 107-21-1

Chemical Formula: $C_2H_6O_2$ Molecular Weight: 62.07

Synonyms: 1,2-dihydroxyethane; ethane-1,2-diol; 1,2-ethanediol; ethylene alcohol; ethylene dihydrate; glycol; glycol alcohol; 2-hydroxyethanol; monoethylene glycol

Ethylene glycol is a major constituent of motor vehicle antifreeze-coolant fluids and is also found in other commercial products including hydraulic brake fluids, adhesives, printer's inks, and wood stains. It is used in the manufacture of polyester films and fibers, polyethylene terephthalate (PET) solid state resins, plasticizers, elastomers, cellophane, and other products. Previous 13-week and 2-year studies of ethylene glycol in F344 rats were considered adequate to evaluate the toxicology and carcinogenicity of ethylene glycol in this species and strain; therefore, the present studies were conducted in mice only. Toxicology and carcinogenesis studies were conducted by administering ethylene glycol (greater than 99% pure) in feed to male and female B6C3F$_1$ mice for 13 weeks and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma L5178Y cells, and Chinese hamster ovary cells.

13-Week Studies: Groups of 10 male and 10 female mice received feed containing 0, 3,200, 6,300, 12,500, 25,000 or 50,000 ppm ethylene glycol. All mice survived to the end of the studies. Final mean body weights of dosed male and female mice and feed consumption of dosed males were similar to those of the controls. Feed consumption of dosed females was significantly greater than that of controls. Absolute and relative organ weights of mice administered ethylene glycol were generally similar to those of controls throughout the study. No chemical-related clinical findings were observed.

Chemical-related kidney and liver lesions, seen only in 25,000 and 50,000 ppm male mice, consisted of nephropathy and centrilobular hepatocellular hyaline degeneration (cytoplasmic accumulation of non-birefringent, eosinophilic, globular, or crystalline material resembling erythrocyte fragments).

2-Year Studies: Groups of 60 mice received diets containing ethylene glycol for up to 103 weeks (males: 0, 6,250, 12,500, or 25,000 ppm; females: 0, 12,500, 25,000, or 50,000 ppm). These concentrations correspond to daily doses of approximately 1,500, 3,000, or 6,000 mg/kg body weight for male mice and 3,000, 6,000, or 12,000 mg/kg for females. Dietary concentrations greater than 50,000 ppm have the potential to affect the nutritional value of the feed. Interim evaluations were performed on six males and nine or ten females from each dose group at 15 months.

Survival, Body Weights, Feed Consumption, and Clinical Findings in the 2-Year Studies:
At the end of the 2-year studies, survival rates of male and female mice exposed to ethylene glycol were similar to those of controls. Mean body weights and feed consumption of exposed male and female groups were also similar to those of controls.

No clinical findings associated with the administration of ethylene glycol were observed.

Pathology Findings: No chemical-related neoplasms were observed in male or female mice in these studies. Hepatocellular hyaline degeneration was seen in mid- and high-dose male and high-dose female mice. Pulmonary arterial medial hyperplasia was observed at an increased incidence in exposed females but not in exposed males. Incidence and severity of nephropathy were not affected by treatment in either sex. Small numbers of oxalate-like crystals, calculi, or both were noted in renal tubules, urethrae, and/or urinary bladders in a few high-dose male mice.

Genetic Toxicology: Ethylene glycol did not induce gene mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, trifluorothymidine resistance in mouse L5178Y lymphoma cells, or sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells. All tests were conducted with and without exogenous metabolic activation (S9).

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of ethylene glycol in male B6C3F$_1$ mice receiving 6,250, 12,500, or 25,000 ppm, or in female B6C3F$_1$ mice receiving 12,500, 25,000, or 50,000 ppm. Administration of ethylene glycol resulted in hepatocellular hyaline degeneration in male mice fed diets containing 12,500 or 25,000 ppm and in female mice fed diets containing 50,000 ppm. An increased incidence of medial hyperplasia of small pulmonary arteries and arterioles occurred in female mice fed diets containing 12,500, 25,000, or 50,000 ppm ethylene glycol.

* Explanation of Levels of Evidence of Carcinogenic Activity appears on page 7. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appear on page 9.

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of Ethylene Glycol

Variable	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses	0, 6,250, 12,500, or 25,000 ppm in feed	0, 12,500, 25,000, or 50,000 ppm in feed
Body weights	Dosed groups similar to controls	Dosed groups similar to controls
2-year survival rates	29/54, 32/54, 32/54, 23/54	33/50, 30/50, 30/51, 37/50
Nonneoplastic effects	Liver: hepatocyte hyaline degeneration (0/54, 0/53, 24/53, 36/54)	Liver: hepatocyte hyaline degeneration (0/50, 0/50, 1/51, 26/50) Lung: medial hyperplasia of the pulmonary arterioles (3/50, 10/50, 10/51, 23/50)
Neoplastic effects	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence
Genetic toxicology		
Salmonella typhimurium gene mutations:	Negative with and without S9 in strains TA98, TA100, TA1535, and TA1537	
L5178Y mouse lymphoma gene mutations:	Negative with and without S9	
Sister chromatid exchanges		
Chinese hamster ovary cells *in vitro*:	Negative with and without S9	
Chromosomal aberrations		
Chinese hamster ovary cells *in vitro*:	Negative with and without S9	

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence including: animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that because of major flaws cannot be evaluated (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence of carcinogenic activity** is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence of carcinogenic activity** is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence of carcinogenic activity** describes studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence of carcinogenic activity** is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study of carcinogenic activity** is demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement is selected for a particular experiment, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. This should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on ethylene glycol on July 10, 1991, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On July 10, 1991, the draft Technical Report on the toxicology and carcinogenesis studies of ethylene glycol received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. M.M. McDonald, NIEHS, introduced the toxicology and carcinogenesis studies of ethylene glycol by discussing uses of the chemical and rationale for the study, describing the experimental design, reporting survival and body weight effects, and reviewing nonneoplastic lesions which developed in mice. The proposed conclusions were *no evidence of carcinogenic activity* in male or female mice.

Dr. C.D. Klaassen, a principal reviewer, agreed with the proposed conclusions. He noted that drinking water was not used as the route of administration in the studies because of the concern that ethylene glycol might decrease animals' intake of water. Dr. Klaassen wondered if, conversely, ethylene glycol might actually increase water consumption. He requested that there be a statement included in the report as to why studies were not performed with rats.

Dr. D.W. Hayden, the second principal reviewer, also agreed with the proposed conclusions, and asked why ethylene glycol was not administered by gavage during the studies. Dr. McDonald said the chemical was administered in the feed to most closely approximate the route of human exposure. Dr. Hayden stated that a reference to hepatocellular erythrophagocytosis should be modified, citing inconclusive evidence that ethylene glycol promoted erythrocyte inclusions. Dr. McDonald said the modification would be made. Dr. Hayden asked if other chemicals studied by the NTP with structures similar to ethylene glycol and its metabolites were associated with the presence of hepatocellular hyaline degeneration. Dr. McDonald said that degeneration was observed in studies of doxylamine, pentachloroanisole, and polybrominated biphenyls, but that none of the three were structurally related to ethylene glycol.

Dr. G.P. Carlson, the third principal reviewer, also agreed with the proposed conclusions. Dr. Carlson asked why drinking water was not used as the route of administration in the study, since the chemical's sweet taste might have encouraged increased consumption. Dr. McDonald commented that ethylene glycol is known to cause severe progressive renal disease in species other than mice. During the final stages of the disease, she noted, polyuria would be expected to lead to excess consumption of water and thus to a resultant overdosage of ethylene glycol. Dr. Carlson asked if information existed on bioavailability of ethylene glycol from feed. Dr. McDonald said no such data existed, adding she believed the high incidence of chemically associated systemic lesions supported adequate bioavailability.

Mr. L.S. Beliczky commented that occupational exposure to ethylene glycol would likely be by inhalation or dermal absorption. He noted that epichlorohydrin was among the two percent impurities and this should be referenced. Dr. J. Haartz, NIOSH, reported that occupational exposure to ethylene glycol is rather extensive, citing a potential workplace exposure figure of more than 1.5 million individuals.

Dr. Klaassen moved that the Technical Report on ethylene glycol be accepted with the revisions discussed and with the conclusions as written for male and female mice, *no evidence of carcinogenic activity*. Dr. P.T. Bailey seconded the motion, which was accepted unanimously with 10 votes.

INTRODUCTION

$HOCH_2CH_2OH$

Ethylene Glycol

CAS No. 107-21-1

Chemical Formula: $C_2H_6O_2$ Molecular Weight: 62.07

Synonyms: 1,2-dihydroxyethane; ethane-1,2-diol; 1,2-ethanediol; ethylene alcohol; ethylene dihydrate; glycol; glycol alcohol; 2-hydroxyethanol; monoethylene glycol

Chemical and Physical Properties

Ethylene glycol is a clear, colorless liquid which is hygroscopic, odorless, sweet-tasting, and relatively nonvolatile. It is soluble in water and several organic solvents such as acetone and aliphatic alcohols. Some chemical and physical properties of ethylene glycol are presented in Table 1 (NPIRI, 1974; CSDSRIC, 1985; *Hazardous Chemicals Data Book*, 1986; *Merck Index*, 1989).

Production and Use

Ethylene glycol is manufactured on a large-scale basis primarily by hydration of ethylene oxide (*Kirk-Othmer*, 1982). Domestic U.S. production of ethylene glycol was over 5.3 billion pounds in 1989 (Kiefer, 1989).

Ethylene glycol has numerous industrial and commercial applications. Since ethylene glycol

TABLE 1
Some Chemical and Physical Properties of Ethylene Glycol

Physical state	colorless, slightly viscous, hygroscopic liquid
Boiling point (at 760 mm)	197° C
Freezing point (at 760 mm)	-13° C
Specific gravity (at 4° C)	1.12
Vapor pressure (at 20° C)	0.05 mm Hg
Vapor density (at 15°-32° C)	2.14
Evaporation rate	0.004 g/cm^2-sec
Flash point	111° C

lowers the freezing point of water, a major use is in antifreeze-coolant mixtures for motor vehicles; it is also found in heat-transfer fluids (Clark *et al.*, 1979; Marshall *et al.*, 1981) and airport runway deicing fluids (*Merck Index*, 1989). Ethylene glycol is also a component of hydraulic brake fluids, printer's inks, wood stains, adhesives, and pesticides (*Kirk-Othmer*, 1982). Ethylene glycol is used in the manufacture of polyester fiber and films, polyethylene terephthalate (PET) solid-state resins, glyoxal, safety explosives, plasticizers, and elastomers; as a solvent for borates in electrolytic capacitors; as a softening agent for cellophane; and as an industrial humectant (*Kirk-Othmer*, 1982; *Merck Index*, 1989).

ENVIRONMENTAL EXPOSURE

Ethylene glycol may enter the environment in waste water from its production and following spills and improper disposal of ethylene glycol-containing commercial products (*Handbook of Environmental Data on Organic Chemicals*, 1977; Christian and Moorehead, 1985; *Reviews of Environmental Contaminants and Toxicology*, 1988). With the growing popularity of residential and commercial underground heat-pump systems, leakage of ethylene glycol-containing heat-transfer fluids may be another source of environmental contamination (Løkke, 1984; *Reviews of Environmental Contaminants and Toxicology*, 1988).

Ethylene glycol readily undergoes aerobic and anaerobic biodegradation in water (Evans and David, 1974; Pitter, 1976; Child and Willetts, 1978; Dwyer and Tiedje, 1983). Its terrestrial fate is unknown, but rapid biodegradation is likely and adsorption in various soil types is known to be limited (Løkke, 1984). Due to its relatively low vapor pressure, evaporation from water or soil is unlikely. Its low octanol/water partition coefficient (log P=1.36; *Handbook of Environmental Data on Organic Chemicals*, 1977) and bioaccumulation factors in activated sludge, green algae, and fish (Freitag *et al.*, 1985) suggest that bioconcentration in aquatic organisms is unlikely.

HUMAN EXPOSURE

Acute human exposure to ethylene glycol usually occurs when single, often large, doses are ingested to mimic ethanol intoxication (Bowen *et al.*, 1978) or in suicide attempts (Parry and Wallach, 1974; Baud *et al.*, 1988). Accidental ingestion, especially by children, has also occurred (Moriarty and McDonald, 1974). Antifreeze-coolant fluid is the most common source of ethylene glycol, but toxicity has also resulted from contamination of potable water systems by ethylene glycol-containing heat-transfer fluids (*Morbidity and Mortality Weekly*, 1987). Based on a survey conducted from 1981 to 1983, the National Institute for Occupational Safety and Health has estimated that more than 1.5 million workers are potentially exposed to ethylene glycol (NIOSH, 1990). Ocular or dermal exposure would most likely occur in industrial settings (Sykowski, 1951, as quoted in McDonald *et al.*, 1973). Occupational inhalation exposure can also result if ethylene glycol vapors or mists are generated by heating or violent agitation (Triosi, 1950, as quoted in Grant, 1974). A threshold-limit value of 50 ppm (127 mg/m^3) has been set by the American Conference of Governmental Industrial Hygienists (ACGIH) (1990) based on published reports (Winek *et al.*, 1978). A permissible exposure limit (PEL) of 50 ppm ethylene glycol has been set by the Occupational Safety and Health Administration (OSHA) (Fed. Regist., 1989).

METABOLISM

Following administration by various routes, ethylene glycol is rapidly absorbed, distributed, and cleared. Plasma half-lives in dogs given ethylene glycol intravenously ranged from 3.0 to 4.4 hours (Martis *et al.*, 1982); plasma half-lives in rats or dogs given ethylene glycol by water gavage were 1.7 and 3.4 hours, respectively (Hewlett *et al.*, 1989). In Sprague-Dawley rats given single, oral doses of 6 or 9 mL/kg body weight, blood levels peaked 1 to 2 hours after dosing; ethylene glycol had virtually disappeared from the blood after 12 hours (Winek *et al.*, 1978). When rats were exposed to ethylene glycol vapors or condensation aerosols by nose-only inhalation, plasma half-lives ranged from 34 to 39 hours and 75% to 80% of the initial body burden was widely distributed in animals examined immediately after exposure (Marshall and Cheng, 1983).

Rates of metabolism and excretion vary with species, dose, and route of administration. Generally, metabolism begins immediately after administration, and excretion of most of the parent compound and metabolites is complete 12 to 48 hours after dosing (McChesney *et al.*, 1971; Winek *et al.*, 1978; Hewlett *et al.*, 1989; Lenk *et al.*, 1989). The major

excretory end products in rats, rabbits, dogs, and rhesus (*Macaca mulatta*) and pigtail (*M. nemestrina*) monkeys are carbon dioxide in exhaled air, and glycolate and unchanged ethylene glycol in the urine (Gessner *et al.*, 1961; McChesney and Golberg, 1972; Clay and Murphy, 1977; Chou and Richardson, 1978; Marshall, 1982; Marshall and Cheng, 1983; Hewlett *et al.*, 1989; Lenk *et al.*, 1989). Depending on the species, variable quantities of other metabolites such as glyoxylate, hippurate, and oxalate may also be excreted in the urine (Gessner *et al.*, 1961; Gessner and Williams, 1961; McChesney *et al.*, 1971; McChesney and Golberg, 1972; Richardson, 1973; Riley *et al.*, 1982).

Metabolism occurs primarily in the liver and kidney (Parry and Wallach, 1974). The initial step is alcohol dehydrogenase-catalyzed conversion of the parent compound to glycolaldehyde (Gessner *et al.*, 1960; Coen and Weiss, 1966; *Patty's Industrial Hygiene and Toxicology*, 1982). Subsequent metabolic steps are not as well characterized. Systemic toxic effects of ethylene glycol, such as metabolic acidosis and renal tubule necrosis, are generally attributed to the action of metabolites rather than of the parent compound, but the precise roles of various metabolites are uncertain (Jacobsen and McMartin, 1986).

Glycolate may play an important role in the development of metabolic acidosis (Clay and Murphy, 1977; Gabow *et al.*, 1986; Gabow, 1988; Jacobsen *et al.*, 1988). Male, but not female, rats fed 1.0% to 2.0% glycolic acid in the diet for up to 35 weeks exhibited increased mortality and developed renal lesions similar to those observed in chronic ethylene glycol intoxication (Silbergeld and Carter, 1959). Despite contrary assertions in earlier literature, there is no clear causal relationship between oxalate precipitation alone and renal damage in humans and other animals (Bove, 1966; Parry and Wallach, 1974; Jacobsen and McMartin, 1986).

Acute Toxicity

Susceptibility to ethylene glycol intoxication can vary with species, sex, and individual (Gessner *et al.*, 1960; Kersting and Nielsen, 1966; Lyon *et al.*, 1966; Clay and Murphy, 1977; NTP, 1990). Minimal lethal doses and LD_{50} values for several species are presented in Table 2. Ethylene glycol intoxication via antifreeze-coolant fluid ingestion is common in domestic dogs and cats (Grauer and Thrall, 1982).

Acute toxicity via the oral route has been extensively studied clinically and experimentally (Hanzlik *et al.*, 1931; Holck, 1937; Kersting and Nielsen, 1966; Lyon *et al.*, 1966; Borden and Bidwell, 1968; Roberts and Seibold, 1969; Sanyer *et al.*, 1973; Clay and Murphy, 1977; Winek *et al.*, 1978; Brown *et al.*, 1983; Cieciura *et al.*, 1983; Gabow *et al.*, 1986; Marshall and Doty, 1990; Smith *et al.*, 1990). In human and veterinary medicine, acute oral ethylene glycol intoxication has been divided into three clinical stages (Berman *et al.*, 1957; Moriarty and McDonald, 1974; Parry and Wallach, 1974; Grauer and Thrall, 1982). During the initial stage, central nervous system signs (ataxia, convulsions, and coma) predominate and are attributed to metabolic acidosis and aldehyde metabolite buildup. Increased anion gap, serum hyperosmolality, hyperglycemia and/or elevated blood urea nitrogen are often present in both clinical and experimental cases (Gabow *et al.*, 1986; Dial *et al.*, 1989; Burkhart and Kulig, 1990; Khera, 1990). These metabolic derangements are often fatal. If the patient survives, a second, somewhat poorly defined, cardiopulmonary stage may occur, characterized by tachycardia, tachypnea, pulmonary edema, and/or cardiac failure. The third stage consists of oliguric or anuric renal failure, which may be reversible with appropriate therapy. This stage is usually associated with characteristic light microscopic and ultrastructural renal changes including tubular epithelial degeneration and necrosis and oxalate crystal deposition in the kidney, lower urinary tract, and other organs. A fourth stage of ethylene glycol intoxication, characterized by cranial nerve deficits, has been recently identified in humans (Factor and Lava, 1987; Anderson, 1990).

To approximate the usual clinical situation, most experimental studies of acute oral ethylene glycol toxicity have employed high doses almost certain to cause severe disease and/or death (Kersting and Nielsen, 1966; Beckett and Shields, 1971; Szablowska and Selye, 1971; Sanyer *et al.*, 1973; Rushton *et al.*, 1981). For this reason, only limited data are available on No Observed Effect Levels (NOELs) or possible toxic effects of lower doses. In most experimental animal species, major morphologic effects occur in the kidney and lower urinary tract.

TABLE 2
Selected Minimal Lethal Doses and LD_{50} Values for Ethylene Glycol in Several Species

Species	Route of Administration	Dose (g/kg)	Reference
Minimal Lethal Doses			
Rat	Oral	0.1	Borden and Bidwell, 1968
	Intramuscular	4.4	Hanzlik *et al.*, 1931
	Intravenous	2.8	Hanzlik *et al.*, 1931
	Subcutaneous	5.0	Gessner *et al.*, 1961
Rabbit	Intramuscular	7.4	Hanzlik *et al.*, 1931
	Intravenous	4.9-6.2	Hanzlik *et al.*, 1931
	Subcutaneous	5.0	Gessner *et al.*, 1961
Dog	Oral	6.7-7.4	Kersting and Nielsen, 1966; Sanyer *et al.*, 1973
Cat	Subcutaneous	1.0	Gessner *et al.*, 1961
Human	Oral	1.57	Andrews and Snyder, 1986
LD_{50} Values			
Mouse	Oral	14.7	Laug *et al.*, 1939
	Oral	15.4	Bornmann, 1954
Rat	Oral	6.2	Laug *et al.*, 1939
	Oral	8.54	Smyth *et al.*, 1941
	Oral	4.0	Clark *et al.*, 1979
	Subcutaneous	5.3	Mason *et al.*, 1971
Guinea Pig	Oral	8.3	Laug *et al.*, 1939
	Oral	7.4	Smyth *et al.*, 1941

Male rats are quite susceptible to ethylene glycol toxicity. Male Porton rats receiving 1.0% ethylene glycol in drinking water for 3 weeks had gross evidence of renal oxalate deposition (Rofe *et al.*, 1986). In another study male rats given 1.0% ethylene glycol in the drinking water died after about 12 weeks; limited histopathologic evaluation revealed renal tubule degeneration and oxalate deposits and centrilobular hepatocellular degeneration (Hanzlik *et al.*, 1947). In drinking water studies, similar renal lesions were observed in male rats that received 0.25% ethylene glycol for 4 weeks (Gershoff and Andrus, 1962) or 1.0% to 2.0% ethylene glycol for 13 weeks (Robinson *et al.*, 1990).

In contrast, higher doses of ethylene glycol are generally required to produce typical toxic effects in female rats. Female rats given 2.92% ethylene glycol in drinking water died after 6 days; limited histopathologic evaluation did not reveal any microscopic lesions (Holck, 1937). Female Sprague-Dawley rats given concentrations of 1.0%, 3.0%, or 5.0% ethylene glycol in drinking water for 2 weeks had oxalate deposits in the kidney, but only females receiving 3.0% and 5.0% in the study died or had any clinical findings (Lyon *et al.*, 1966). Similarly, renal lesions and/or mortality occurred in female Sprague-Dawley rats given doses of 1.0% to 2.0% ethylene glycol in drinking water for 90 days, but not in those receiving 0.5% to 1.0% ethylene glycol (Robinson *et al.*, 1990).

When ethylene glycol was fed to male and female F344 rats at concentrations of 3,200, 6,300, 12,500,

25,000, or 50,000 ppm for 13 weeks, 4 of the 10 male rats given 50,000 ppm ethylene glycol died (Melnick, 1984). Surviving males in the 25,000 and 50,000 ppm groups had moderate to severe renal tubule degeneration, necrosis, and regeneration, as well as renal interstitial fibrosis and oxalate crystals in the kidney, lower urinary tract, and/or brain. All female rats in the study survived; mild renal lesions without oxalate precipitation were observed only in the female 50,000 ppm group. Body weights and body weight gains were significantly decreased in pregnant F344 rats and CD rats receiving ethylene glycol by gavage at doses of 2,500 or 5,000 mg/kg body weight daily on gestation days 6 through 15 (NTP, 1984; Myers *et al.*, 1988).

Macaques (*M. mulatta*, *M. irus*, and *M. radiata*) given ethylene glycol in drinking water at doses from 1 to 132 mg/kg body weight for 6 to 13 days had renal lesions and/or azotemia, although oxalate precipitation in the kidney and other tissues was noted only in animals given doses of 15 mL/kg or more (Roberts and Seibold, 1969).

When pregnant CD-1 mice were given ethylene glycol by gavage at doses of from 50 to 1,550 mg/kg body weight daily on gestation days 6 through 15, no treatment-related changes in maternal body weights, liver or kidney weights, water consumption, or clinical findings were noted, and no histopathologic lesions were seen in kidneys of high-dose dams (Tyl *et al.*, 1989b). However, in pregnant New Zealand white rabbits given doses from 100 to 2,000 mg/kg daily by gavage on days 6 through 19 of gestation, maternal mortality and typical renal lesions occurred in the high-dose group (NTP, 1990).

Morphologic lesions have occasionally been noted outside the urinary system following oral ethylene glycol administration. Electroretinographic abnormalities and retinal and uveal oxalate deposition were seen in New Zealand white rabbits given drinking water containing 4.0% ethylene glycol (Rossa and Weber, 1990). Bone marrow hypocellularity and depression of marrow granulocyte-macrophage progenitor colony (CFU-C) formation occurred in male and female $B6C3F_1$ mice up to 14 days after administration of ethylene glycol in water by gavage at doses of 200 to 1,000 mg/kg (Hong *et al.*, 1988). These changes were most pronounced at the higher doses. No other gross or histopathologic lesions or hematologic changes were observed in treated mice (the doses selected were not expected to cause severe systemic toxicity).

In general, ethylene glycol is considerably less toxic when given by routes other than feed (*Patty's Industrial Hygiene and Toxicology*, 1982). Renal lesions were not observed when rats were given ethylene glycol by intramuscular injection at a dose level of 180 mg/kg body weight daily for 40 days (Hanzlik *et al.*, 1931). Mild chemosis, lacrimation, and iridal flare were found in rabbit eyes exposed topically or intraocularly to high concentrations of ethylene glycol (Hanzlik *et al.*, 1931; Latven and Molitor, 1939; McDonald *et al.*, 1972, 1973, 1977; Clark *et al.*, 1979). Dermal applications of pure ethylene glycol did not irritate rabbit skin (Clark *et al.*, 1979), but intradermal injections of ethylene glycol in guinea pigs did result in skin irritation (Latven and Molitor, 1939).

Continuous whole-body inhalation exposure of Sprague-Dawley and Long-Evans rats, guinea pigs, New Zealand white rabbits, beagle dogs, and squirrel monkeys (*Saimiri sciureus*) to ethylene glycol at 12 mg/m^3 resulted only in moderate to severe chemosis, lacrimation, or corneal opacity in rats and rabbits; no toxic findings were seen when these species had repeated exposure at 10 or 57 mg/m^3 for 6 weeks (Coon *et al.*, 1970). No toxic findings were noted in rats and mice exposed to ethylene glycol vapor, 0.3 mg/L, for 16 weeks (Wiley *et al.*, 1938a,b). Only mild toxic effects (increased liver and/or kidney weights and reduced weight gains) occurred when pregnant CD rats were exposed to whole body aerosols of ethylene glycol at 2,500 mg/m^3 daily or when pregnant CD-1 mice were exposed to whole body or nose-only aerosols of 1,000 to 2,500 mg/m^3 daily (Tyl *et al.*, 1989a,b). Nystagmus, sometimes accompanied by fainting, occurred in female workers exposed to unknown concentrations of ethylene glycol vapors (Triosi, 1950, as quoted in Grant, 1974) and mild contact ocular irritation has been reported in humans (Sykowski, 1951, as quoted in McDonald *et al.*, 1973). Human volunteers exposed to ethylene glycol aerosols tolerated doses from 3 to 67 mg/m^3, but considered 140 mg/m^3 subjectively irritating and 200 mg/m^3 intolerable (Wills *et al.*, 1974).

CHRONIC TOXICITY

Chronic oral administration of ethylene glycol to rats has produced similar results in several studies. In an early study, male rats given 1.0% ethylene glycol in the drinking water died after 12 weeks; limited histopathologic evaluation revealed characteristic renal changes and marked centrilobular hepatocellular degeneration (Hanzlik *et al.*, 1947). Similarly, another early report described decreased survival; renal tubule degeneration, oxalate crystal deposition, and oxalate urolithiasis; and mild hepatocellular centrilobular atrophy and fatty change in male albino rats fed a diet containing 1.0% or 2.0% ethylene glycol for up to 2 years; female rats were not as severely affected (Morris *et al.*, 1942).

More recently, similar dose-related and sex-related changes including increased incidences of kidney lesions and decreased survival were observed in male and female Sprague-Dawley rats fed diets containing from 0.5% to 4.0% ethylene glycol for up to 2 years (Blood, 1965). Male and female F344 rats were fed 0.4, 0.2, or 1.0 g ethylene glycol per kg of body weight daily for up to 2 years (DePass *et al.*, 1986a). Only high-dose male rats developed urinary system lesions, progressing from renal tubule dilatation and oxalate crystalluria at 6 months to severe chronic nephritis and oxalate lithiasis by 15 months, at which time mortality was 100%. In dosed female rats, mild hepatocellular fatty change was noted. Increased incidences of neoplasia attributable to treatment were not observed.

In an early study (Hanzlik *et al.*, 1947), most male white mice given 3.0% ethylene glycol in the diet died within 12 weeks, but most males given 1.0% survived 7 months; some of the females given 1.5% ethylene glycol survived 1 year. Limited histopathologic evaluation revealed renal tubule "calcification and crystalline deposits." However, in a more recent study (DePass *et al.*, 1986a), no clinical findings or pathologic lesions were seen in male and female CD-1 mice fed 0.4 to 1.0 g ethylene glycol per kg body weight in the diet for 2 years. Likewise, in rhesus monkeys, there were no clinical or pathologic findings in two males given 0.2% and one female given 0.5% ethylene glycol in feed for 3 years (Blood *et al.*, 1962).

Subcutaneous ethylene glycol inoculation of 30 to 1,000 mg/kg twice weekly in male and female F344 rats for 1 year did not result in any toxic or carcinogenic effects (Mason *et al.*, 1971).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Although mortality was increased, no teratogenic changes were noted in chick embryos exposed to ethylene glycol (Gebhardt, 1968; Ameenuddin and Sunde, 1984). *In vitro* exposure of rat embryos to 40 μL ethylene glycol/mL culture medium resulted in developmental abnormalities including absence of yolk sac circulation, absent hindlimb buds, and central nervous system abnormalities (Grafton and Hansen, 1987).

In vivo studies in rats and mice indicate that only oral administration of ethylene glycol (versus cutaneous or aerosol exposure) results in reproductive and teratogenic changes (Myers *et al.*, 1988; Tyl *et al.*, 1988a,b, 1989a,b; Longzhan *et al.*, 1989; Bates *et al.*, 1990; Khera, 1990; Marr *et al.*, 1990). In several reproductive studies in rats and/or mice, decreased litter size, reduced pup birth weight, reduced pup survival, or fetal craniofacial and/or axial skeletal anomalies were noted following oral ethylene glycol administration (Maronpot *et al.*, 1983; NTP, 1984, 1988; Schuler *et al.*, 1984; Lamb *et al.*, 1985; Price *et al.*, 1985; DePass *et al.*, 1986b; Tyl *et al.*, 1989b). In general, mice exhibited more severe reproductive abnormalities at lower doses than did rats. The NOEL for developmental toxicity, including teratogenicity, in CD-1 mice has been determined to be 150 mg/kg (Tyl *et al.*, 1989b).

Although maternal toxicity and mortality occurred at 2,000 mg/kg, no evidence of teratogenicity or embryotoxicity was seen when pregnant New Zealand white rabbits were administered ethylene glycol at doses of 100 to 2,000 mg/kg body weight daily on gestation days 6 through 19 (NTP, 1990).

GENETIC TOXICOLOGY

Ethylene glycol is not genotoxic *in vitro*, and the results of *in vivo* studies are either conflicting or not reported in detail. Results from several mutagenicity studies in *Salmonella typhimurium* were uniformly negative (McCann *et al.*, 1975; Clark *et al.*, 1979; Pfeiffer and Dunkelberg, 1980; Zeiger *et al.*, 1987) and no growth inhibition due to DNA damage was observed in repair-deficient *Escherichia coli* (McCarroll *et al.*, 1981). Negative results were also obtained in a gene mutation test in

yeast, *Saccharomyces pombe* (Abbondandolo *et al.*, 1980). Additional negative results were reported for induction of aneuploidy in the fungus *Neurospora* (Griffiths, 1979, 1981), and for induction of sperm inactivation or dominant lethal mutations, as measured by infertility, in the parasitic wasp, *Bracon hebetor* (LaChance and Leverich, 1969). In plants, no induction of polyploidy was reported in *Allium cepa* root tips grown for 6 days in ethylene glycol solutions (Kabarity *et al.*, 1980). Meiotic disruption was reportedly induced in maize microsporocytes following treatment with ethylene glycol (Maguire, 1974), but no information on dosing or chemical purity was presented. Human embryonic fibroblasts treated with ethylene glycol in the absence of exogenous metabolic activation (dosing information not provided) exhibited no increase in chromosomal aberrations (Oya *et al.*, 1986).

Ethylene glycol was reported to induce chromosomal aberrations in male rat bone marrow cells harvested 50 hours after single gavage administration of 1,200 mg/kg ethylene glycol, and dominant lethal mutations were reportedly induced in the offspring of male rats treated with 120 or 1,200 mg/kg ethylene glycol by gavage at the late spermatid stage (Barilyak and Kozachuk, 1985). However, the purity of ethylene glycol was not provided, control values were unacceptably low, and no primary data were included. For these reasons, the validity of these results is questionable. No dominant lethal mutations or reproductive abnormalities were noted in F344 rats given ethylene glycol in the feed at doses of 0.4 to 1.0 g/kg body weight per day for three generations (DePass *et al.*, 1986b).

Mutagenicity information is available on a single metabolite, glycolate. Tested at a dose of 500 μg/plate, it was negative for induction of gene mutation in *S. typhimurium* strain TA100 with and without S9 activation (Yamaguchi and Nakagawa, 1983).

Study Rationale

Ethylene glycol was nominated for toxicology and carcinogenesis studies to the National Toxicology Program (NTP) through an interagency agreement between the NTP and the Environmental Protection Agency to provide toxicologic testing of chemicals under the Comprehensive Environmental Response, Compensation and Liability Act of 1980 (Superfund). Due to the large production volume and numerous commercial uses of ethylene glycol, there is high potential for widespread workplace and general exposure, as well as environmental contamination.

Recent bioassays with F344 rats (DePass *et al.*, 1986a) were considered adequate to evaluate the chronic toxicity and carcinogenicity of ethylene glycol in this species and strain. Therefore, the present studies were carried out only with $B6C3F_1$ mice.

Oral administration was chosen because most human exposure would be expected to occur by this route. The gavage route was not selected because administration of large bolus doses would not closely approximate the low-level chronic exposure expected for the general human population. Dosed feed was selected because of concerns that with drinking water exposure, the anticipated renal toxicity might have resulted in polydipsia and polyuria which could have affected the amounts of ethylene glycol consumed and/or excreted.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Ethylene glycol was obtained from Ashland Chemical Company (Columbus, OH) in one lot (lot A021180). Identity, purity, and stability analyses were performed by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and confirmed by the study laboratory (Appendix E).

The chemical, a colorless liquid, was identified as ethylene glycol by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity was found to be greater than 99% by elemental analyses, Karl Fischer water analysis, thin-layer chromatography, and gas chromatography. Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for ethylene glycol. Stability studies by gas chromatography indicated that ethylene glycol was stable as a bulk chemical for 2 weeks at temperatures up to 60° C when stored protected from light. The identity and stability of the bulk chemical was confirmed periodically at the study laboratory with infrared spectroscopy and gas chromatography. Identity was confirmed and no change in purity was observed.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing appropriate amounts of ethylene glycol and feed in a blender (Table E1). Studies to determine the homogeneity and stability of the dosed feed preparations were conducted by the analytical chemistry laboratory. Gas chromatographic methods were used to confirm homogeneity as well as the stability of dose formulations stored protected from light for 2 weeks at 25° C. During the studies, the dose formulations were stored at 5° C before use and at room temperature during use for up to 14 days.

Periodic analyses of the dose formulations of ethylene glycol were conducted at the study laboratory and the analytical chemistry laboratory using gas chromatography. During the 13-week studies, the dose formulations were analyzed at the initiation and the mid-point of the studies (Table E2). During the 2-year studies, the dose formulations were analyzed at least once every 8 weeks (Table E3), and 98% (97/99) of the dose formulations were within 10% of the target concentrations. Results of periodic referee analyses performed by the analytical chemistry laboratory were in good agreement with the results obtained by the study laboratory (Table E4).

13-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to ethylene glycol and to determine the appropriate doses for the 2-year studies.

Male and female B6C3F$_1$ mice were obtained from Harlan Industries (Indianapolis, IN). The animals were quarantined for 19 days and the average age was 63 days when the studies began. Five animals of each sex were randomly selected and killed prior to study initiation for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on the serum of five control animals of each sex in accordance with the protocols of the NTP Sentinel Animal Program (Appendix H).

Groups of 10 mice of each sex were fed diets containing 0, 3,200, 6,300, 12,500, 25,000, or 50,000 ppm ethylene glycol. Beginning on day 1, the appropriate feed was available *ad libitum* for 92 to 96 consecutive days.

Animals were housed five per cage. Water was available *ad libitum*. Animals were observed twice daily and weighed once weekly and at the end of the study. Clinical findings were recorded weekly.

At the end of the study, blood samples were collected by cardiac puncture. Urine samples were collected by expressing the urinary bladder. Table 3 contains the complete list of clinical pathology

analyses performed on animals in the 13-week studies of ethylene glycol.

During necropsy, the organs and tissues of all animals were examined for gross lesions. Organ weights were recorded for the brain, heart, right kidney, liver, lungs, and thymus in all mice, and the right testis of all males. Tissues for microscopic examination were embedded in paraffin, sectioned to a thickness of 4 to 6 μm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all mice that received 0 or 50,000 ppm. The kidneys and livers of males that received 12,500 or 25,000 ppm were examined microscopically. Table 3 lists the tissues and organs that were examined microscopically.

2-YEAR STUDIES

Study Design

Groups of 60 male mice were fed diets containing 0, 6,250, 12,500 or 25,000 ppm ethylene glycol in feed. Groups of 60 female mice were given diets containing 0, 12,500, 25,000 or 50,000 ppm ethylene glycol in feed. The appropriate feed was available *ad libitum* for 103 weeks. Ten mice per dose group were designated for interim evaluations (organ weights, hematology, clinical chemistry, and histopathology) after 15 months of chemical administration. However, because of early deaths in males, six males per group instead of ten were evaluated at 15 months.

Source and Specification of Animals

The B6C3F$_1$ mice used in these studies were obtained from Frederick Cancer Research Facility (Frederick, MD). All animals were quarantined for 19 days, then five mice of each sex were randomly selected for parasite evaluation and gross observation of evidence of disease. The average ages of the animals were 62 days (males) and 55 days (females) when dosing began. Animal health was monitored by serologic analyses during the course of the studies in accordance with the protocols of the NTP Sentinel Animal Program (Appendix H).

Animal Maintenance

Male mice were housed five per cage for 54 weeks, then individually until study end. Female mice were housed five per cage for 67 weeks, then individually until study end. Feed and water were available *ad libitum*. Cages were rotated within racks and racks were rotated within rooms every 2 weeks. Further details on animal maintenance are given in Table 3.

Clinical Examinations and Pathology

All animals were observed twice daily and clinical findings were recorded at each weight check. Individual body weights were obtained weekly through week 13, monthly thereafter, and at the end of the study. After 15 months, 6 male and 9 to 10 female mice from each dose group were evaluated. Organ weights were recorded for the brain, right kidney, and liver of all animals evaluated at 15 months. Blood samples were collected by cardiac puncture. Table 3 contains the complete list of the clinical pathology analyses performed on animals at the 15-month interim evaluations in the 2-year studies of ethylene glycol.

Necropsy was performed on all animals. During necropsy, all organs and tissues were examined for gross lesions. Tissues for microscopic examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned to a thickness of 4 to 6 μm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all control and high-dose mice and all animals that died early in the low- and mid-dose groups. For all other low- and mid-dose mice, organs and tissues examined included all gross lesions, kidney, liver, and thyroid gland in all mice, lung in females, and urinary bladder in males.

Samples of formalin-fixed liver from selected high-dose male and female mice were post-fixed in Fowler's solution (Fowler *et al.*, 1983) for 2 days, post-fixed in 1.0% osmium, dehydrated in ethanol, and infiltrated with Epon 812. Resulting blocks were thin-sectioned (approximately 90 nm), mounted on 100-mesh copper-rhodium grids, stained with 2.7% lead citrate and 5.0% uranyl acetate, and examined with a Philips 400 transmission electron microscope.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System (TDMS). The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit

for accuracy of labeling and animal identification and for thoroughness of tissue trimming. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. A quality assessment pathologist reviewed kidney and liver from male and female mice, thyroid gland and testis from male mice, and lung, ovary, adrenal gland, and mesentery from female mice for accuracy and consistency of lesion diagnosis.

The quality assessment report and slides were submitted to the Pathology Working Group (PWG) chair. Representative examples of potential chemical-related nonneoplastic lesions and neoplasms of liver, kidney, lung, ovary, and thyroid gland, and examples of disagreements in diagnosis between the laboratory and quality assessment pathologists were selected by the PWG chair for review by the PWG. The PWG included the quality assessment pathologist as well as other pathologists experienced in rodent toxicologic pathology who examined these tissues without knowledge of dose group or previously rendered diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the final diagnosis was changed to reflect the opinion of the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Animals were censored from the survival analyses at the time they were found dead from other than natural causes; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table tests to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A5, B1, and B5 are given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of all nonneoplastic lesions and most neoplasms (Tables A2 and B2) are also given as the ratio of the number of affected animals to the number of animals with the site examined microscopically. However, when macroscopic examination was required to detect neoplasms (e.g., skin, intestine, Harderian gland, and mammary gland) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Neoplasm Incidences

The majority of lesions in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which assumed that the diagnosed lesions were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, lesion prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The dosed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When lesions are incidental, this comparison of the time-specific lesion prevalences also provides a comparison of the time-specific lesion incidences (McKnight and Crowley, 1984).

In addition to logistic regression, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal lesions, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971;

Gart *et al.*, 1979), procedures based on the overall proportion of lesion-bearing animals.

Tests of significance include pairwise comparisons of each dosed group with controls and a test for an overall dose-response trend. Continuity-corrected tests were used in the analysis of lesion incidence, and reported P values are one sided. The procedures described above also were used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, see Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test was used, a procedure based on the overall proportion of affected animals.

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 that had approximately normal distributions were analyzed using the multiple comparison procedures of Williams (1971, 1972) and Dunnett (1955). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-response trends and to determine whether a trend-sensitive test (Williams' test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response trend (Dunnett's test).

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, historical control data can be helpful in the overall assessment of lesion incidence. Consequently, control lesion incidences from the NTP historical control database (Haseman *et al.*, 1984, 1985) are included in the NTP reports for lesions appearing to show compound-related effects.

Quality Assurance Methods

The 13-week and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as study records were submitted to the NTP Archives, they were audited by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and preliminary review draft of the NTP Technical Report were conducted. Audit procedures are presented in the reports, which are on file at the NIEHS. The audit findings were reviewed and assessed by the NTP staff so that all had been resolved or were otherwise addressed during the preparation of the Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of ethylene glycol was assessed by testing its ability to induce mutations in various strains of *Salmonella typhimurium*, trifluorothymidine resistance in mouse L5178Y lymphoma cells, and sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. The protocols and results for these studies are given in Appendix C.

The genetic toxicity studies of ethylene glycol are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the structure of the chemical and its responses in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemically induced DNA damage and to predict carcinogenicity in animals, based on the electrophilic theory of chemical carcinogenesis and the somatic mutation theory (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

Of the four *in vitro* tests evaluated by the NTP to date (mutagenicity in *S. typhimurium*, mutagenicity in mouse lymphoma cells, chromosomal aberrations in Chinese hamster ovary cells or sister chromatid exchanges in Chinese hamster ovary cells), there is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *S. typhimurium*, and carcinogenicity in rats and mice or at multiple tissue sites (Ashby and Tennant, 1991). The other *in vitro* tests do not correlate well with carcinogenicity in rodents (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Mutagenicity in *S. typhimurium* was the most predictive for rodent carcinogenicity (89% of the mutagens were carcinogens in rats and/or mice), while

mutations in mouse lymphoma cells or chromosomal aberrations or sister chromatid exchanges in Chinese hamster ovary cells were less predictive of carcinogenicity; 63% of chemicals inducing mutations in mouse lymphoma cells, 73% of chemicals inducing chromosomal aberrations, and 64% of chemicals inducing sister chromatid exchanges were carcinogenic in rodents. Moreover, no battery of tests that included the *S. typhimurium* test improved the predictability of the *S. typhimurium* test alone. The predictivity of a positive response in bone marrow chromosome aberration or micronucleus tests is not yet defined. Refer to the articles cited above for details regarding the correlation of structural alerts (or absence thereof), mutagenicity, and carcinogenicity results of 301 chemicals in the NTP database.

TABLE 3
Experimental Design and Materials and Methods in the Feed Studies of Ethylene Glycol

13-Week Studies	2-Year Studies
Study Laboratory Southern Research Institute (Birmingham, AL)	Same as 13-week studies
Strain and Species B6C3F$_1$ Mice	Same as 13-week studies
Animal Source Harlan Industries (Indianapolis, IN)	Frederick Cancer Research Facility (Frederick, MD)
Time Held Before Study 19 days	Same as 13-week studies
Average Age When Placed on Study 63 days	Males: 62 days Females: 55 days
Date of First Dose 25 May 1981	28 September 1982
Duration of Dosing Day 1 to day of sacrifice (days 92-96), dosed feed available *ad libitum*	Day 1 to day 721, dosed feed available *ad libitum*
Date of Last Dose 28 August 1981	17 September 1984
Necropsy Dates 24-28 August 1981	Interim: 3-5 January 1984 Terminal: 27 September-4 October 1984
Method of Sacrifice Thoracotomy	CO_2 asphyxiation
Average Age When Killed 156 days	Males: Interim - 527 days Terminal - 797 days Females: Interim - 520 days Terminal - 790 days
Size of Study Groups 10 males and 10 females	60 males and 60 females

TABLE 3
Experimental Design and Materials and Methods in the Feed Studies of Ethylene Glycol (continued)

13-Week Studies	2-Year Studies
Method of Animal Distribution Animals grouped by weight intervals. Animals assigned to cages. A table of random numbers was used to assign treatment groups to cages.	Same as 13-week studies
Animals per Cage 5	Males: 5, changed to 1 per cage on 13 October 1983 Females: 5, changed to 1 per cage on 13 January 1984
Method of Animal Identification Earmark	Earmark and toe clip
Diet NIH-07 Open formula mash diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as 13-week studies
Maximum Storage Time for Feed 90 days from milling	Same as 13-week studies
Water Birmingham Water Works (Birmingham, AL), available *ad libitum*	Same as 13-week studies
Cages Solid-bottom polycarbonate (Lab Products, Inc., Garfield, NJ)	Same as 13-week studies
Bedding BetaChips® (Northeastern Products Corp., Warrensburg, NY), changed twice weekly	Same as 13-week studies except changed once weekly after animals housed individually
Cage Filters Reemay spun-bonded polyester filters (Snow Filtration, Cincinnati, OH), changed once every 2 weeks	Same as 13-week studies
Racks Stainless steel (Lab Products, Inc., Garfield, NJ), changed once every 2 weeks	Same as 13-week studies
Animal Room Environment Temperature: 20°-22° C Relative humidity: 37-58% Fluorescent light: 12 hours/day Room air changes: minimum of 15 changes/hour	Temperature 21°-23° C Relative humidity: 51%-54% Fluorescent light: 12 hours/day Room air changes: minimum of 15 changes/hour
Doses 0, 3,200, 6,300, 12,500, 25,000 or 50,000 ppm ethylene glycol in feed	Males: 0, 6,250, 12,500, or 25,000 ppm ethylene glycol in feed Females: 0, 12,500, 25,000, or 50,000 ppm ethylene glycol in feed
Type and Frequency of Observation Observed twice/day; weighed once/week and at termination; clinical findings recorded weekly	Observed twice/day; weighed once/week through week 13, once/month thereafter, and at sacrifice; clinical findings recorded at each weigh period and at sacrifice

TABLE 3
Experimental Design and Materials and Methods in the Feed Studies of Ethylene Glycol (continued)

13-Week Studies	2-Year Studies
Necropsy Examinations	
Necropsy performed on all animals. Organ weights recorded for the brain, heart, right kidney, liver, lungs, and thymus of all animals, and the right testis of all males.	Necropsy performed on all animals. Organ weights recorded for the brain, right kidney, and liver of all animals evaluated at 15 months.
Clinical Pathology	
Blood samples were collected from all animals. *Hematology*: hematocrit, hemoglobin, erythrocytes, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, total and differential leukocyte counts. *Clinical chemistry*: blood urea nitrogen, creatinine, sodium, potassium, chloride, partial carbon dioxide, calcium, phosphorus (inorganic), total protein, albumin, albumin/globulin ratio, total bilirubin, and pH. *Urinalysis*: glucose, protein, specific gravity, pH, urobilinogen, bilirubin, blood (hemoglobin), ketones, and nitrite.	Blood samples were collected from animals evaluated at 15 months. *Hematology*: hematocrit, hemoglobin, erythrocytes, total and differential leukocyte counts. *Clinical chemistry*: blood urea nitrogen, creatinine, total bilirubin, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and sorbitol dehydrogenase.
Histopathology	
Complete histopathology was performed on all control and high-dose animals. In addition to tissue masses, gross lesions, and associated regional lymph nodes, the following organs and/or tissues were included: adrenal gland, brain, colon, esophagus, femur including marrow, gallbladder, heart, kidney, liver, lung, mammary gland, mandibular and mesenteric lymph nodes, nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, small intestine, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. The liver and kidney of males in the 12,500 and 25,000 ppm groups were also examined.	Complete histopathology was performed on all high-dose and control males and females at the 15-month interim evaluations. Complete histopathology was also performed on all control and high-dose mice at the end of the 2-year studies and on animals that died before the end of the 2-year studies. In addition to tissue masses and gross lesions, the following organs and/or tissues were examined: adrenal gland, brain, epididymis, esophagus, femur including marrow, gallbladder, heart, kidney, large intestine (cecum, colon, rectum), liver, lung, mammary gland, mandibular or mesenteric lymph nodes, nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicles, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. For all low- and mid-dose mice, organs and tissues examined at the end of the 2-year studies included all gross lesions, kidney, liver, and thyroid gland in males and females; lung in females; and urinary bladder in males.

RESULTS

13-WEEK STUDIES

Data from the literature were adequate for selecting doses for the 13-week feed studies; therefore, 14-day repeated dose studies were not conducted. Doses for the 13-week studies were 0, 3,200, 6,300, 12,500, 25,000, or 50,000 ppm ethylene glycol in the feed. Some of the findings described below have been previously reported (Melnick, 1984).

All animals survived to the end of the studies. The mean body weight gains of male groups that received 12,500 or 50,000 ppm were significantly less than those of the controls (Table 4). No chemical-related clinical findings were observed; fighting was observed among all exposed and control male mice. No biologically significant changes in final mean body weights, absolute or relative organ weights (Table D1), or hematology or clinical chemistry parameters were noted in any dosed group. Results from all serologic analyses for murine viruses were negative (Appendix H).

TABLE 4
Survival and Mean Body Weights of Mice in the 13-Week Feed Studies of Ethylene Glycol

Concentration (ppm)	Survival[a]	Body Weights and Weight Changes[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	23.0 ± 0.3	32.5 ± 0.6	9.5 ± 0.5	
3,200	10/10	22.8 ± 0.4	32.4 ± 0.8	9.6 ± 0.6	100
6,300	10/10	23.1 ± 0.4	32.4 ± 1.0	9.3 ± 0.8	100
12,500	10/10	23.0 ± 0.4	30.2 ± 1.0	7.2 ± 0.9*	93
25,000	10/10	22.8 ± 0.4	31.1 ± 0.6	8.3 ± 0.6	96
50,000	10/10	23.0 ± 0.3	30.4 ± 0.6	7.4 ± 0.6*	94
Female					
0	10/10	18.7 ± 0.3	26.1 ± 0.8	7.4 ± 0.7	
3,200	10/10	18.1 ± 0.3	25.1 ± 0.6	7.0 ± 0.5	96
6,300	10/10	18.0 ± 0.3	25.2 ± 0.7	7.2 ± 0.6	97
12,500	10/10	18.3 ± 0.3	25.8 ± 0.8	7.5 ± 0.7	99
25,000	10/10	18.3 ± 0.4	25.6 ± 0.6	7.3 ± 0.3	98
50,000	10/10	18.1 ± 0.4	24.9 ± 0.6	6.8 ± 0.4	95

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes given as mean ± standard error

Treatment-related histopathologic lesions were noted only in the kidneys and livers of male mice that received 25,000 or 50,000 ppm (Table 5). Hyaline degeneration in the liver occurred in the centrilobular hepatocytes. Affected cells contained cytoplasmic accumulations of nonbirefringent, eosinophilic (hyaline), globular, or crystalline material which resembled erythrocytes in size, shape, and tinctorial properties. In some cases, only one or two hepatocytes around central veins were affected; in more severe cases, affected hepatocytes were present in several layers of the hepatic cords adjacent to central veins. Nephropathy was characterized by several renal tissue changes that included tubule dilatation, cytoplasmic vacuolation, or regenerative hyperplasia of tubule epithelial cells. These changes were focal, randomly distributed, and of minimal to mild severity.

In one male that received 50,000 ppm, a small birefringent crystal resembling an oxalate crystal was present in the wall of a meningeal artery. In females, no treatment-related lesions were seen in any organ.

Dose Selection Rationale: In male mice, potentially progressive renal lesions were seen in the 25,000 and 50,000 ppm groups and significantly decreased mean weight gain occurred in the 12,500 and 50,000 ppm male groups. Also, ethylene glycol is known to be more toxic to males than females in other rodent species (Blood, 1965; DePass *et al.*, 1986a). Therefore, a high dose of 25,000 ppm and lower doses of 6,250 and 12,500 ppm were selected for male mice in the 2-year feed studies.

Because greater quantities might affect nutritional adequacy, 50,000 ppm is generally recommended as the maximum concentration of a test compound which should be administered in the diet. For this reason, 50,000 ppm ethylene glycol was selected as the high dose for females in the 2-year studies, even though no clinical or pathologic changes were seen at 50,000 ppm or lower doses in the 13-week studies. The lower doses selected for female mice in the 2-year feed studies were 12,500 and 25,000 ppm.

TABLE 5
Selected Nonneoplastic Lesions in Male Mice in the 13-Week Feed Study of Ethylene Glycol

	0 ppm	3,200 ppm	6,300 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Liver: Hyaline Degeneration[a]						
Overall rates[b]	0/10	–[c]	–	0/10	10/10**	10/10**
Kidney: Nephropathy[d]						
Overall rates	0/10	–	–	0/10	1/10	5/10*

* Significantly different (P≤0.05) from the control group by the Fisher exact test
** P≤0.01
[a] Diagnostic term used by the study pathologist for this lesion was degeneration.
[b] Number of affected animals/number of animals necropsied or number of animals with tissues examined microscopically
[c] Not examined histopathologically
[d] Diagnostic term used by the study pathologist for this lesion was nephrosis.

2-YEAR STUDIES

Survival

There were no statistically significant differences in survival between dosed and control groups (Table 6 and Figure 1). Of the high-dose males, 65% (35/54) survived to 18 months. Because of several early deaths due to extensive fighting, male mice were housed individually after week 54; female mice were housed individually after week 67.

Body Weights, Feed Consumption, and Clinical Findings

The mean body weights of exposed and control male and female mice were similar (Figure 2 and Tables 7 and 8). No treatment-related clinical findings or gross lesions were noted. Results from all serologic analyses for murine viruses were negative (Appendix H). Feed consumption by exposed male and female mice was similar to that by controls (Tables F1 and F2). For male mice, dietary levels of 0, 6,250, 12,500, and 25,000 ppm resulted in average daily ethylene glycol consumption levels of approximately 1,500, 3,000, or 6,000 mg/kg body weight. For females, dietary levels of 0, 12,500, 25,000, or 50,000 ppm resulted in average daily ethylene glycol consumption levels of approximately 3,000, 6,000, or 12,000 mg/kg body weight.

TABLE 6
Survival of Mice in the 2-Year Feed Studies of Ethylene Glycol[a]

Male	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Animals initially in study	60	60	60	60
15-Month interim evaluation	6	6	6	6
Natural deaths	15	8	11	16
Moribund	10	14	11	15
Animals surviving to study termination	29	32	32	23
Percent probability of survival at end of study[b]	55	60	61	45
Mean survival (days)[c]	581	642	592	524
Survival analyses[d]	P=0.136	P=0.455N	P=0.706N	P=0.306

Female	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	9	10
Natural deaths	8	11	9	1
Moribund	9	9	12	12
Animals surviving to study termination	33	30	30	37
Percent probability of survival at end of study	66	60	59	75
Mean survival (days)	651	645	638	656
Survival analyses	P=0.371N	P=0.649	P=0.554	P=0.541N

[a] First day of sacrifice: 731
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice).

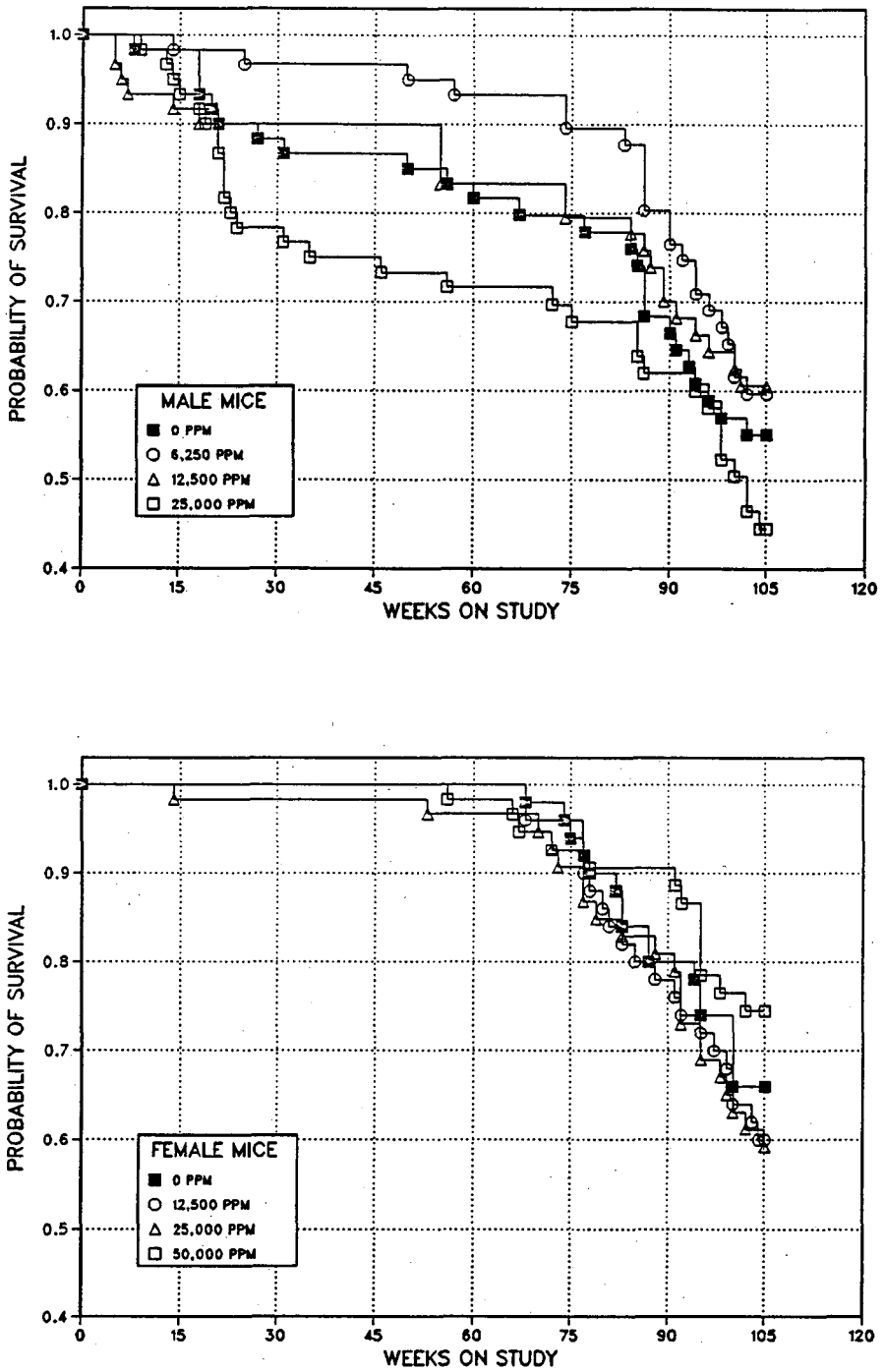


FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Mice Administered Ethylene Glycol in Feed for 2 Years

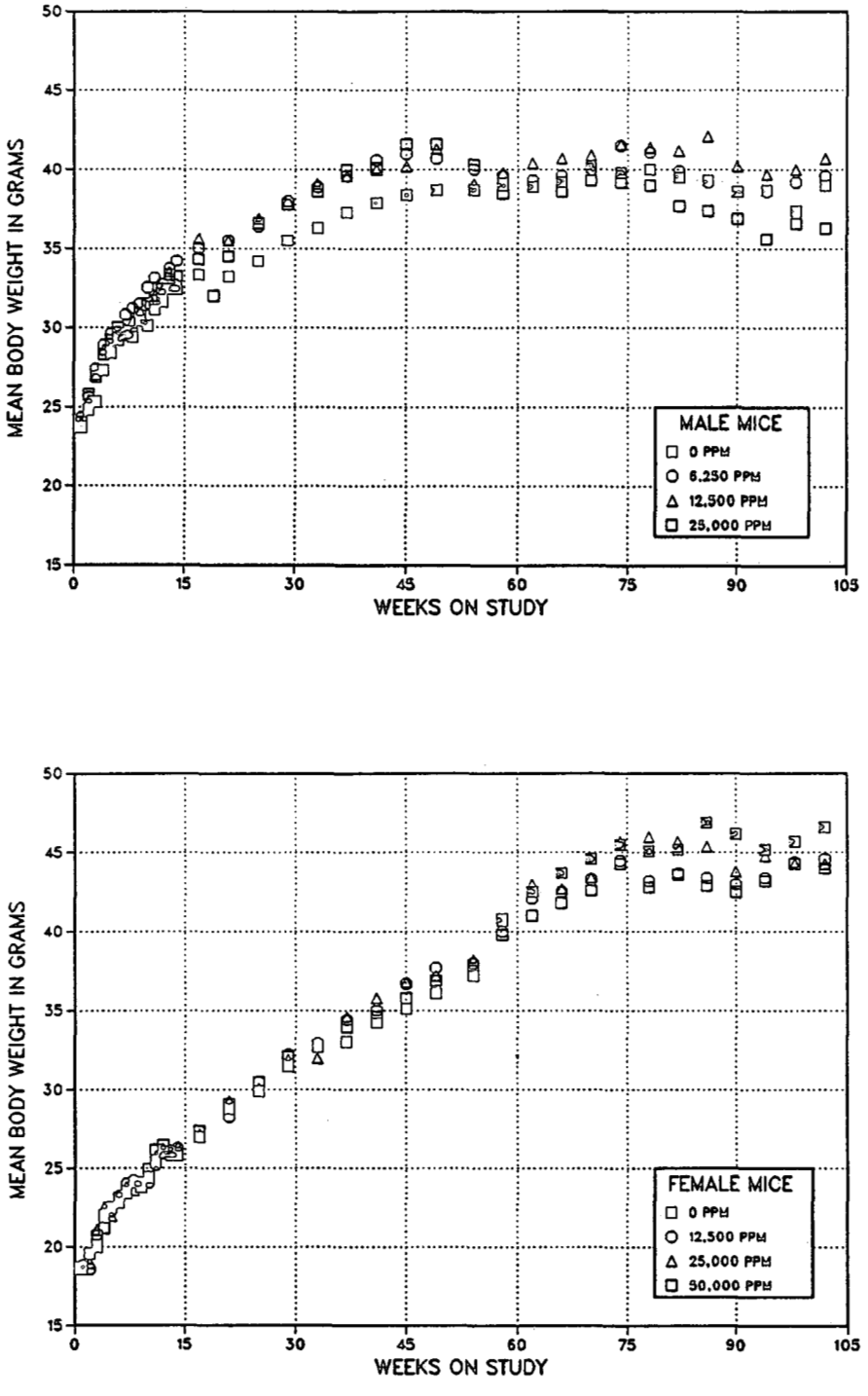


FIGURE 2
Growth Curves for Male and Female Mice Administered Ethylene Glycol in Feed for 2 Years

TABLE 7
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of Ethylene Glycol

Weeks on Study	0 ppm		6,250 ppm			12,500 ppm			25,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.7	60	24.3	103	60	24.0	101	60	24.0	101	60
2	24.8	60	25.6	103	60	25.4	102	60	25.8	104	60
3	25.3	60	27.4	108	60	27.0	107	60	26.9	106	60
4	27.3	60	28.9	106	60	28.6	105	60	28.3	104	60
5	28.4	60	29.6	104	60	28.7	101	60	29.2	103	60
6	29.2	60	29.7	102	60	29.5	101	58	30.0	103	60
7	29.6	60	30.8	104	60	29.9	101	57	29.5	100	60
8	29.4	60	31.2	106	60	30.4	103	56	29.6	101	60
9	30.4	59	31.5	104	60	30.9	102	56	30.9	102	60
10	30.1	59	32.5	108	60	31.4	104	56	31.5	105	59
11	31.1	59	33.1	106	60	32.0	103	56	31.7	102	59
12	31.6	59	32.7	104	60	32.3	102	56	32.4	103	59
13	32.4	59	33.7	104	60	33.5	103	56	33.3	103	59
14	32.4	59	34.2	106	60	32.7	101	56	33.2	103	58
17	33.3	59	35.0	105	59	35.6	107	55	34.3	103	56
21	33.2	55	35.5	107	59	35.5	107	54	34.5	104	54
25	34.2	54	36.4	106	59	36.9	108	54	36.6	107	47
29	35.5	53	38.0	107	58	38.0	107	54	37.8	107	47
33	36.3	52	38.9	107	58	39.1	108	54	38.6	106	46
37	37.3	52	39.6	106	58	39.6	106	54	40.0	107	45
41	37.9	52	40.6	107	58	40.0	106	54	40.1	106	45
45	38.4	52	41.0	107	58	40.2	105	54	41.6	108	45
49	38.7	52	40.7	105	58	41.3	107	54	41.6	108	44
54	38.7	51	40.0	103	57	39.1	101	54	40.3	104	44
58	39.0	50	39.6	102	56	39.8	102	50	38.5	99	43
62	38.9	49	39.3	101	56	40.4	104	50	38.9	100	43
66	39.2	49	39.6	101	56	40.7	104	50	38.6	99	43
70[a]	40.1	42	40.2	100	50	40.9	102	44	39.3	98	37
74	39.8	42	41.5	104	50	41.6	105	44	39.2	99	36
78	40.0	41	41.1	103	48	41.4	104	42	39.0	98	35
82	39.5	41	39.9	101	48	41.2	104	42	37.7	95	35
86	39.3	39	39.2	100	47	42.1	107	41	37.4	95	33
90	38.6	36	38.6	100	42	40.2	104	37	36.9	96	32
94	38.7	33	38.6	100	40	39.7	103	36	35.6	92	32
98	37.4	31	39.2	105	37	40.0	107	34	36.6	98	30
102	39.0	30	39.6	102	33	40.7	104	32	36.3	93	26
Terminal sacrifice		29			32			32			23
Mean for weeks											
1-13	28.7		30.1	105		29.5	103		29.5	103	
14-52	35.7		38.0	106		37.9	106		37.8	106	
53-102	39.1		39.7	102		40.6	104		38.0	97	

[a] Interim evaluation occurred during week 70.

TABLE 8
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of Ethylene Glycol

Weeks on Study	0 ppm		12,500 ppm			25,000 ppm			50,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.7	60	18.8	101	60	18.9	101	60	18.6	100	60
2	19.6	60	18.6	95	60	19.0	97	60	19.6	100	60
3	20.0	60	20.8	104	60	21.1	106	60	20.7	104	60
4	22.0	60	22.1	101	60	22.5	102	60	21.2	96	60
5	22.5	60	22.3	99	60	22.0	98	60	22.5	100	60
6	22.8	60	23.2	102	60	23.3	102	60	23.1	101	60
7	23.5	60	24.0	102	60	23.8	101	60	23.4	100	60
8	24.1	60	24.2	100	60	24.2	100	60	23.6	98	60
9	23.8	60	24.1	101	60	24.1	101	60	23.9	100	60
10	24.4	60	24.1	99	60	24.5	100	60	24.9	102	60
11	25.4	60	25.2	99	60	25.8	102	60	26.2	103	60
12	25.9	60	26.1	101	60	26.3	102	60	26.5	102	60
13	25.8	60	26.1	101	60	26.2	102	60	26.0	101	60
14	25.8	60	26.3	102	60	26.4	102	60	26.0	101	60
17	27.0	60	27.4	102	60	27.2	101	59	27.4	102	60
21	28.8	60	28.3	98	60	29.3	102	59	29.1	101	60
25	29.9	60	30.5	102	60	30.1	101	59	30.5	102	60
29	31.5	60	32.2	102	60	31.9	101	59	32.1	102	60
33	32.8	60	32.9	100	60	32.0	98	59	32.7	100	60
37	33.0	60	34.4	104	60	34.6	105	59	33.9	103	60
41	34.2	60	35.0	102	60	35.8	105	59	34.6	101	60
45	35.1	60	36.7	105	60	36.8	105	59	35.8	102	60
49	36.1	60	37.7	104	60	37.2	103	59	36.9	102	60
54	37.2	60	38.0	102	60	38.2	103	58	37.7	101	60
58	40.8	60	40.0	98	60	40.7	100	58	39.8	98	59
62	42.5	60	42.1	99	60	43.1	101	58	41.0	97	59
66	43.7	60	42.5	97	60	42.7	98	58	41.8	96	59
70[a]	44.6	49	43.3	97	48	43.4	97	49	42.6	96	47
73	45.5	49	44.5	98	48	45.7	100	47	44.3	97	46
77	45.1	46	43.2	96	46	46.0	102	44	42.8	95	46
81	45.2	45	43.7	97	42	45.7	101	43	43.6	97	45
85	46.9	42	43.4	93	40	45.4	97	42	42.9	92	45
89	46.2	40	43.0	93	39	43.8	95	41	42.5	92	45
93	45.2	40	43.4	96	37	44.8	99	37	43.2	96	43
97	45.7	37	44.4	97	35	44.3	97	35	44.3	97	39
102	46.6	33	44.6	96	32	44.3	95	31	44.0	94	38
Terminal sacrifice		33			30			30			37
Mean for weeks											
1-13	23.0		23.0	100		23.2	101		23.1	100	
14-52	31.4		32.1	102		32.1	102		31.9	102	
53-102	44.2		42.8	97		43.7	99		42.3	96	

[a] Interim evaluation occurred during week 70.

Pathology and Statistical Evaluation

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred at an incidence of at least 5% in at least one study group, and historical incidences for certain neoplasms are presented in Appendixes A for male mice and B for female mice.

No treatment-related neoplasms were observed in male or female mice at the 15-month interim evaluations or at the end of the 2-year studies. Several treatment-related or biologically significant nonneoplastic lesions were seen in exposed mice at interim evaluations and in the 2-year studies.

Liver: The incidence of hepatocellular hyaline degeneration was increased in exposed male and female mice at the 15-month interim evaluations (Table 9). At the end of the 2-year studies, incidences of centrilobular hepatocyte hyaline degeneration were increased in dosed male and female mice and were considered clearly related to ethylene glycol administration. Severity did not increase with dose. Under light microscopy, this lesion appeared similar to hyaline degeneration seen in the 13-week studies and the 15-month interim evaluations, and consisted of cytoplasmic accumulations of nonbirefringent, eosinophilic, granular to globular material resembling erythrocytes in size, shape, and tinctorial properties (Plate 1).

TABLE 9
Selected Nonneoplastic Lesions in Male and Female Mice in the 2-Year Feed Studies of Ethylene Glycol

Male	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
15-Month Interim Evaluation				
Liver: Hyaline degeneration				
Overall rates[a]	0/6	3/6	2/6	6/6
Kidney: Nephropathy				
Overall rates	2/6	2/6	5/6	6/6*
2-Year Study				
Liver: Hyaline degeneration				
Overall rates	0/54	0/53	24/53**	36/54**

Female	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
15-Month Interim Evaluation				
Liver: Hyaline degeneration				
Overall rates	0/10	0/10	3/9	10/10
Lung: Arterial medial hyperplasia				
Overall rates	2/10	0/10	0/9	3/10
2-Year Study				
Liver: Hyaline degeneration				
Overall rates	0/50	0/50	1/51	26/50**
Lung: Arterial medial hyperplasia				
Overall rates	3/50	10/50*	10/51*	23/50**

* Significantly different ($P \leq 0.05$) from the control group by logistic regression test
** $P \leq 0.01$
[a] Number of affected animals/number of animals necropsied or number of animals with tissues examined microscopically

This material did not stain when the following stains were applied to representative liver sections from high-dose male and female mice: periodic acid-Schiff (PAS), Ziehl-Neelsen (acid-fast), Heidenhain-Mallory (protein), toluidine blue (metachromasia), and Prussian blue (iron). When examined by transmission electron microscopy, irregularly shaped, pleomorphic, nonmembrane bound, intracytoplasmic inclusions were seen in affected hepatocytes. These inclusions were composed of crystalline parallel arrays of alternating electron-dense and electron-lucent linear structures (8.5 to 11 nm) with 11 to 13 nm periodicity (Plates 3 and 4). Hepatocellular erythrophagocytosis was diagnosed in two high-dose female mice (Table B5) and consisted of subcapsular hepatocytes whose cytoplasm was packed with intact erythrocytes.

Urinary System: The incidence of nephropathy was increased in male mice at the 15-month interim evaluations (Table 9); however, there were no treatment-related changes in the incidence or severity of nephropathy in male or female mice at the end of the 2-year studies (Tables A5 and B5).

In several high-dose males, small numbers of pale yellow to clear crystals morphologically compatible with oxalate were seen in the renal cortical tubules (8 mice), urethral lumens (12 mice), and/or renal pelvis (1 mouse) (Table A5). These crystals were birefringent when examined under polarized light. Renal tubule dilatation was often seen in association with renal tubule crystals. Calculi composed of oxalate-like material were detected grossly or microscopically in four high-dose males. Except for one male with both urethral crystals and gross calculus and another male with pelvic crystals and microscopic calculus, there were no simultaneous occurrences of crystals and calculi. The incidence of urethral suppurative inflammation was increased in high-dose males and that of urinary bladder chronic inflammation was increased in all exposed males (Table A5).

Except for two deaths at weeks 35 and 46, all male mice with renal tubule crystals died by week 31. Urethral and/or urinary bladder inflammation was also present in most of these animals. The causes of death or reasons for moribund kill in these males were severe gross and microscopic urogenital and skin lesions including ulcers of the penis and prepuce; alopecia, ulcers, or inflammation of skin in the posterior body; and seminal vesicle and prostate gland inflammation. These lesions were probably related to fight wound trauma and ascending secondary infections. Infections and trauma may have resulted in dehydration, urine stasis, urinary pH alterations, and other physiological changes which could have provided microenvironments favorable for oxalate precipitation in the renal tubules. Therefore, it is uncertain if the presence of this oxalate-like material was a direct toxic result of ethylene glycol administration.

All but three males with urethral or pelvic crystals or urinary bladder calculi survived to study end or were killed in a moribund condition at week 84 or later. In these mice, gross and microscopic urogenital and skin lesions possibly related to fight wound trauma were absent or of low severity.

Lung: At the 15-month interim evaluations, two control and three high-dose females had medial hyperplasia of small pulmonary arteries and/or arterioles (Table 9). At the end of the 2-year studies, exposed females had an increased incidence of medial hyperplasia of the small pulmonary arteries and/or arterioles (Table 9 and Plate 2), but severity did not increase with dose. Affected vessels were distributed randomly and had minimal to mild circumferential thickening of mural smooth muscle; it was unclear if this thickening was due to smooth muscle hypertrophy, to hyperplasia, or to both. The vessels were sometimes surrounded by lymphocytic infiltrates. The increased incidence of medial hyperplasia was considered to be clearly related to ethylene glycol administration.

Alveolar/bronchiolar adenomas (0/50, 4/50, 4/51, 1/50) and combined adenomas and carcinomas (1/50, 6/50, 6/51, 1/50) were marginally increased in low- and mid-dose females (Table B3). The historical control incidence of combined alveolar/bronchiolar adenomas and carcinomas in female mice from recent NTP dosed feed studies is 70/870 (8%, range 2% to 26%; Table B4a). No increased incidences of alveolar epithelial hyperplasia were noted in exposed female mice. Since the incidences of these neoplasms were within the historical control range and these increases were not dose-related, these neoplasms were not considered directly related to ethylene glycol administration.

Thyroid Gland: The incidence, but not the severity, of follicular cell hyperplasia increased in exposed females (8/49, 16/50, 22/51, 14/50; Table B5).

Follicular cell adenomas occurred in one control, one low-dose, one mid-dose, and three high-dose females (Table B3). The historical incidence for combined thyroid follicular cell adenomas and carcinomas in control female mice in recent NTP feed studies is 22/850 (3%, range 0% to 9%). Because the incidences of follicular cell adenomas and carcinomas were within the historical control range, the increased incidences of hyperplasia were not dose-related, and the severity of hyperplasia did not increase in exposed females, the thyroid follicular cell proliferative changes were not considered to be related to ethylene glycol administration.

Harderian Gland: In male mice, two harderian gland adenomas occurred in the mid-dose group, and two adenomas and one carcinoma occurred in the high-dose group (Table A1). The historical incidences for combined harderian gland adenomas and carcinomas in control male mice from recent NTP feed studies is 48/872 (6%, range 0% to 20%; Table A4). Because the incidences of harderian gland adenomas and carcinomas were only marginally increased and were within the historical control range, these neoplasms were not considered to be related to ethylene glycol administration.

All Organs: In female mice, the incidence of combined lymphomas had a statistically significant decrease (17/50, 13/50, 9/51, 7/50; Table B3). The historical incidence of combined lymphomas in control female mice from recent NTP feed studies is 259/870 (30%, range 10% to 44%; Table B4b). The relationship of this decrease to ethylene glycol administration is uncertain.

GENETIC TOXICITY

Negative results were obtained in all *in vitro* genotoxicity assays with ethylene glycol. Ethylene glycol was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 when tested with a preincubation protocol at concentrations up to 10,000 μg/plate in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Table C1; Zeiger *et al.*, 1987). Ethylene glycol (concentrations up to 5,000 μg/mL) was concluded to be negative, with and without Aroclor 1254-induced male Fischer rat liver S9 activation enzymes, in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y cells (Table C2; McGregor *et al.*, 1991). In the absence of S9, inconsistent responses were seen among the three trials; a positive response was obtained in one trial, but this was not reproduced in the other two trials performed without S9. The results of two trials with S9 were negative. Results of *in vitro* cytogenetic tests for induction of sister chromatid exchanges and chromosomal aberrations with ethylene glycol in Chinese hamster ovary cells were negative with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9 (Tables C3 and C4). In the sister chromatid exchange and the chromosomal aberration tests, doses up to 5,000 μg/mL ethylene glycol were tested. Little or no toxicity was seen in all tests.

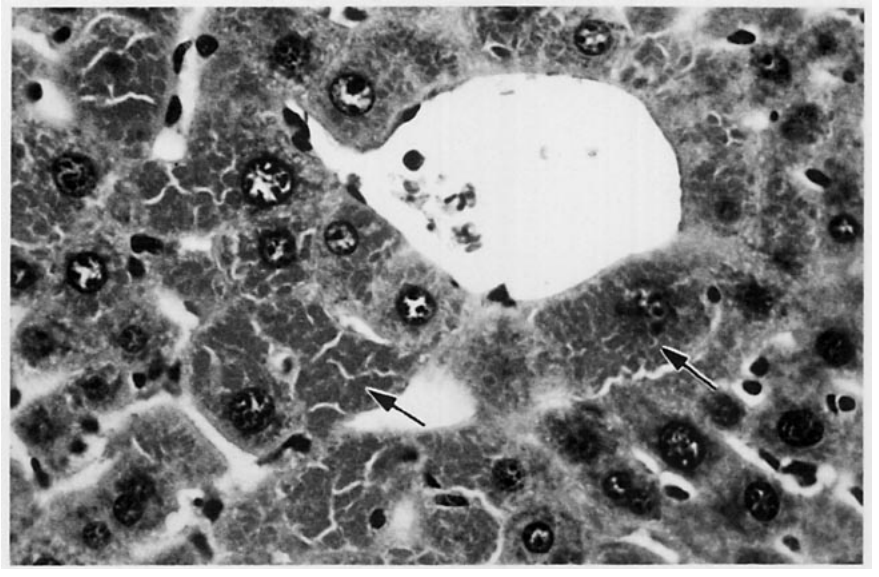


PLATE 1
Hepatocellular hyaline degeneration in the liver of a male B6C3F$_1$ mouse given 2,500 ppm of ethylene glycol in feed for 2 years. Hepatocellular cytoplasm is packed with coarsely granular, eosinophilic material (arrows). H&E 395x.

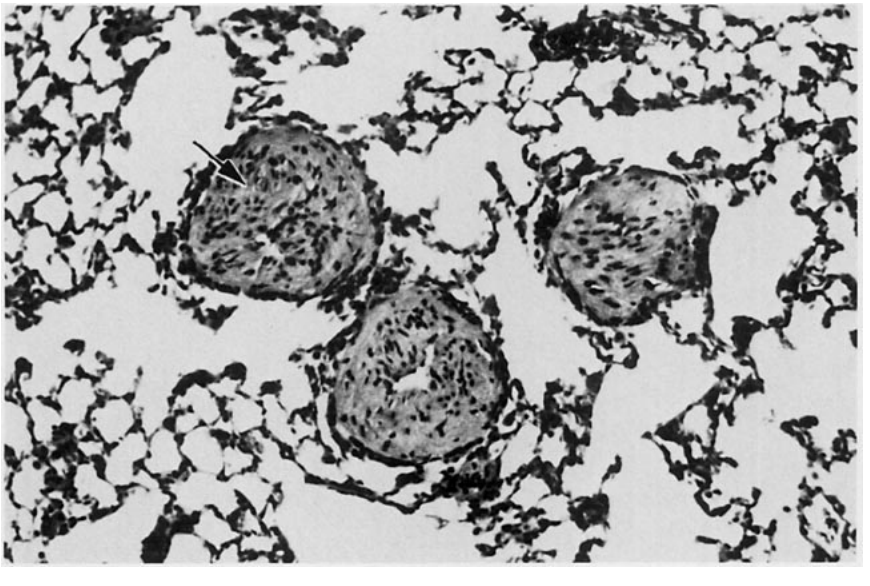


PLATE 2
Pulmonary arterial hyperplasia (arrow) in the lung of a female B6C3F$_1$ mouse given 5,000 ppm ethylene glycol in feed for 2 years. H&E 150x.

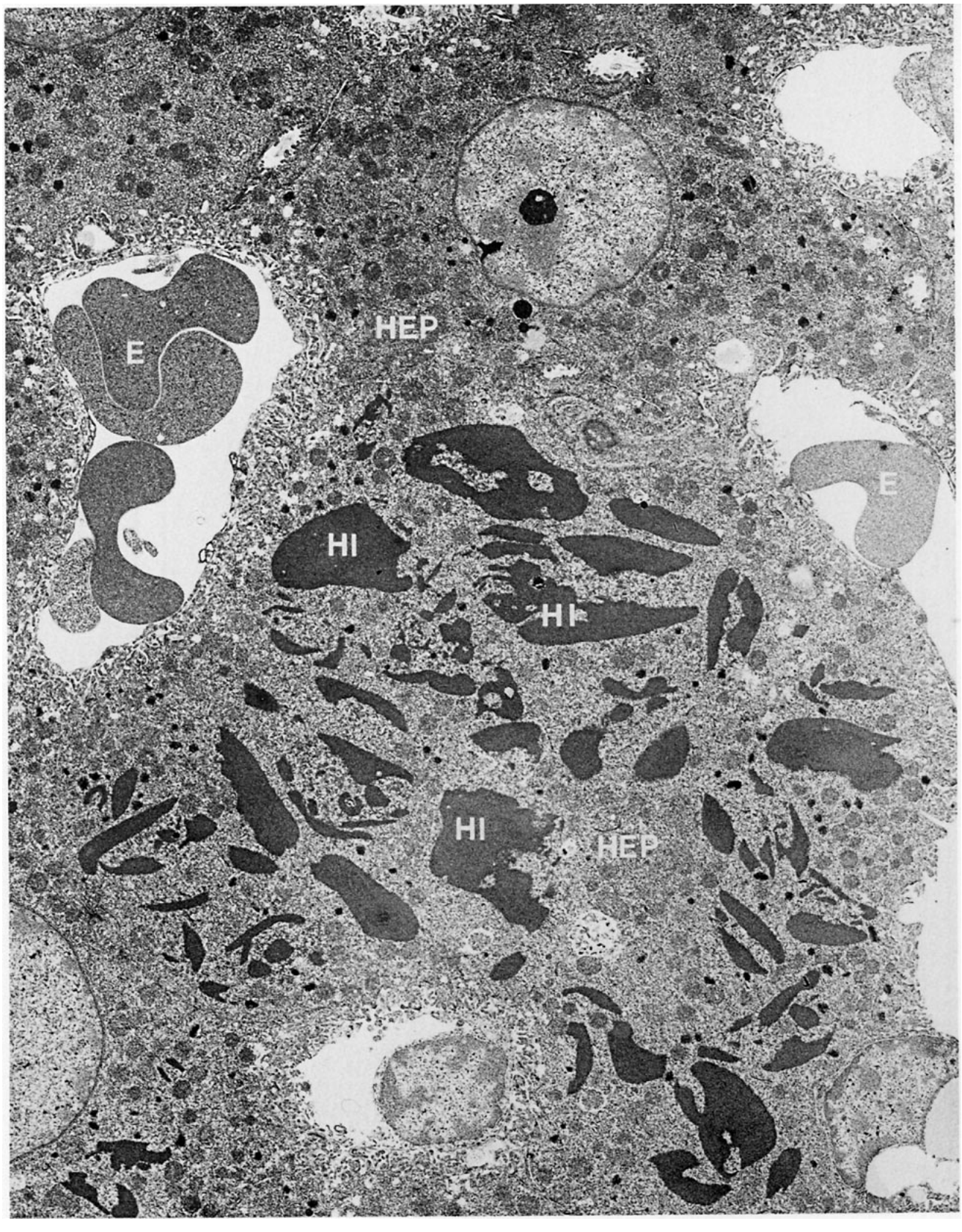


PLATE 3

Liver of a male $B6C3F_1$ mouse given 2,500 ppm ethylene glycol in feed for 2 years. Hepatocyte (HEP) cytoplasm is packed with irregularly-shaped inclusions (HI) corresponding to the eosinophilic material seen by light microscopy (PLATE 1). Note erythrocytes (E) in adjacent capillaries. × 5,400.

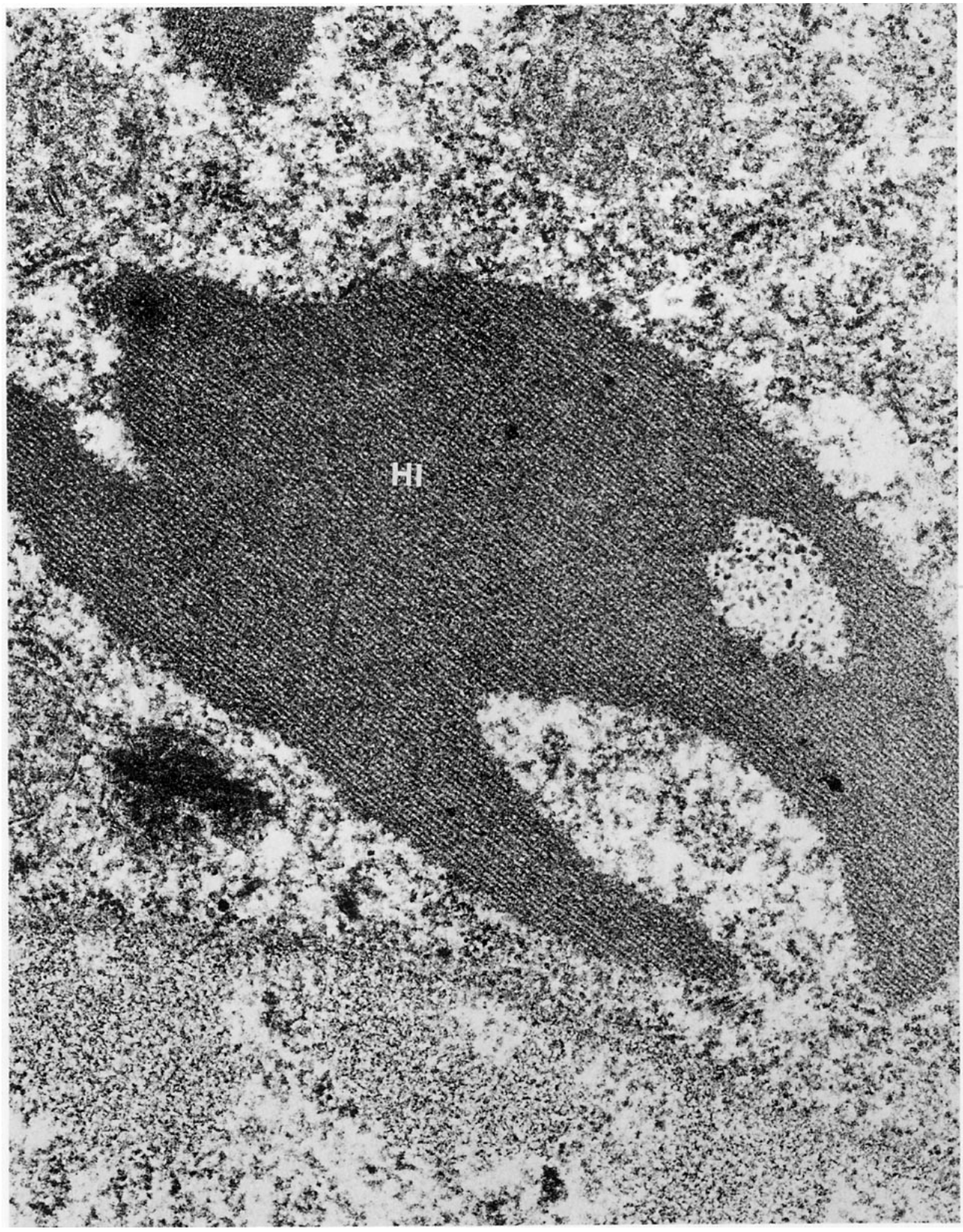


PLATE 4
Hepatocellular inclusion (HI) in the liver of a male $B6C3F_1$ mouse given 2,500 ppm ethylene glycol in feed for 2 years. Note the crystalline arrays of parallel linear structures (8.5 nm to 11 nm) with 11 nm to 13 nm periodicity. x 54,000.

DISCUSSION AND CONCLUSIONS

Ethylene glycol has numerous commercial and industrial applications. It is a component of motor vehicle antifreeze-coolant fluids and many other products. It is also used in the manufacture of polyester fibers, glyoxal, resins, and other substances, and as a solvent, humectant, and softening agent. Ethylene glycol was selected for study because its very large volume production (over five billion pounds in 1989) and easy availability present a high potential for widespread general and workplace exposure and environmental contamination.

Oral administration was chosen to most closely approximate the major route of human exposure. Dosed feed was selected because of concern that with drinking water exposure, polydipsia and/or polyuria resulting from anticipated renal toxicity might affect the amounts of ethylene glycol consumed and excreted.

The present studies were carried out only in B6C3F$_1$ mice because a recent bioassay in F344 rats (DePass *et al.*, 1986a) was considered adequate to evaluate the chronic toxicity and carcinogenicity of ethylene glycol in this species and strain. In that study, male and female F344 rats were administered 0.2, 0.4, or 1.0 g ethylene glycol/kg body weight in feed daily for 2 years. Decreased survival was seen only in high-dose male rats and was attributed to severe renal disease. No treatment-related neoplastic effects were observed in rats.

Repeated-dose studies were not performed because the data available in the literature were considered adequate for dose selection for the 13-week studies. In the 13-week studies, male and female B6C3F$_1$ mice were administered 0, 3,200, 6,300, 12,500, 25,000, or 50,000 ppm ethylene glycol in feed. All animals survived to the end of the study and final mean body weights of exposed mice did not differ significantly from those of the controls. Only male mice in the 25,000 and 50,000 ppm groups exhibited histopathologic lesions (nephropathy and hepatocellular hyaline degeneration).

The renal lesions observed in male mice in the two highest dose groups were considered potentially progressive and life threatening. In other species, males have been shown to be more susceptible than females to ethylene glycol-mediated chronic nephrotoxicity (Morris *et al.*, 1942; Blood, 1965; DePass *et al.*, 1986a). For these reasons, dietary concentrations of ethylene glycol selected for male mice in the 2-year studies were 0, 6,250, 12,500, or 25,000 ppm. Although exposed female mice exhibited no toxic or pathologic effects in the 13-week studies, substitution of more than 50,000 ppm of the diet with a test compound for 2 years might have compromised nutritional adequacy (NCI, 1976; Conner and Newberne, 1984). Therefore, dietary concentrations of ethylene glycol selected for female mice in the 2-year studies were 0, 12,500, 25,000, or 50,000 ppm.

In the 2-year studies, survival of exposed mice and controls was similar (males: 0 ppm, 29/54; 6,250 ppm, 32/54; 12,500 ppm, 32/54; 25,000 ppm, 23/54; females: 0 ppm, 33/50; 12,500 ppm, 30/50; 25,000 ppm, 30/51; 50,000 ppm, 37/50). At 18 months, 65% (35/54) of the high-dose males and 78% (42/54) of the mid-dose males were alive. The survival of control and exposed mice of each sex was considered adequate for evaluation of the carcinogenic potential of ethylene glycol.

Final mean body weights of dosed groups did not vary significantly from those of the controls.

No chemical-related neoplasms were observed in male or female mice in the 15-month interim evaluations or in the 2-year studies. However, chemical-related nonneoplastic lesions occurred in the liver, lung, and/or urinary tract.

Centrilobular hepatocellular hyaline degeneration was first seen in exposed male mice in the 13-week studies and occurred at a high incidence in mid- and high-dose males in the 2-year studies. However, in female mice, a high incidence of hyaline degeneration was seen only in the high-dose group in the 2-year studies, suggesting that female mice might be less susceptible to development of this lesion than males. The lesion was not considered life threatening in either sex.

Hyaline degeneration appeared to be morphologically distinct from other hepatocellular lesions such as "mild fatty metamorphosis" (DePass *et al.*, 1986a), "fatty degeneration" (Morris *et al.*, 1942), or "hydropic degeneration" (Hanzlik *et al.*, 1947) occasionally noted in rats given ethylene glycol. However, changes histologically similar to hyaline degeneration have occasionally been observed in livers of $B6C3F_1$ mice and less commonly in F344 rats (NTP, 1992); the relationship of hyaline degeneration to chemical administration in these cases is uncertain.

Hepatocellular hyaline degeneration was characterized by intracytoplasmic accumulations of granular to globular, nonbirefringent, eosinophilic material with dimensions and tinctorial properties similar to those of erythrocytes. Transmission electron microscopy (TEM) revealed that the eosinophilic material in affected hepatocytes consisted of smoothly contoured to angular intracytoplasmic electron-dense inclusions which resembled the phagocytized erythrocyte fragments seen in Kaposi's sarcoma cells (Schenk, 1986; Kao *et al.*, 1990).

The liver is a major site of ethylene glycol metabolism (Richardson, 1973), and ethylene glycol and its metabolites are known to affect oxidative phosphorylation, glucose metabolism, and other hepatocyte metabolic pathways *in vitro* (Coen and Weiss, 1966; Bachmann and Golberg, 1971; Mannering and Van Harken, 1974; Rajagopal and Ramakrishnan, 1978; Ebel, 1980). Therefore, it would not be entirely unexpected that possible ethylene glycol-mediated effects on the hepatocyte phagocytic potential (Gregory *et al.*, 1991) might result in uptake of normal or crystal-containing erythrocytes.

The hepatocellular crystalline arrays observed in the present studies differed from the amorphous to fine granularity of normal erythrocyte cytoplasm and of intra-erythrocytic hemoglobin crystals (Ghadially, 1982) and Heinz bodies (Christopher *et al.*, 1990). They did not resemble the Mallory bodies seen in alcoholic hepatitis and other liver diseases of humans (Cotran *et al.*, 1989). However, various proteins, including apoferritin and its iron-containing derivative ferritin, are known to undergo crystallization artifactually or under different physiological conditions (Kent and Bahu, 1979; Ghadially, 1982). The inclusions were apparently not membrane-bound, as might be expected for the contents of phagolysosomes. Although inclusions were seen only within hepatocytes, the possibility that the crystalline arrays were formed prior to hepatocellular uptake could not be excluded (Kao *et al.*, 1990). Thus, although some evidence supports an erythrocyte origin, the exact identity of the inclusions, and whether they represent a toxic effect, remains undetermined.

Pulmonary arterial medial hyperplasia occurred with a chemical-related increased incidence in female mice in the 2-year studies. In the present studies, affected mice did not exhibit other vascular changes such as intimal proliferation and fibrosis, elastic membrane reduplication and adventitial fibrosis, which are associated with pulmonary hypertension in humans (Spencer, 1985; Cotran *et al.*, 1989). Additionally, affected mice did not display clinical signs of peripheral or pulmonary hypertensive disease such as dypsnea, cyanosis, and syncope.

Therefore, the pathogenesis of pulmonary arterial medial hyperplasia in the present studies remains undetermined. Important glycoprotein and enzyme components of the major vasopressor-regulating renin-angiotensin system are produced in the liver and lung (Webb and Cockcroft, 1990); treatment-related liver and lung lesions were present in female mice in the 2-year studies. Although direct evidence is lacking, the development of pulmonary arterial hyperplasia might be linked to subtle ethylene glycol-mediated primary or secondary perturbations of the renin-angiotensin system. Alternately, the *in vitro* effects of ethylene glycol on muscle contractile proteins have been well documented (Clarke *et al.*, 1984; Johnson, 1986; Maruyama *et al.*, 1989; Mushtaq and Greene, 1989). However, it is unknown whether pulmonary arterial hyperplasia was related to direct ethylene glycol toxic effects on vascular smooth muscle.

The incidence and severity of nephropathy were not influenced by ethylene glycol administration in either sex. Other treatment-related urinary system lesions occurred only in males. Small numbers of oxalate-like crystals and/or calculi were noted in kidneys, urethras, and/or urinary bladders in some high-dose male mice.

Most of the males with urethral crystals, urinary bladder calculi, or both survived to study termination or were killed moribund late in the 2-year studies (week 84 or later) and generally did not

exhibit severe skin and urogenital lesions of the types associated with fight wound trauma and secondary ascending urinary tract infections. However, most of the mice with renal tubule crystals died or were killed moribund early in the 2-year studies (before week 31). These male mice did exhibit skin or urogenital lesions typically associated with fight wounds (fighting was widespread in all male mice groups until individual housing was instituted after week 54). In these cases, the skin or urogenital lesions were considered severe enough to have caused early death or morbidity. Such pronounced traumatic and inflammatory lesions may have resulted in dehydration, urine stasis, urine pH alterations, and other physiologic derangements which could have favored oxalate precipitation. It is uncertain what role these possible complicating factors may have played in development of detectable oxalate-like deposits. Therefore, it remains undetermined if ethylene glycol administration was directly related to the occurrence of renal tubule crystals.

Although ethylene glycol toxicity has not been investigated extensively in mice, available evidence indicates that mice are less susceptible than other species to ethylene glycol intoxication. In previous studies, renal lesions in mice have been absent, or occurred later and were less severe than those seen in rats receiving similar or lower dietary concentrations of ethylene glycol (Hanzlik *et al.*, 1947; DePass *et al.*, 1986a).

Overall, the results of the present studies are consistent with these earlier reports. The mechanisms responsible for this apparent species difference in toxicity are unknown. However, metabolism and excretion of ethylene glycol is known to vary among species. For example, rats, guinea pigs, chinchilla rabbits, cats, and rhesus monkeys given ethylene glycol excrete varying proportions of the parent compound and such metabolites as oxalate, glycolate, glyoxalate, and hippurate (Gessner *et al.*, 1961; Gessner and Williams, 1961; McChesney *et al.*, 1971; McChesney and Goldberg, 1972). In the present studies, variations in the types or proportions of toxic metabolites peculiar to $B6C3F_1$ mice may have played a pathogenic role. Alternately, absorption of ethylene glycol and disposition of its metabolites may differ in $B6C3F_1$ mice compared to other species and strains.

Similarly, differences in absorption, metabolism, and excretion of ethylene glycol between male and female $B6C3F_1$ mice may have played a role in the sex distribution of several nonneoplastic lesions in the present studies. This hypothesis is supported by previous findings that males and females of the same species can produce different proportions of ethylene glycol metabolites (Richardson, 1965), and that male rats exhibit increased susceptibility to ethylene glycol-associated renal toxicity compared to female rats (Morris *et al.*, 1942; Blood, 1965; DePass *et al.*, 1986a).

It is possible that male mice in the 2-year studies could have tolerated slightly higher doses. If potential nutritional deficiency had not been a factor, female mice in the 2-year studies probably could have tolerated higher doses. However, the doses used in the 2-year studies resulted in increased incidences of treatment-related nonneoplastic lesions in the liver of male mice and in the liver and lung of females. Therefore, the doses employed were considered adequate to evaluate the carcinogenic potential of ethylene glycol.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity*° of ethylene glycol in male $B6C3F_1$ mice receiving 6,250, 12,500, or 25,000 ppm, or in female $B6C3F_1$ mice receiving 12,500, 25,000, or 50,000 ppm. Administration of ethylene glycol resulted in hepatocellular hyaline degeneration in male mice fed diets containing 12,500 or 25,000 ppm and in female mice fed diets containing 50,000 ppm. An increased incidence of medial hyperplasia of small pulmonary arteries and arterioles occurred in female mice fed diets containing 12,500, 25,000, or 50,000 ppm ethylene glycol.

° Explanation of Levels of Evidence of Carcinogenic Activity appears on page 7. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appear on page 9.

REFERENCES

Abbondandolo, A., Bonatti, S., Corsi, C., Corti, G., Fiorio, R., Leporini, C., Mazzaccaro, A., Nieri, R., Barale, R., and Loprieno, N. (1980). The use of organic solvents in mutagenicity testing. *Mutat. Res.* 79, 141-150.

Ameenuddin, S., and Sunde, M.L. (1984). Sensitivity of chick embryo to various solvents used in egg injection studies. *Proc. Soc. Exp. Biol. Med.* 175, 176-178.

American Conference of Governmental Industrial Hygienists (ACGIH) (1990). 1990-1991 Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. p. 21. ACGIH, Cincinnati, OH.

Anderson, B. (1990). Facial-auditory nerve oxalosis. *Am. J. Med.* 88, 87-88. (Letter to the editor.)

Andrews, L.S., and Snyder, R.S. (1986). 3rd ed. Toxic effects of solvents and vapors. In *Casarett and Doull's Toxicology* (C.D. Klaassen, M.O. Amdur, and J. Doull, Eds.), pp. 636-668. Macmillan, New York.

Armitage, P. (1971). *Statistical Methods in Medical Research*, pp. 362-365. John Wiley and Sons, New York.

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity, and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* 257, 229-306.

Bachmann, E., and Golberg, L. (1971). Reappraisal of the toxicology of ethylene glycol. III. Mitochondrial effects. *Food Cosmet. Toxicol.* 9, 39-55.

Barilyak, I.R., and Kozachuk, S.Y. (1985). Mutagenic effect of various alcohols in experiment. *Tsitol. Genet.* 19, 436-442.

Bates, H.K., Price, C.J., George, J.D., Marr, M.C., Kimmell, C.A., Morrissey, R.E., and Schwetz, B.A. (1990). Postnatal effects of *in utero* exposure to ethylene glycol (EG) in the CD® rat. *Toxicologist* 10, 38. (Abstr.)

Baud, F.J., Galliot, M., Astier, A., Bien, D.V., Garnier, R., Likforman, J., and Bismuth, C. (1988). Treatment of ethylene glycol poisoning with intravenous 4-methylpyrazole. *N. Engl. J. Med.* 319, 97-100.

Beckett, S.D., and Shields, R.P. (1971). Treatment of acute ethylene glycol (antifreeze) toxicosis in the dog. *J. Am. Vet. Med. Assoc.* 158, 472-476.

Berman, L.B., Schreiner, G.E., and Feys, J. (1957). The nephrotoxic lesion of ethylene glycol. *Ann. Intern. Med.* 46, 611-619.

Blood, F.R. (1965). Chronic toxicity of ethylene glycol in the rat. *Food Cosmet. Toxicol.* 3, 229-234.

Blood, F.R., Elliott, G.A., and Wright, M.S. (1962). Chronic toxicity of ethylene glycol in the monkey. *Toxicol. Appl. Pharmacol.* 4, 489-491.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Borden, T.A., and Bidwell, C.D. (1968). Treatment of acute ethylene glycol poisoning in rats. *Invest. Urol.* 6, 205-210.

Bornmann, G. (1954). Grundwirkungen der Glykole und ihre Bedeutung für die Toxizität. *Arzneimittelforschung* 4, 643-646.

Bove, K.E. (1966). Ethylene glycol toxicity. *Am. J. Clin. Pathol.* 45, 46-50.

Bowen, D.A.L., Minty, P.S.B., and Sengupta, A. (1978). Two fatal cases of ethylene glycol poisoning. *Med. Sci. Law* **18**, 102-107.

Brown, C.G., Trumbull, D., Klein-Schwartz, W., and Walker, J.D. (1983). Ethylene glycol poisoning. *Ann. Emerg. Med.* **12**, 501-506.

Burkhart, K.K., and Kulig, K.W. (1990). The other alcohols: methanol, ethylene glycol, and isopropanol. *Emerg. Med. Clin. North Am.* **8**, 913-928.

Child, J., and Willetts, A. (1978). Microbial metabolism of aliphatic glycols: bacterial metabolism of ethylene glycol. *Biochim. Biophys. Acta* **538**, 316-327.

Chou, J.Y., and Richardson, K.E. (1978). The effect of pyrazole on ethylene glycol toxicity and metabolism in the rat. *Toxicol. Appl. Pharmacol.* **43**, 33-44.

Christian, K.L., and Moorehead, W.P. (1985). Ethylene dichloride/ethylene glycol spill in a major water resource in British Columbia. *J. Environ. Health* **47**, 192-196.

Christopher, M.M., White, J.G., Eaton, J.W. (1990). Erythrocyte pathology of Heinz body-mediated hemolysis of cats. *Vet. Pathol.* **27**, 299-310.

Cieciura, L., Kidawa, Z., Orkisz, S., and Trznadel, K. (1983). Ultrastructural appearances of nephron damage in acute poisoning with ethylene glycol. *Proc. Eur. Dial. Transplant Assoc.* **20**, 636-640.

Clark, C.R., Marshall, T.C., Merickel, B.S., Sanchez, A., Brownstein, D.G., and Hobbs, C.H. (1979). Toxicological assessment of heat transfer fluids proposed for use in solar energy applications. *Toxicol. Appl. Pharmacol.* **51**, 529-535.

Clarke, M.L., Rodger, C.D., and Tregear, R.T. (1984). Modification of crossbridge states by ethylene glycol in insect flight muscle. *J. Muscle Res. Cell Motil.* **5**, 81-96.

Clay, K.L., and Murphy, R.C. (1977). On the metabolic acidosis of ethylene glycol intoxication. *Toxicol. Appl. Pharmacol.* **39**, 39-49.

Code of Federal Regulations (CFR) **21**, Part 58.

Coen, G., and Weiss, B. (1966). Oxidation of ethylene glycol to glycolaldehyde by mammalian tissues. *Enzymol. Biol. Clin. (Basel)* **6**, 288-296.

Compendium of Safety Data Sheets for Research and Industrial Chemicals (CSDSRIC) Part II (1985). (L.H. Keith and D.B. Walters, Eds.), pp. 840-841. VCH Publishers, Deerfield Beach, FL.

Conner, M.W., and Newberne, P.M. (1984). Drug-nutrient interactions and their implications for safety evaluation. *Fundam. Appl. Toxicol.* **4**, 5,341-5,356.

Coon, R.A., Jones, R.A., Jenkins, L.J., Jr., and Siegel, J. (1970). Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol. *Toxicol. Appl. Pharmacol.* **16**, 646-655.

Cotran, R.S., Kumar, V., and Robbins, S.L., (1989). 4th ed. *Robbins Pathologic Basis of Disease*, pp. 764-765, 945-946. W.B. Saunders Co., Philadelphia.

Cox, D.R. (1972). Regression models and life tables. *J. R. Stat. Soc.* **B34**, 187-220.

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology*. (W.G. Flamm and R.J. Lorentzen, Eds.), pp. 13-59. Princeton Scientific, Princeton, NJ.

DePass, L.R., Garman, R.H., Woodside, M.D., Giddens, W.E., Maronpot, R.R., and Weil, C.S. (1986a). Chronic toxicity and oncogenicity studies of ethylene glycol in rats and mice. *Fundam. Appl. Toxicol.* **7**, 547-565.

DePass, L.R., Woodside, M.D., Maronpot, R.R., and Weil, C.S. (1986b). Three-generation reproduction and dominant lethal mutagenesis studies of ethylene glycol in the rat. *Fundam. Appl. Toxicol.* **7**, 566-572.

Dial, S.M., Thrall, M.A., and Hamar, D.W. (1989). 4-Methylpyrazole as treatment for naturally acquired ethylene glycol intoxication in dogs. *J. Am. Vet. Med. Assoc.* **195**, 73-76.

Dinse, G.E., and Haseman, J.K. (1986). Logistic regression analysis of incidental-tumor data from animal carcinogenicity experiments. *Fundam. Appl. Toxicol.* **6**, 44-52.

Dinse, G.E., and Lagakos, S.W. (1983). Regression analysis of tumor prevalence data. *Appl. Statist.* 32, 236-248.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* 50, 1,095-1,121.

Dwyer, D.F., and Tiedje, J.M. (1983). Degradation of ethylene glycol and polyethylene glycols by methanogenic consortia. *Appl. Environ. Microbiol.* 46, 185-190.

Ebel, R.E. (1980). Perturbation of the hepatic microsomal monooxygenase system by ethylene glycol. *Fed. Proc.* 39, 948. (Abstr.)

Evans, W.H., and David, E.J. (1974). Biodegradation of mono-, di-, and triethylene glycols in river waters under controlled laboratory conditions. *Water Research* 8, 97-100.

Factor, S.A., and Lava, N.S. (1987). Ethylene glycol intoxication: a new stage in the clinical syndrome. *N.Y. State J. Med.* 87, 179-180.

Federal Register (1989). 54, 2,462.

Fowler, B.A., Kardish, R.M., and Woods, J.S. (1983). Alteration of hepatic microsomal structure and function by indium chloride. *Lab. Invest.* 48, 471-478.

Freitag, D., Ballhorn, L., Geyer, H., and Korte, F. (1985). Environmental hazard profile of organic chemicals: an experimental method for the assessment of the behaviour of organic chemicals in the ecosphere by means of simple laboratory tests with ^{14}C labelled chemicals. *Chemosphere* 14, 1,589-1,616.

Gabow, P.A. (1988). Ethylene glycol intoxication. *Am. J. Kidney Dis.* 11, 277-279.

Gabow, P.A., Clay, K., Sullivan, J.B., and Lepoff, R. (1986). Organic acids in ethylene glycol intoxication. *Ann. Intern. Med.* 105, 16-20.

Galloway, S.M., Bloom, A.D., Resnick, M., Margolin, B.H., Nakamura, F., Archer, P., and Zeiger, E. (1985). Development of a standard protocol for *in vitro* cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. *Environ. Mutagen.* 7, 1-51.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.* 10 (Suppl. 10), 1-175.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *J. Natl. Cancer Inst.* 62, 957-974.

Gebhardt, D.O.E. (1968). The teratogenic action of propylene glycol (propanediol-1,2) and propanediol-1,3 in the chick embryo. *Teratology* 1, 153-162.

Gershoff, S.N., and Andrus, S.B. (1962). Effect of vitamin B_6 and magnesium on renal deposition of calcium oxalate induced by ethylene glycol administration. *Proc. Soc. Exp. Biol. Med.* 109, 99-102.

Gessner, P.K., and Williams, R.T. (1961). The metabolism of ^{14}C-ethanediol (ethylene glycol). *Pure Appl. Chem.* 3, 281-283.

Gessner, P.K., Parke, D.V., and Williams, R.T. (1960). Studies in detoxication 80. The metabolism of glycols. *Biochem. J.* 74, 1-5.

Gessner, P.K., Parke, D.V., and Williams, R.T. (1961). Studies in detoxication 86. The metabolism of ^{14}C-labelled ethylene glycol. *Biochem. J.* 79, 482-489.

Ghadially, F.N. (1982). 2nd ed. *Ultrastructural Pathology of the Cell and Matrix*, p. 720. Butterworths, London.

Grafton, T.F., and Hansen, D.K. (1987). *In vitro* embryotoxic effects of ethylene glycol in rats. *Teratogenesis Carcinog. Mutagen.* **7**, 483-489.

Grant, W.M. (1974). 2nd ed. *Toxicology of the Eye.* p. 475-478. C.C. Thomas, Springfield, IL.

Grauer, G.F., and Thrall, M.A. (1982). Ethylene glycol (antifreeze) poisoning in the dog and cat. *J. Am. Animal Hospital Assoc.* **18**, 492-497.

Gregory, S., Barczynski, L., and Wing, E. (1991). Hepatocyte-mediated clearance of bacteria from the bloodstream. *Fed. Am. Soc. Exp. Biol.* **5**, A1095. (Abstr.)

Griffiths, A.J.F. (1979). *Neurospora* prototroph selection system for studying aneuploid production. *Environ. Health Perspect.* **31**, 75-80.

Griffiths, A.J.F. (1981). *Neurospora* and environmentally induced aneuploidy. In *Progress in Mutation Research*: Evaluation of short-term tests for carcinogens (F.J. Serres and J. Ashby, Eds.) Vol. 1, pp. 187-199. Elsevier North Holland, Inc., New York.

Handbook of Environmental Data on Organic Chemicals (1977). 2nd ed. (K. Verschueren, Ed.), p. 322-323. Van Nostrand Rheinhold Company, New York.

Hanzlik, P.J., Seidenfeld, M.A., and Johnson, C.C. (1931). General properties, irritant and toxic actions of ethylene glycol. *J. Pharmacol. Exp. Ther.* **41**, 387-406.

Hanzlik, P.J., Lawrence, W.S., and Laqueur, G.L. (1947). Comparative chronic toxicity of diethylene glycol monoethyl ether (carbitol) and some related glycols: results of continued drinking and feeding. *J. Ind. Hyg. Toxicol.* **29**, 233-241.

Haseman, J.K. (1984). Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ. Health Perspect.* **58**, 385-392.

Haseman, J.K., Huff, J., and Boorman, G.A. (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.* **12**, 126-135.

Haseman, J.K., Huff, J.E., Rao, G.N., Arnold, J.E., Boorman, G.A., and McConnell, E.E. (1985). Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N x C3H/HeN)F_1 (B6C3F_1) mice. *JNCI* **75**, 975-984.

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., and Zeiger, E. (1983). *Salmonella* mutagenicity test results for 250 chemicals. *Environ. Mutagen.* **5** (Suppl. 1), 3-142.

Hazardous Chemicals Data Book (1986). (G. Weiss, Ed.), p. 499. Noyes Data Corporation, Park Ridge, NJ.

Hewlett, T.P., Jacobsen, D., Collins, T.D., and McMartin, K.E. (1989). Ethylene glycol and glycolate kinetics in rats and dogs. *Vet. Hum. Toxicol.* **31**, 116-120.

Holck, H.G.O. (1937). Glycerin, ethylene glycol, propylene glycol and diethylene glycol: Report on feeding experiments in rats. *J. Am. Med. Assoc.* **109**, 1517-1520.

Hong, H.L., Canipe, J., Jameson, C.W., and Boorman, G.A. (1988). Comparative effects of ethylene glycol and ethylene glycol monomethyl ether exposure on hematopoiesis and histopathology in B6C3F_1 mice. *J. Environ. Pathol. Toxicol. Oncol.* **8**, 27-38.

Jacobsen, D., and McMartin, K.E. (1986). Methanol and ethylene glycol poisonings: Mechanism of toxicity, clinical course, diagnosis and treatment. *Med. Toxicol.* **1**, 309-334.

Jacobsen, D., Hewlett, T.P., Webb, R., Brown, S.T., Ordinario, A.T., and McMartin, K.E. (1988). Ethylene glycol intoxication: Evaluation of kinetics and crystalluria. *Am. J. Med.* **84**, 145-152.

Johnson, R.E. (1986). Effect of ethylene glycol and Ca^{2+} on the binding of Mg^{2+} · adenyl-5′-yl imidodiphosphate to rabbit skeletal myofibrils. *J. Biol. Chem.* **261**, 728-732.

Jonckheere, A.R. (1954). A distribution-free k-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kabarity, A., El-Bayoumi, A., and Habib, A. (1980). C-Tumors and polyploidy induced by some alkaloids of opium and cannabis. *Cytologia* (Tokyo) 45, 497-506.

Kao, G.F., Johnson, F.B., Sulica, V.I. (1990). The nature of hyaline (eosinophilic) globules and vascular slits in Kaposi's sarcoma. *Am. J. Dermatopathol.* 12, 256-267.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation of incomplete observations. *J. Am. Stat. Assoc.* 53, 457-481.

Kent, G., and Bahu, R.M. (1979). Iron overload. In *Pathology of the Liver* (R.N.M. MacSween, P.P. Anthony, and P.J. Scheuer, Eds.), pp. 148-163. Churchill Livingstone, Edinburgh.

Kersting, E.J., and Nielsen, S.W. (1966). Experimental ethylene glycol poisoning in the dog. *Am. J. Vet. Res.* 27, 574-582.

Khera, K.S. (1990). Ethyleneglycol-induced maternal acidosis and hyperosmolality: A possible cause of fetal malformations in rats. *Toxicologist* 10, 224. (Abstr.)

Kiefer, D.M. (1989). Competition heats up as demand for chemicals cools. *Chem. Eng. News* 67, 24-26.

Kirk-Othmer Encyclopedia of Chemical Technology (1982). 3rd ed., Vol. 11, pp. 933-945. John Wiley and Sons, New York.

LaChance, L.E., and Leverich, A.P. (1969). Chemosterilant studies on *Bracon* sperm. II. Studies of selected compounds for induction of dominant lethal mutations or sperm inactivation. *Ann. Entomol. Soc. Am.* 62, 790-796.

Lamb, J.C., Maronpot, R.R., Gulati, D.K., Russell, V.S., Hommel, L., and Sabharwal, P.S. (1985). Reproductive and developmental toxicity of ethylene glycol in the mouse. *Toxicol. Appl. Pharmacol.* 81, 100-112.

Latven, A.R., and Molitor, H. (1939). Comparison of the toxic, hypnotic and irritating properties of eight organic solvents. *J. Pharmocol. Exp. Ther.* 65, 89-94.

Laug, E.P., Calvery, H.O., Morris, H.J., and Woodard, G. (1939). The toxicology of some glycols and derivatives. *J. Ind. Hyg. Toxicol.* 21, 173-201.

Lenk, W., Löhr, D., and Sonnenbichler, J. (1989). Pharmacokinetics and biotransformation of diethylene glycol and ethylene glycol in the rat. *Xenobiotica* 19, 961-979.

Løkke, H. (1984). Leaching of ethylene glycol and ethanol in subsoils. *Water Air Soil Pollut.* 22, 373-387.

Longzhan, Y., Zheng, L., Lihua, S., and Kemin, B. (1989). Teratotogenic and genotoxic effects of ethylene glycol (EG). *Environ. Mol. Mutagen.* 14, 119. (Abstr.)

Lyon, E.S., Borden, T.A., and Vermeulen, C.W. (1966). Experimental oxalate lithiasis produced with ethylene glycol. *Invest. Urol.* 4, 143-151.

Maguire, M.P. (1974). Chemically induced abnormal chromosome behavior at meiosis in maize. *Chromosoma* 48, 213-223.

Mannering, G.J., and Van Harken, D. (1974). Stimulation of peroxidative methanol oxidation by ethylene glycol and its metabolites in the intact rat, perfused liver and liver homogenate. In *Alcohol and Aldehyde Metabolizing Systems* (R.G. Thurman, T. Yonetani, J.R. Williamson, and B. Chance, Eds.), p. 532. Academic Press, New York.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* 10, 71-80.

Maronpot, R.R., Zelenak, J.P., Weaver, E.V., and Smith, N.J. (1983). Teratogenicity study of ethylene glycol in rats. *Drug Chem. Toxicol.* 6, 579-594.

Marr, M.C., Price, C.J., Myers, C.B., Morrissey, R.E., and Schwetz, B.A. (1990). Developmental stages of the CD® rat skeleton after maternal exposure to ethylene glycol. *Teratology* 41, 576. (Abstr.)

Marshall, D.A., and Doty, R.L. (1990). Taste responses of dogs to ethylene glycol, propylene glycol, and ethylene glycol-based antifreeze. *J. Am. Vet. Med. Assoc.* 197, 1599-1602.

Marshall, T.C. (1982). Dose-dependent disposition of ethylene glycol in the rat after intravenous administration. *J. Toxicol. Environ. Health* **10**, 397-409.

Marshall, T.C., and Cheng, Y.S. (1983). Deposition and fate of inhaled ethylene glycol vapor and condensation aerosol in the rat. *Fundam. Appl. Toxicol.* **3**, 175-181.

Marshall, T.C., Clark, C.R., Brewster, D.W., and Henderson, T.R. (1981). Toxicological assessment of heat transfer fluids proposed for use in solar energy applications, II. *Toxicol. Appl. Pharmacol.* **58**, 31-38.

Martis, L., Kroes, R., Darby, T.D., and Woods, E.F. (1982). Disposition kinetics of ethylene oxide, ethylene glycol, and 2-chlorethanol in the dog. *J. Toxicol. Environ. Health* **10**, 847-856.

Maruyama, T., Kometani, K., and Yamada, K. (1989). Modification of the contractile properties of rabbit skeletal muscle by ethylene glycol. *J. Biochem.* (Tokyo) **105**, 1009-1013.

Mason, M.M., Cate, C.C., and Baker, J. (1971). Toxicology and carcinogenesis of various chemicals used in the preparation of vaccines. *Clin. Toxicol.* **4**, 185-204.

McCann, J., Choi, E., Yamasaki, E., and Ames, B.N. (1975). Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals. *Proc. Natl. Acad. Sci. USA* **72**, 5135-5139.

McCarroll, N.E., Piper, C.E., and Keech, B.H. (1981). An *E. coli* microsuspension assay for the detection of DNA damage induced by direct-acting agents and promutagens. *Environ. Mutagen.* **3**, 429-444.

McChesney, E.W., and Golberg, L. (1972). Reappraisal of the toxicology of ethylene glycol. IV. The metabolism of labelled glycollic and glyoxylic acids in the Rhesus monkey. *Food Cosmet. Toxicol.* **10**, 655-670.

McChesney, E.W., Golberg, L., Parekh, C.K., Russell, J.C., and Min, B.H. (1971). Reappraisal of the toxicology of ethylene glycol. II. Metabolism studies in laboratory animals. *Food Cosmet. Toxicol.* **9**, 21-38.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

McDonald, T.O., Roberts, M.D., and Borgmann, A.R. (1972). Ocular toxicity of ethylene chlorohydrin and ethylene glycol in rabbit eyes. *Toxicol. Appl. Pharmacol.* **21**, 143-150.

McDonald, T.O., Kasten, K., Hervey, R., Gregg, S., Borgmann, A.R., and Murchison, T. (1973). Acute ocular toxicity of ethylene oxide, ethylene glycol, and ethylene chlorohydrin. *Bull. Parenter. Drug Assoc.* **27**, 153-164.

McDonald, T.O., Kasten, K., Hervey, R., Gregg, S., and Britton, B. (1977). Acute ocular toxicity for normal and irritated rabbit eyes and subacute ocular toxicity for ethylene oxide, ethylene chlorohydrin, and ethylene glycol. *Bull. Parenter. Drug Assoc.* **31**, 25-32.

McGregor, D.B., Brown, A.G., Howgate, S., McBride, D., Riach, C., and Caspary, W.J. (1991). Responses of the L5178Y mouse lymphoma cell forward mutation assay. V. 27 coded chemicals. *Environ. Mol. Mutagen.* **17**, 196-219.

McKnight, B., and Crowley, J. (1984). Tests for differences in tumor incidence based on animal carcinogenesis experiments. *J. Am. Stat. Assoc.* **79**, 639-648.

Melnick, R.L. (1984). Toxicities of ethylene glycol and ethylene glycol monoethyl ether in Fischer 344/N rats and B6C3F$_1$ mice. *Environ. Health Perspect.* **57**, 147-155.

The Merck Index (1989). 11th ed. (S. Budavari, Ed.), p. 599. Merck and Company, Rahway, NJ.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer*. (H.H. Yiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-628. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Morbidity and Mortality Weekly (1987). Ethylene glycol intoxication due to contamination of water systems. 36, 611-614.

Moriarty, R.W., and McDonald, R.H., Jr. (1974). The spectrum of ethylene glycol poisoning. *Clin. Toxicol.* 7, 583-596.

Morris, H.J., Nelson, A.A., and Calvery, H.O. (1942). Observations on the chronic toxicities of propylene glycol, ethylene glycol, diethylene glycol, ethylene glycol mono-ethyl-ether and diethylene glycol mono-ethyl-ether. *J. Pharmacol. Exp. Ther.* 74, 266-273.

Mushtaq, E., and Greene, L.E. (1989). Effect of ethylene glycol on the interaction of different myosin subfragment 1 · nucleotide complexes with actin. *Biochemistry* 28, 6,478-6,482.

Myers, C.B., Marr, M.C., and Sleet, R.B. (1988). A comparison of developmental toxicity in Fischer 344 (F-344) and CD rats exposed to ethylene glycol (EG). *Teratology* 37, 479. (Abstr.)

National Cancer Institute (NCI) (1976). Guidelines for Carcinogen Bioassay in Small Rodents. Technical Report Series No. 1. NIH Publication No. 76-801. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Institute for Occupational Safety and Health (NIOSH) (1990). National Occupational Exposure Survey (NOES) (1981-1983), unpublished provisional data as of 1 July 1990.

National Institutes of Health (NIH) (1978). Open Formula Rat and Mouse Ration (NIH-07). NIH Publication No. 11-1335. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

NPIRI *Raw Materials Data Handbook* (1974). (J.M. Fetsko, Ed.). Organic Solvents. Vol. 1, p. 49. National Printing Ink Research Institute, Bethlehem, PA.

National Toxicology Program (NTP) (1984). Teratologic Evaluation of Ethylene Glycol (CAS No. 107-21-1) Administered to CD Rats on Gestational Days 6 Through 15. NTP-84-159. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

National Toxicology Program (NTP) (1988). Developmental Toxicity Evaluation of Ethylene Glycol (CAS No. 107-21-1) in CD Rats. NTP-88-079. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

National Toxicology Program (NTP) (1990). Developmental Toxicity Evaluation of Ethylene Glycol (CAS No. 107-21-1) Administered to New Zealand White Rabbits by Gavage on Gestational Days 6 Through 19. NTP-89-CTER-129. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

National Toxicology Program (NTP) (1992). Toxicology and Carcinogenesis Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (CAS No. 7336-20-1) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). Technical Report Series No. 412. NIH Publication No. 92-3143. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

Oya, Y., Yamamoto, K., and Tonomura, A. (1986). The biological activity of hydrogen peroxide: I. Induction of chromosome-type aberrations susceptible to inhibition by scavengers of hydroxyl radicals in human embryonic fibroblasts. *Mutat. Res.* 172, 245-253.

Parry, M.F., and Wallach, R. (1974). Ethylene glycol poisoning. *Am. J. Med.* 57, 143-150.

Patty's Industrial Hygiene and Toxicology (1982). 3rd ed. (G.D. Clayton and F.E. Clayton, Eds.), Vol. 2C, pp. 3817-3908. John Wiley and Sons, New York.

Pfeiffer, E.H., and Dunkelberg, H. (1980). Mutagenicity of ethylene oxide and propylene oxide and of the glycols and halohydrins formed from them during the fumigation of foodstuffs. *Food Cosmet. Toxicol.* **18**, 115-118.

Pitter, P. (1976). Determination of biological degradability of organic substances. *Water Res.* **10**, 231-235.

Price, C.J., Kimmel, C.A., Tyl, R.W., and Marr, M.C. (1985). The developmental toxicity of ethylene glycol in rats and mice. *Toxicol. Appl. Pharmacol.* **81**, 113-127.

Rajagopal, G., and Ramakrishnan, S. (1978). Effect of ethylene glycol toxicity on hepatic carbohydrate metabolism in rats. *Toxicol. Appl. Pharmacol.* **46**, 507-515.

Reviews of Environmental Contaminants and Toxicology (1988). *Ethylene glycol* (G.W. Ware, Ed.), Vol. 106, pp. 133-141.

Richardson, K.E. (1965). Endogenous oxalate synthesis in male and female rats. *Toxicol. Appl. Pharmacol.* **7**, 507-515.

Richardson, K.E. (1973). The effect of partial hepatectomy on the toxicity of ethylene glycol, glycolic acid, glyoxylic acid and glycine. *Toxicol. Appl. Pharmacol.* **24**, 530-538.

Riley, J.H., Stahr, H.M., O'Brien, S., and Riley, M.G.I. (1982). Urine and tissue oxalate and hippurate levels in ethylene glycol intoxication in the dog. *Vet. Hum. Toxicol.* **24**, 331-334.

Roberts, J.A., and Seibold, H.R. (1969). Ethylene glycol toxicity in the monkey. *Toxicol. Appl. Pharmacol.* **15**, 624-631.

Robinson, M., Pond, C.L., Laurie, R.D., Bercz, J.P., Henningsen, G., and Condie, L.W. (1990). Subacute and subchronic toxicity of ethylene glycol administered in drinking water to Sprague-Dawley rats. *Drug Chem. Toxicol.* **13**, 43-70.

Rofe, A.M., Bais, R., and Conyers, R.A.J. (1986). The effect of dietary refined sugars and sugar alcohols on renal calcium oxalate deposition in ethylene glycol-treated rats. *Food Chem. Toxicol.* **24**, 397-403.

Rossa, V., and Weber, U. (1990). Effect of ethylene glycol on rabbit retinas. *Ophthalmologica* **200**, 98-103.

Rushton, H.G., Spector, M., Rodgers, A.L., Hughson, M., and Magura, C.E. (1981). Developmental aspects of calcium oxalate tubular deposits and calculi induced in rat kidneys. *Invest. Urol.* **19**, 52-57.

Sadtler Standard Spectra. IR No. 2166 and NMR No. 6406M. Sadtler Research Laboratories, Philadelphia.

Sanyer, J.L., Oehme, F.W., and McGavin, M.D. (1973). Systematic treatment of ethylene glycol toxicosis in dogs. *J. Am. Vet. Med. Assoc.* **34**, 527-534.

Schenk, P. (1986). Erythrophagocytosis in Kaposi's sarcoma in acquired immunodeficiency syndrome. *J. Oto-Rhino-Laryngol Related Specialties* **48**, 167-173.

Schuler, R.L., Hardin, B.D., Niemeier, R.W., Booth, G., Hazelden, K., Piccirillo, V., and Smith, K. (1984). Results of testing fifteen glycol ethers in a short-term *in vivo* reproductive toxicity assay. *Environ. Health Perspect.* **57**, 141-146.

Silbergeld, S., and Carter, H.E. (1959). Toxicity of glycolic acid in male and female rats. *Arch. Biochem. Biophys.* **84**, 183-187.

Smith, B.J., Anderson, B.G., Smith, S.A., and Chew, D.J. (1990). Early effects of ethylene glycol on the ultrastructure of the renal cortex in dogs. *J. Am. Vet. Med. Assoc.* **51**, 89-96.

Smyth, H.F., Jr., Seaton, J., and Fischer, L. (1941). The single dose toxicity of some glycols and derivatives. *J. Ind. Hyg. Toxicol.* **23**, 259-268.

Spencer, H. (1985). 4th ed. Chronic pulmonary hypertension. In *Pathology of the Lung*, Vol. 2, pp. 631-704. Pergamon Press, Oxford.

Straus, D.S. (1981). Somatic mutation, cellular differentiation, and cancer causation. *JNCI* 67, 233.

Szablowska, M., and Selye, H. (1971). Hormonal influences upon ethylene glycol poisoning. *Arch. Environ. Health* 23, 13-17.

Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* 62, 679-682.

Tennant, R.W., and Ashby, J. (1991). Classification according to chemical structure, mutagenicity to *Salmonella* and level of carcinogenicity of a further 39 chemicals tested for carcinogenicity by the U.S. National Toxicology Program. *Mutat. Res.* 257, 209-227.

Tennant, R.W., Margolin, B.H., Shelby, M.D. Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. *Science* 236, 933-941.

Tyl, R.W., Ballantyne, B., Fisher, L.C., Fait, D.L., Klonne, D.R., Pritts, I.M., and Dodd, D.E. (1988a). Developmental toxicity evaluation of ethylene glycol (EG) aerosol by nose-only (NO) or whole-body (WB) exposure in CD®-1 mice. *Toxicologist* 8, 242. (Abstr.)

Tyl, R.W., Fisher, L.C., Kubena, M.F., Vrbanic, M.A., and Losco, P.E. (1988b). Developmental toxicity evaluation of ethylene glycol (EG) applied cutaneously to CD®-1 mice. *Teratology* 37, 498. (Abstr.)

Tyl, R.W., Ballantyne, B., Fisher, L.C., Fait, D.L., Savine, T.A., Klonne, D.R., Pritts, I.M., and Dodd, D.E. (1989a). Developmental toxicity of ethylene glycol (EG) aerosol by whole-body exposure in CD rats and CD-1 mice. *Toxicologist* 9, 270. (Abstr.)

Tyl, R.W., Fisher, L.C., Kubena, M.F., Losco, P.E., and Vrbanic, M.A. (1989b). Determination of a developmental toxicity "no observable effect level" (NOEL) for ethylene glycol (EG) by gavage in CD-1 mice. *Teratology* 39, 487. (Abstr.)

Webb, D.J., and Cockcroft, J.R. (1990). Circulating and tissue renin-angiotensin systems: The role of the endothelium. In *The Endothelium: An Introduction to Current Research* (J.B. Warren, Ed.), pp. 65-80. Wiley-Liss, Inc., New York.

Wiley, F.H., Hueper, W.C., Bergen, D.S., and Blood, F.R. (1938a). The formation of oxalic acid from ethylene glycol and related solvents. *J. Ind. Hyg. Toxicol.* 20, 269-277.

Wiley, F.H., Hueper, W.C., and von Oettingen, W.F. (1938b). The toxicity and potential dangers of ethylene glycol. *J. Ind. Hyg. Toxicol.* 18, 123-126.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* 27, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* 28, 519-531.

Wills, J.H., Coulston, F., Harris, E.S., McChesney, E.W., Russell, J.C., and Serrone, D.M. (1974). Inhalation of aerosolized ethylene glycol by man. *Clin. Toxicol.* 7, 463-476.

Winek, C.L., Shingleton, D.P., and Shanor, S.P. (1978). Ethylene and diethylene glycol toxicity. *Clin. Toxicol.* 13, 297-324.

Yamaguchi, T., and Nakagawa, K. (1983). Mutagenicity of and formation of oxygen radicals by trioses and glyoxal derivatives. *Agric. Biol. Chem.* 47, 2461-2465.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., and Speck, W. (1987). *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. *Environ. Mutagen.* 9 (Suppl. 9), 1-109.

Zeiger, E., Haseman, J.K., Shelby, M.D. Margolin, B.H., and Tennant, R.W. (1990). Evaluation of four *in vitro* genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. *Environ. Mol. Mutagen.* 16 (Suppl. 18), 1-14.

APPENDIX A
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR FEED STUDY OF ETHYLENE GLYCOL

TABLE A1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Ethylene Glycol[a]

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-month interim evaluation	6	6	6	6
Early deaths				
Natural deaths	15	8	11	16
Moribund	10	14	11	15
Survivors				
Terminal sacrifice	29	32	32	23
Animals examined microscopically	54	54	54	54
Alimentary System				
Gallbladder	(51)	(19)	(16)	(48)
Sarcoma	1 (2%)			
Intestine small, duodenum	(53)	(20)	(18)	(46)
Polyp adenomatous		1 (5%)		
Intestine small, ileum	(53)	(17)	(17)	(45)
Intestine small, jejunum	(54)	(18)	(19)	(50)
Polyp adenomatous	2 (4%)			1 (2%)
Liver	(54)	(53)	(53)	(54)
Cholangiocarcinoma	1 (2%)			
Hemangiosarcoma, multiple, two	1 (2%)			
Hepatocellular carcinoma	10 (19%)	15 (28%)	10 (19%)	9 (17%)
Hepatocellular carcinoma, multiple, two		1 (2%)	2 (4%)	
Hepatocellular carcinoma, multiple, four		1 (2%)		
Hepatocellular adenoma	9 (17%)	6 (11%)	5 (9%)	8 (15%)
Hepatocellular adenoma, multiple, two				2 (4%)
Serosa, sarcoma	1 (2%)			
Mesentery	(5)	(3)	(1)	(7)
Cholangiocarcinoma, metastatic, liver	1 (20%)			
Hemangiosarcoma	1 (20%)			
Hepatocellular carcinoma, metastatic, liver		1 (33%)		
Sarcoma	1 (20%)			
Pancreas	(54)	(23)	(21)	(54)
Salivary glands	(53)	(21)	(20)	(54)
Stomach, forestomach	(54)	(20)	(21)	(54)
Mast cell neoplasm benign	1 (2%)			
Stomach, glandular	(54)	(20)	(21)	(54)
Tooth	(21)	(4)	(1)	(18)
Cardiovascular System				
Heart	(54)	(21)	(22)	(54)
Adenocarcinoma, metastatic, uncertain primary site	1 (2%)			
Endocrine System				
Adrenal gland, cortex	(54)	(20)	(21)	(53)
Adenoma	2 (4%)			
Capsule, cholangiocarcinoma, metastatic, liver	1 (2%)			
Capsule, sarcoma	1 (2%)			
Subcapsular, adenoma	1 (2%)			3 (6%)
Subcapsular, adenoma, multiple	1 (2%)			

TABLE A1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Ethylene Glycol
(continued)

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Endocrine System (continued)				
Adrenal gland, medulla	(54)	(21)	(21)	(53)
Pheochromocytoma benign	1 (2%)	1 (5%)		
Pituitary gland	(52)	(21)	(17)	(49)
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(53)	(53)	(54)	(53)
Follicular cell, adenoma			1 (2%)	
Follicular cell, carcinoma	1 (2%)			1 (2%)
General Body System				
Tissue NOS	(2)			
Sarcoma	1 (50%)			
Genital System				
Epididymis	(54)	(21)	(22)	(54)
Hemangioma				1 (2%)
Prostate	(53)	(20)	(22)	(54)
Sarcoma	1 (2%)			
Seminal vesicle	(52)	(23)	(23)	(54)
Sarcoma	1 (2%)			
Testes	(54)	(22)	(22)	(54)
Interstitial cell, adenoma		1 (5%)		1 (2%)
Hematopoietic System				
Bone marrow	(54)	(21)	(22)	(54)
Hemangiosarcoma	1 (2%)			
Lymph node	(54)	(33)	(27)	(54)
Lymph node, mandibular	(45)	(19)	(17)	(51)
Spleen	(54)	(53)	(53)	(53)
Hemangiosarcoma	2 (4%)		1 (2%)	
Thymus	(53)	(19)	(19)	(51)
Integumentary System				
Skin	(54)	(45)	(43)	(54)
Basosquamous neoplasm benign	1 (2%)			
Mast cell neoplasm benign	1 (2%)			
Subcutaneous tissue, fibroma	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Subcutaneous tissue, fibrosarcoma	3 (6%)	8 (18%)	5 (12%)	1 (2%)
Subcutaneous tissue, fibrosarcoma, multifocal			1 (2%)	
Subcutaneous tissue, fibrosarcoma, metastatic, skin				1 (2%)
Subcutaneous tissue, histiocytic sarcoma	2 (4%)			
Subcutaneous tissue, sarcoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Subcutaneous tissue, sarcoma, multiple				1 (2%)
Subcutaneous tissue, schwannoma malignant				1 (2%)
Subcutaneous tissue, schwannoma NOS		1 (2%)		

TABLE A1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Ethylene Glycol
(continued)

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Musculoskeletal System				
Skeletal muscle	(1)			
Diaphragm, cholangiocarcinoma, metastatic, liver	1 (100%)			
Nervous System				
None				
Respiratory System				
Lung	(54)	(25)	(32)	(54)
Adenocarcinoma, metastatic, uncertain primary site	1 (2%)			
Alveolar/bronchiolar adenoma	6 (11%)	2 (8%)	7 (22%)	3 (6%)
Alveolar/bronchiolar adenoma, multiple, two			1 (3%)	
Alveolar/bronchiolar adenoma, multiple, greater than five	1 (2%)			
Alveolar/bronchiolar carcinoma	1 (2%)	1 (4%)	2 (6%)	
Alveolar/bronchiolar carcinoma, multiple, two		1 (4%)		
Cholangiocarcinoma, metastatic, liver	1 (2%)			
Fibrosarcoma, metastatic, multiple, skin				1 (2%)
Hepatocellular carcinoma, metastatic, multiple, four, liver			1 (3%)	
Hepatocellular carcinoma, metastatic, multiple, five, liver		1 (4%)		
Hepatocellular carcinoma, metastatic, multiple, greater than five, liver			1 (3%)	
Mediastinum, fibrosarcoma, metastatic, skin				1 (2%)
Mediastinum, hepatocellular carcinoma, metastatic, liver			1 (3%)	
Pleura, fibrosarcoma, metastatic, multiple, skin				1 (2%)
Nose	(54)	(21)	(21)	(54)
Special Senses System				
Harderian gland			(2)	(3)
Adenoma			2 (100%)	2 (67%)
Carcinoma				1 (33%)
Urinary System				
Kidney	(54)	(53)	(53)	(54)
Carcinoma, metastatic, uncertain primary site			1 (2%)	
Cholangiocarcinoma, metastatic, liver	1 (2%)			
Hepatocellular carcinoma, metastatic, liver		1 (2%)		
Capsule, sarcoma	1 (2%)			
Renal tubule, adenoma	1 (2%)			1 (2%)
Urinary bladder	(53)	(53)	(53)	(54)

TABLE A1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Ethylene Glycol (continued)

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Systemic Lesions				
Multiple organs[b]	(54)	(54)	(54)	(54)
Histiocytic sarcoma	2 (4%)			
Lymphoma malignant histiocytic				1 (2%)
Lymphoma malignant lymphocytic				1 (2%)
Lymphoma malignant mixed	6 (11%)	3 (6%)	5 (9%)	6 (11%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	34	36	31	33
Total primary neoplasms	67	46	44	47
Total animals with benign neoplasms	17	11	13	19
Total benign neoplasms	29	14	17	24
Total animals with malignant neoplasms	22	28	23	20
Total malignant neoplasms	38	31	27	23
Total animals with metastatic neoplasms	2	2	2	1
Total metastatic neoplasms	7	3	4	4
Total animals with malignant neoplasms-uncertain primary site	1		1	
Total animals with neoplasms uncertain-benign or malignant		1		
Total uncertain neoplasms		1		

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm

Number of Days on Study	0 5 4	1 2 0	1 2 3	1 2 6	1 3 6	1 4 5	1 8 6	2 1 3	3 5 0	3 8 6	4 1 5	4 6 9	5 3 8	5 8 5	5 9 5	5 9 8	6 0 2	6 0 2	6 3 0	6 3 7	6 4 9	6 5 4	6 6 7	6 8 2	7 0 9	7 3 1	7 3 1	7 3 1	7 3 1
Carcass ID Number	0 7 1	1 1 1	1 8 1	1 5 1	1 1 2	1 4 1	1 1 3	1 1 4	4 4 3	9 9 5	0 2 1	0 5 5	0 6 2	0 1 5	0 5 4	1 0 5	0 7 5	0 9 2	0 4 4	1 0 4	1 2 3	0 7 4	0 6 4	0 3 5	0 9 3	0 1 1	0 1 2	0 1 3	0 2 2
Alimentary System																													
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	M	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma													X																
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																													
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma													X																
Hemangiosarcoma, multiple, two																													
Hepatocellular carcinoma													X	X	X			X				X	X		X				
Hepatocellular adenoma											X															X			
Serosa, sarcoma													X																
Mesentery		+											+	+															
Cholangiocarcinoma, metastatic, liver														X															
Hemangiosarcoma																													
Sarcoma													X																
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor benign																													X
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																		+			+			+	+		+	
Cardiovascular System																													
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, uncertain primary site																						X							

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	
Carcass ID Number	0 2 3	0 2 4	0 2 5	0 3 1	0 3 2	0 3 3	0 3 4	0 4 2	0 4 5	0 5 2	0 5 3	0 6 1	0 6 3	0 6 5	0 7 2	0 7 3	0 8 2	0 8 3	0 8 4	0 8 5	0 9 4	0 9 1	1 0 1	1 0 2	1 0 3	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Sarcoma																										1
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, ileum	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Polyp adenomatous								X							X											2
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Cholangiocarcinoma																										1
Hemangiosarcoma, multiple, two						X																				1
Hepatocellular carcinoma			X		X										X											10
Hepatocellular adenoma		X						X		X									X		X		X	X		9
Serosa, sarcoma																										1
Mesentery			+	+																						5
Cholangiocarcinoma, metastatic, liver																										1
Hemangiosarcoma				X																						1
Sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Mast cell tumor benign																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Tooth	+		+	+		+	+		+			+	+	+	+	+					+	+	+	+	+	21
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Adenocarcinoma, metastatic, uncertain primary site																										1

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm
(continued)

Number of Days on Study	0 5 4	1 2 0	1 2 3	1 2 6	1 3 6	1 4 5	1 8 6	2 1 3	3 5 0	3 8 6	4 1 5	4 6 9	5 3 8	5 8 5	5 9 5	5 9 8	6 0 2	6 0 2	6 3 0	6 3 7	6 4 9	6 5 4	6 6 7	6 8 2	7 0 9	7 3 1	7 3 1	7 3 1	7 3 1
Carcass ID Number	0 7 1	1 1 1	0 8 1	0 5 1	1 1 2	0 4 1	1 1 3	1 1 4	0 4 3	0 9 5	0 2 1	0 5 5	0 6 2	0 1 5	0 5 4	1 0 5	0 7 5	0 9 2	1 4 4	1 0 4	1 2 3	0 7 4	0 6 4	0 3 5	0 9 3	0 1 1	0 1 2	0 1 3	0 2 2
Endocrine System																													
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																													
Capsule, cholangiocarcinoma, metastatic, liver														X															
Capsule, sarcoma													X																
Subcapsular, adenoma																													
Subcapsular, adenoma, multiple																									X				
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																													
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars intermedia, adenoma																						X							
Thyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, carcinoma																											X		
General Body System																													
Tissue NOS													+																
Sarcoma													X																
Genital System																													
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis							+				+	+		+								+							
Preputial gland								+		+		+	+											+	+				
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma													X																
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Sarcoma													X																
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																													
Blood																							+						
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																													
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	M	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																													
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	0 2 3	0 2 4	0 2 5	0 3 1	0 3 2	0 3 3	0 3 4	0 4 2	0 4 5	0 5 2	0 5 3	0 6 1	0 6 3	0 6 5	0 7 2	0 7 3	0 8 2	0 8 3	0 8 4	0 8 5	0 9 4	0 9 1	1 0 1	1 0 2	1 0 3	**Total Tissues/ Tumors**
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Adenoma																			X		X					2
Capsule, cholangiocarcinoma, metastatic, liver																										1
Capsule, sarcoma																										1
Subcapsular, adenoma													X													1
Subcapsular, adenoma, multiple																										1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Pheochromocytoma benign																								X		1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pituitary gland	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Follicular cell, carcinoma																										1
General Body System																										
Tissue NOS															+											2
Sarcoma																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Penis																										5
Preputial gland			+		+			+	+													+		+		12
Prostate	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Sarcoma																										1
Seminal vesicle	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Sarcoma																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Hematopoietic System																										
Blood																										1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Hemangiosarcoma				X																						1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	M	+	M	+	+	+	+	+	45
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Hemangiosarcoma		X		X																						2
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm
(continued)

Number of Days on Study	0 5 4	1 2 0	1 2 3	1 2 6	1 3 6	1 4 5	1 8 6	2 1 3	3 5 0	3 8 6	4 1 5	4 6 9	5 3 8	5 8 5	5 9 5	5 9 8	6 0 2	6 0 2	6 3 0	6 3 7	6 4 9	6 5 4	6 6 7	6 8 2	7 0 9	7 3 1	7 3 1	7 3 1	7 3 1
Carcass ID Number	0 7 1	1 1 1	0 8 1	0 5 1	1 1 2	0 4 1	1 1 3	0 1 4	0 4 3	0 9 5	0 2 1	0 5 5	0 6 2	0 1 5	0 5 4	1 0 5	0 7 5	0 9 2	0 4 4	1 0 4	1 2 3	0 7 4	0 6 4	0 3 5	0 9 3	0 1 1	0 1 2	0 1 3	0 2 2
Integumentary System																													
Mammary gland	+	M	+	M	+	M	+	+	+	+	+	+	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor benign																													X
Subcutaneous tissue, fibroma																													
Subcutaneous tissue, fibrosarcoma																X					X			X					
Subcutaneous tissue, histiocytic sarcoma																			X				X						
Subcutaneous tissue, sarcoma									X																				
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle														+															
Diaphragm, cholangiocarcinoma, metastatic, liver														X															
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, uncertain primary site																						X							
Alveolar/bronchiolar adenoma																													
Alveolar/bronchiolar adenoma, multiple, greater than five																													
Alveolar/bronchiolar carcinoma																													
Cholangiocarcinoma, metastatic, liver														X															
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																													
None																													
Urinary System																													
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma, metastatic, liver														X															
Capsule, sarcoma													X																
Renal tubule, adenoma																						X							
Urethra					+																								
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	0 2 3	0 2 4	0 2 5	0 3 1	0 3 2	0 3 3	0 3 4	0 4 2	0 4 5	0 5 2	0 5 3	0 6 1	0 6 3	0 6 5	0 7 2	0 7 3	0 8 2	0 8 3	0 8 4	0 8 5	0 9 4	0 9 1	1 0 1	1 0 2	1 0 3		**Total Tissues/ Tumors**
Integumentary System																											
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	+	M	+	M		13
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		54
Mast cell tumor benign																		X	X								1
Subcutaneous tissue, fibroma																	X	X									2
Subcutaneous tissue, fibrosarcoma																											3
Subcutaneous tissue, histiocytic sarcoma																											2
Subcutaneous tissue, sarcoma																											1
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		54
Skeletal muscle																											1
Diaphragm, cholangiocarcinoma, metastatic, liver																											1
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		54
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		54
Adenocarcinoma, metastatic, uncertain primary site																											1
Alveolar/bronchiolar adenoma				X						X						X					X			X	X	6
Alveolar/bronchiolar adenoma, multiple, greater than five								X																			1
Alveolar/bronchiolar carcinoma								X																			1
Cholangiocarcinoma, metastatic, liver																											1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		54
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		54
Special Senses System																											
None																											
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		54
Cholangiocarcinoma, metastatic, liver																											1
Capsule, sarcoma																											1
Renal tubule, adenoma																											1
Urethra																											1
Urinary bladder	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm
(continued)

Number of Days on Study	0	1	1	1	1	1	1	2	3	3	4	4	5	5	5	5	6	6	6	6	6	6	6	7	7	7	7	7	
	5	2	2	2	3	4	8	1	5	8	1	6	3	8	9	9	0	0	3	3	4	5	6	8	0	3	3	3	
	4	0	3	6	6	5	6	3	0	6	5	9	8	5	5	8	2	2	0	7	9	4	7	2	9	1	1	1	
Carcass ID Number	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	
	7	1	8	5	1	4	1	1	4	9	2	5	6	1	5	0	7	9	4	0	2	7	6	3	9	1	1	1	
	1	1	1	1	2	1	3	4	3	5	1	5	2	5	4	5	5	2	4	4	3	4	4	5	3	1	2	3	
Systemic Lesions																													
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Histiocytic sarcoma																			X				X						
Lymphoma malignant mixed																				X					X				

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	
Carcass ID Number	0 2 3	0 2 4	0 2 5	0 3 1	0 3 2	0 3 3	0 3 4	0 4 2	0 4 5	0 5 2	0 5 3	0 6 1	0 6 3	0 6 5	0 7 2	0 7 3	0 8 2	0 8 3	0 8 4	0 8 5	0 9 4	0 9 1	1 0 1	1 0 2	1 0 3	Total Tissues/ Tumors
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Histiocytic sarcoma																										2
Lymphoma malignant mixed			X			X									X					X						6

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 6,250 ppm

Number of Days on Study	0 9 8	1 7 5	3 4 6	3 9 4	5 1 4	5 1 4	5 7 8	5 9 9	5 9 9	6 0 1	6 0 1	6 2 4	6 2 7	6 4 1	6 5 4	6 5 4	6 6 7	6 8 2	6 9 3	6 9 7	6 9 7	7 0 9	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2
Carcass ID Number	4 0 1	4 0 3	4 0 1	4 8 2	3 7 5	4 6 1	3 8 2	4 2 1	4 4 4	3 5 5	4 4 2	4 3 5	3 9 2	3 8 1	3 8 3	3 9 3	4 4 1	4 6 2	5 2 2	3 3 3	3 4 4	1 1 1	7 7 1	7 7 2	7 7 3	7 7 4	8 4 4	9 1 4	9 4 4
Alimentary System																													
Esophagus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Gallbladder	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine large	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine large, cecum	A	A	A	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine large, colon	A	+	A	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine large, rectum	A	+	A	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine small	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine small, duodenum	A	+	A	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Polyp adenomatous																													
Intestine small, ileum	A	A	A	M	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine small, jejunum	A	A	A	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Liver	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma										X	X				X	X	X		X	X	X			X				
Hepatocellular carcinoma, multiple, two																													
Hepatocellular carcinoma, multiple, four														X															
Hepatocellular adenoma								X																		X			
Mesentery				+									+				+												
Hepatocellular carcinoma, metastatic, liver																	X												
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+			
Salivary glands	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Stomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Stomach, forestomach	A	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Stomach, glandular	A	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Tooth													+			+			+			+							
Cardiovascular System																													
Heart	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Endocrine System																													
Adrenal gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+			
Adrenal gland, cortex	A	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Adrenal gland, medulla	A	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+				+			
Pheochromocytoma benign																										X			
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Parathyroid gland	A	+	+	+	+	+	M	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+							
Pituitary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 6,250 ppm
(continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	3 9 5	4 0 2	4 0 4	4 0 5	4 1 2	4 1 3	4 1 4	4 1 5	4 2 2	4 2 3	4 2 4	4 2 5	4 3 1	4 3 2	4 3 3	4 4 4	4 4 5	4 5 1	4 5 3	4 5 5	4 6 3	4 6 4	4 6 5	4 7 1	4 7 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus																										21
Gallbladder																										19
Intestine large																										20
Intestine large, cecum																										18
Intestine large, colon																										19
Intestine large, rectum																										19
Intestine small				+																						21
Intestine small, duodenum				+																						20
Polyp adenomatous				X																						1
Intestine small, ileum																										17
Intestine small, jejunum																										18
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Hepatocellular carcinoma							X	X					X	X	X		X									15
Hepatocellular carcinoma, multiple, two																				X						1
Hepatocellular carcinoma, multiple, four																										1
Hepatocellular adenoma			X	X								X										X				6
Mesentery																										3
Hepatocellular carcinoma, metastatic, liver																										1
Pancreas																				+						23
Salivary glands																										21
Stomach																										21
Stomach, forestomach																										20
Stomach, glandular																										20
Tooth																										4
Cardiovascular System																										
Heart																										21
Endocrine System																										
Adrenal gland																										22
Adrenal gland, cortex																										20
Adrenal gland, medulla																										21
Pheochromocytoma benign																										1
Islets, pancreatic																										21
Parathyroid gland																										18
Pituitary gland																										21
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 6,250 ppm
(continued)

Number of Days on Study	0 9 8	1 7 5	3 4 6	3 9 4	5 1 4	5 1 4	5 7 8	5 9 9	5 9 9	6 0 1	6 0 1	6 2 4	6 2 7	6 4 1	6 5 4	6 5 4	6 6 7	6 8 2	6 9 3	6 9 7	6 9 7	7 0 9	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2
Carcass ID Number	4 0 1	4 0 3	4 8 1	4 8 2	3 7 5	4 6 1	3 8 2	4 2 1	4 5 4	3 8 5	4 4 2	4 3 5	3 9 2	3 8 1	3 8 3	3 9 3	4 4 1	4 6 2	4 5 2	4 3 3	4 3 4	4 1 1	3 1 1	3 7 2	3 7 3	3 7 4	3 8 4	3 9 1	3 9 4
General Body System																													
None																													
Genital System																													
Epididymis	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Penis																													
Preputial gland		+								+															+				
Prostate	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Seminal vesicle	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							+
Testes	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Interstitial cell, adenoma																													
Hematopoietic System																													
Blood							+																			+			
Bone marrow	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Lymph node	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+		+		
Lymph node, mandibular	A	+	+	+	+	+	+		+	M	+	+	M	+	+	+	+	+	+	+	+	+							
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+							
Integumentary System																													
Mammary gland	M	M	M	M	+	M	+	+	M	M	M	M	+	+	M	M	M	+	+	+	M	+							
Skin	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Basosquamous tumor benign																													
Subcutaneous tissue, fibroma																													
Subcutaneous tissue, fibrosarcoma					X			X	X			X														X	X		
Subcutaneous tissue, sarcoma						X																							
Subcutaneous tissue, schwannoma NOS														X															
Musculoskeletal System																													
Bone	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Nervous System																													
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 6,250 ppm
(continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	3 9 5	4 0 2	4 0 4	4 0 5	4 1 2	4 1 3	4 1 4	4 1 5	4 2 2	4 2 3	4 2 4	4 2 5	4 3 1	4 3 2	4 3 3	4 4 4	4 4 5	4 5 1	4 5 3	4 5 5	4 6 3	4 6 4	4 6 5	4 7 1	4 7 5	**Total Tissues/ Tumors**
General Body System																										
None																										
Genital System																										
Epididymis																										21
Penis	+																									1
Preputial gland							+				+											+				6
Prostate																										20
Seminal vesicle										+																23
Testes			+																							22
Interstitial cell, adenoma			X																							1
Hematopoietic System																										
Blood																				+						3
Bone marrow																										21
Lymph node						+	+		+		+	+							+	+			+		+	33
Lymph node, mandibular																									+	19
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Thymus																										19
Integumentary System																										
Mammary gland																										9
Skin			+	+	+	+		+	+	+	+	+	+		+	+	M		+	+	+	+	+		+	45
Basosquamous tumor benign											X															1
Subcutaneous tissue, fibroma						X										X										2
Subcutaneous tissue, fibrosarcoma								X												X						8
Subcutaneous tissue, sarcoma																										1
Subcutaneous tissue, schwannoma NOS																										1
Musculoskeletal System																										
Bone																										21
Nervous System																										
Brain																										21

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 6,250 ppm
(continued)

Number of Days on Study	0 9 8	1 7 5	3 4 6	3 9 4	5 1 4	5 1 4	5 7 8	5 9 9	5 9 9	6 0 1	6 0 1	6 2 4	6 2 7	6 4 1	6 5 4	6 5 4	6 6 7	6 8 2	6 9 3	6 9 7	6 9 7	7 0 9	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2
Carcass ID Number	4 0 1	4 0 3	4 0 1	4 8 2	3 7 5	4 6 1	3 8 2	4 2 1	4 5 4	3 8 5	4 4 2	4 3 5	3 9 2	3 8 1	3 8 3	3 9 3	4 4 1	4 6 2	4 5 2	4 3 3	4 3 4	4 1 1	3 7 1	3 7 2	3 7 3	3 7 4	3 8 4	3 9 1	3 9 4
Respiratory System																													
Lung	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
Alveolar/bronchiolar adenoma																							X						
Alveolar/bronchiolar carcinoma																													
Alveolar/bronchiolar carcinoma, multiple, two																							X						
Hepatocellular carcinoma, metastatic, multiple, five, liver																		X											
Nose	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Trachea	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Special Senses System																													
None																													
Urinary System																													
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic, liver																		X											
Urethra				+							+													+					
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																													
Multiple organs		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed							X						X												X				

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 6,250 ppm
(continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	3 9 5	4 0 2	4 0 4	4 0 5	4 1 2	4 1 3	4 1 4	4 1 5	4 2 2	4 2 3	4 2 4	4 2 5	4 3 1	4 3 2	4 3 3	4 4 4	4 4 5	4 5 1	4 5 3	4 5 5	4 6 3	4 6 4	4 6 5	4 7 1	4 7 5	**Total Tissues/ Tumors**
Respiratory System																										
Lung										+								+	+							25
Alveolar/bronchiolar adenoma																			X							2
Alveolar/bronchiolar carcinoma										X																1
Alveolar/bronchiolar carcinoma, multiple, two																										1
Hepatocellular carcinoma, metastatic, multiple, five, liver																										1
Nose																										21
Trachea																										21
Special Senses System																										
None																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Hepatocellular carcinoma, metastatic, liver																										1
Urethra						+																				4
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymphoma malignant mixed																										3

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 12,500 ppm

Number of Days on Study	0 3 1	0 3 2	0 4 2	0 4 6	0 9 3	1 2 1	3 8 3	3 8 4	3 8 4	3 8 4	5 1 3	5 1 7	5 1 4	5 8 8	5 9 7	6 0 4	6 2 0	6 2 2	6 3 3	6 5 4	6 6 7	6 9 7	7 0 5	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2
Carcass ID Number	2 8 1	2 6 1	2 6 2	2 9 1	3 6 1	3 2 1	3 0 5	3 1 3	3 3 2	3 3 3	3 0 3	3 4 4	3 0 4	3 5 4	3 6 5	3 0 2	3 7 1	2 5 4	2 6 4	3 2 4	2 6 4	3 2 5	2 5 3	3 0 1	3 1 1	3 1 2	3 1 4	3 1 5	2 5 1	2 5 2
Alimentary System																														
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Gallbladder	+	+	M	M	+	+	M	A	+	+	+	M	+	+	+	+	+	+	+	M	+	+	A							
Intestine large	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine large, cecum	+	+	+	+	M	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine large, colon	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine large, rectum	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine small	+	+	+	+	M	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine small, duodenum	+	+	+	+	M	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine small, ileum	+	+	+	+	M	+	A	A	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+							
Intestine small, jejunum	+	+	+	+	M	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+							
Liver	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma															X			X	X			X		X		X	X		
Hepatocellular carcinoma, multiple, two												X					X												
Hepatocellular adenoma																														
Mesentery																			+											
Pancreas	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Salivary glands	+	+	+	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Stomach	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Stomach, forestomach	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Stomach, glandular	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Tooth													+																	
Cardiovascular System																														
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Endocrine System																														
Adrenal gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+					
Adrenal gland, cortex	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Adrenal gland, medulla	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Islets, pancreatic	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Pituitary gland	+	+	+	M	M	M	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+							
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																													X	
General Body System																														
None																														

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 12,500 ppm
(continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	2 5 5	2 6 3	2 7 2	2 7 3	2 7 4	2 7 5	2 8 4	2 8 5	2 8 2	2 8 3	2 9 2	2 9 3	2 9 4	2 9 5	3 2 2	3 2 3	3 3 1	3 3 4	3 3 5	3 4 1	3 4 2	3 4 3	3 4 5	3 5 5	3 6 4	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus																										22
Gallbladder																+										16
Intestine large																										21
Intestine large, cecum																										18
Intestine large, colon																										20
Intestine large, rectum																										21
Intestine small																	+									19
Intestine small, duodenum																										18
Intestine small, ileum																										17
Intestine small, jejunum																	+									19
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Hepatocellular carcinoma										X							X						X			10
Hepatocellular carcinoma, multiple, two																										2
Hepatocellular adenoma							X	X	X	X						X										5
Mesentery																										1
Pancreas																						+				21
Salivary glands																										20
Stomach																										21
Stomach, forestomach																										21
Stomach, glandular																										21
Tooth																										1
Cardiovascular System																										
Heart																										22
Endocrine System																										
Adrenal gland																										22
Adrenal gland, cortex																										21
Adrenal gland, medulla																										21
Islets, pancreatic																										20
Parathyroid gland																+										22
Pituitary gland																										17
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Follicular cell, adenoma																										1
General Body System																										
None																										

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 12,500 ppm
(continued)

Number of Days on Study	0 3 1	0 3 2	0 4 2	0 4 6	0 9 3	1 2 1	3 8 3	3 8 4	3 8 4	3 8 4	5 1 3	5 1 7	5 8 4	5 9 8	6 0 7	6 2 0	6 2 2	6 3 3	6 5 4	6 6 7	6 9 7	7 0 5	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2
Carcass ID Number	2 8 1	2 6 1	2 6 2	2 9 1	3 6 1	3 2 1	3 0 5	3 1 3	3 3 2	3 3 3	3 0 3	3 4 4	3 4 4	3 5 4	3 6 5	3 0 2	3 7 1	3 5 4	3 2 4	3 6 4	3 2 5	3 5 3	3 0 1	3 1 1	3 1 2	3 1 4	3 1 5	2 5 1	2 5 2
Genital System																													
Coagulating gland					+																								
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Penis	+						+	+		+																			
Preputial gland		+					+			+																			
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Seminal vesicle	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+						
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Lymph node	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+		+		
Lymph node, mandibular	+	+	+	+	+	+	M	M	M	M	+	+	+	+	+	+	+	+	+	+	+	M							
Spleen	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																						X							
Thymus	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+							
Integumentary System																													
Mammary gland	+	M	+	+	M	M	M	+	M	+	M	M	M	+	M	M	+	+	+	M	+	+							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	
Subcutaneous tissue, fibroma																													
Subcutaneous tissue, fibrosarcoma													X	X			X												
Subcutaneous tissue, fibrosarcoma, multifocal																					X								
Subcutaneous tissue, sarcoma																				X									
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 12,500 ppm
(continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	2 5 5	2 6 3	2 7 2	2 7 3	2 7 4	2 7 5	2 8 4	2 8 5	2 8 2	2 8 3	2 9 2	2 9 3	2 9 4	2 9 5	3 2 2	3 2 3	3 3 1	3 3 4	3 3 5	3 4 1	3 4 2	3 4 3	3 4 5	3 5 5	3 6 4	**Total Tissues/ Tumors**
Genital System																										
Coagulating gland																										1
Epididymis																										22
Penis																										4
Preputial gland			+										+	+						+						7
Prostate																										22
Seminal vesicle																									+	23
Testes																										22
Hematopoietic System																										
Bone marrow																										22
Lymph node								+						+						+			+			27
Lymph node, mandibular																										17
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Hemangiosarcoma																										1
Thymus																										19
Integumentary System																										
Mammary gland																										11
Skin		+	+	+	+		+	+	+			+		+	+	+		+			+	+		+	+	43
Subcutaneous tissue, fibroma							X																			1
Subcutaneous tissue, fibrosarcoma							X											X								5
Subcutaneous tissue, fibrosarcoma, multifocal																										1
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone																										22
Nervous System																										
Brain																										22

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 12,500 ppm
(continued)

Number of Days on Study	0 3 1	0 3 2	0 4 2	0 4 6	0 9 3	1 2 1	3 8 3	3 8 4	3 8 4	3 8 4	5 1 3	5 1 7	5 8 4	5 9 8	6 0 7	6 2 0	6 2 2	6 3 3	6 5 4	6 6 7	6 9 7	7 0 5	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2
Carcass ID Number	2 8 1	2 6 1	2 6 2	2 9 1	3 6 1	3 2 1	3 0 5	3 1 3	3 3 2	3 3 3	3 0 3	3 4 4	4 0 4	4 5 4	5 6 5	5 9 2	5 7 1	5 5 4	5 2 4	5 6 4	5 2 5	5 5 3	5 0 1	5 1 1	5 1 2	5 1 4	5 1 5	5 5 1	5 5 2
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						+
Alveolar/bronchiolar adenoma																													X
Alveolar/bronchiolar adenoma, multiple, two																													
Alveolar/bronchiolar carcinoma																						X							
Hepatocellular carcinoma, metastatic, multiple, four, liver																					X								
Hepatocellular carcinoma, metastatic, multiple, greater than five, liver																X													
Mediastinum, hepatocellular carcinoma, metastatic, liver																					X								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+							
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Special Senses System																													
Harderian gland																													
Adenoma																													
Urinary System																													
Kidney	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uncertain primary site																					X								
Urethra				+		+																							
Urinary bladder	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																													
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed																							X	X			X		

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 12,500 ppm
(continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	2 5 5	2 6 3	2 7 2	2 7 3	2 7 4	2 7 5	2 8 4	2 8 5	2 8 2	2 8 3	2 9 2	2 9 3	2 9 4	2 9 5	3 2 2	3 2 3	3 3 1	3 3 4	3 3 5	3 4 1	3 4 2	3 4 3	3 4 5	3 5 5	3 5 4	**Total Tissues/ Tumors**
Respiratory System																										
Lung								+		+				+		+	+	+		+				+		32
Alveolar/bronchiolar adenoma										X				X		X	X			X				X		7
Alveolar/bronchiolar adenoma, multiple, two								X																		1
Alveolar/bronchiolar carcinoma																				X						2
Hepatocellular carcinoma, metastatic, multiple, four, liver																										1
Hepatocellular carcinoma, metastatic, multiple, greater than five, liver																										1
Mediastinum, hepatocellular carcinoma, metastatic, liver																										1
Nose																										21
Trachea																										22
Special Senses System																										
Harderian gland				+								+														2
Adenoma				X								X														2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, uncertain primary site																										1
Urethra																										2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Lymphoma malignant mixed																		X					X			5

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 25,000 ppm

Number of Days on Study	0 6 0	0 9 1	0 9 8	1 0 1	1 2 6	1 2 7	1 4 4	1 4 5	1 4 9	1 5 0	1 5 4	1 6 1	1 6 8	2 1 5	2 4 1	3 1 7	3 8 6	4 9 8	5 1 9	5 9 0	5 9 5	5 9 7	6 5 4	6 6 7	6 8 1	6 8 1	6 8 2	6 9 7	7 0 9
Carcass ID Number	1 9 1	2 1 1	2 1 2	2 1 3	1 4 1	1 8 2	1 3 3	1 9 2	1 9 3	1 9 4	2 2 2	2 2 3	2 0 1	1 4 2	1 6 2	1 5 2	2 3 1	1 7 1	1 8 5	1 5 5	1 6 3	1 7 2	1 6 4	1 7 3	1 6 5	1 9 5	1 7 4	1 5 3	1 8 1
Alimentary System																													
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	+	+	+	+	+	+	+	+	I	+	+	+	M	A	+	+	+	+	M	M	+	+	+	+	+	+	+
Intestine large	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	+	+	+	+	M	+	A	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	M	M	+	+	+	+	+	M	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine small	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	M	+	+	+	+	+	+	M	+	A	+	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	+	+	+	+	+	+	+	M	M	A	M	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+
Polyp adenomatous																													
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																			X	X			X	X			X		X
Hepatocellular adenoma																X													
Hepatocellular adenoma, multiple, two																											X		
Mesentery					+		+								+			+	+										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth	+														+											+			
Cardiovascular System																													
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																													
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcapsular, adenoma																													
Adrenal gland, medulla	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Pituitary gland	+	+	M	+	M	M	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, carcinoma																													
General Body System																													
None																													

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 25,000 ppm
(continued)

Number of Days on Study	7 0 9	7 2 2	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	1 8 4	2 3 3	1 3 1	1 3 2	1 3 4	1 3 5	1 4 3	1 4 4	1 4 5	1 5 1	1 5 4	1 6 1	1 7 5	1 8 3	2 0 2	2 0 3	2 0 4	2 0 5	2 1 4	2 1 5	2 2 1	2 2 4	2 2 5	2 3 4	2 3 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp adenomatous										X																1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Hepatocellular carcinoma	X						X													X						9
Hepatocellular adenoma					X	X								X	X		X		X						X	8
Hepatocellular adenoma, multiple, two											X															2
Mesentery							+					+														7
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Tooth		+			+				+	+	+	+		+	+	+	+		+		+	+	+		+	18
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Subcapsular, adenoma												X	X								X					3
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	50
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Follicular cell, carcinoma							X																			1
General Body System																										
None																										

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 25,000 ppm
(continued)

Number of Days on Study	0 6 0	0 9 1	0 9 8	1 0 1	1 2 6	1 2 7	1 4 4	1 4 5	1 4 9	1 5 0	1 5 4	1 6 1	1 6 8	2 1 5	2 4 1	3 1 7	3 8 6	4 9 8	5 1 9	5 9 0	5 9 5	5 9 7	6 5 4	6 6 7	6 8 1	6 8 1	6 9 2	7 0 9
Carcass ID Number	1 9 1	2 1 1	2 1 2	2 1 3	1 4 1	1 8 2	1 3 3	1 9 2	1 9 3	1 9 4	2 2 2	2 2 3	2 0 1	2 4 2	1 6 2	1 5 2	2 3 1	1 7 1	1 8 5	1 5 5	1 6 3	1 7 2	1 6 4	1 7 3	1 6 5	1 9 5	1 7 4	1 5 3	1 8 1
Genital System																													
Coagulating gland												+																	
Ductus deferens																										+			
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																													
Penis																													+
Preputial gland							+						+	+								+				+			
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																													
Hematopoietic System																													
Blood																												+	
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																													
Mammary gland	+	M	M	+	+	+	+	+	+	M	+	+	+	+	M	M	+	+	M	M	+	M	M	M	+	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																													
Subcutaneous tissue, fibrosarcoma																												X	
Subcutaneous tissue, fibrosarcoma, metastatic, skin																										X			
Subcutaneous tissue, sarcoma																									X				
Subcutaneous tissue, sarcoma, multiple																					X								
Subcutaneous tissue, schwannoma malignant																		X											
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 25,000 ppm
(continued)

Number of Days on Study	7 0 9	7 2 2	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	1 8 4	2 3 3	1 3 1	1 3 2	1 3 4	1 3 5	1 4 3	1 4 4	1 4 5	1 5 1	1 5 4	1 6 1	1 7 5	1 8 3	2 0 2	2 0 3	2 0 4	2 0 5	2 1 4	2 1 5	2 2 1	2 2 4	2 2 5	2 3 4	2 3 5	Total Tissues/ Tumors
Genital System																										
Coagulating gland																										1
Ductus deferens																										1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Hemangioma																							X			1
Penis	+		+		+		+												+							6
Preputial gland					+				+				+		+		+	+	+							12
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Interstitial cell, adenoma			X																							1
Hematopoietic System																										
Blood																										1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	51
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	51
Integumentary System																										
Mammary gland	M	+	M	M	M	M	M	+	+	+	+	+	+	M	+	M	+	M	+	+	+	M	M	+	+	29
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Subcutaneous tissue, fibroma						X	X																			2
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, fibrosarcoma, metastatic, skin																										1
Subcutaneous tissue, sarcoma																										1
Subcutaneous tissue, sarcoma, multiple																										1
Subcutaneous tissue, schwannoma malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 25,000 ppm
(continued)

Number of Days on Study	0 6 0	0 9 1	0 9 8	1 0 1	1 2 6	1 2 7	1 4 4	1 4 5	1 4 9	1 5 0	1 5 4	1 6 1	1 6 8	2 1 5	2 4 1	3 1 7	3 8 6	4 9 8	5 1 9	5 9 0	5 9 5	5 9 7	6 5 4	6 6 7	6 8 1	6 8 1	6 9 2	7 0 7	7 0 9
Carcass ID Number	1 9 1	2 1 1	2 1 2	2 1 3	1 4 1	1 8 2	1 3 3	1 9 2	1 9 3	1 9 4	2 2 2	2 2 3	2 0 1	1 4 2	1 6 2	1 5 2	2 3 1	1 7 1	1 8 5	1 5 5	1 6 3	1 7 2	1 6 4	1 7 3	1 6 5	1 9 5	7 4 5	5 3 4	8 1 3
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																	X												
Fibrosarcoma, metastatic, multiple, skin																											X		
Mediastinum, fibrosarcoma, metastatic, skin																											X		
Pleura, fibrosarcoma, metastatic, multiple, skin																											X		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																													
Ear							+																						
Harderian gland																											+		
Adenoma																											X		
Carcinoma																													
Urinary System																													
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																													
Urethra	+			+		+	+	+			+					+				+					+	+		+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																													
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic																						X							
Lymphoma malignant lymphocytic																													
Lymphoma malignant mixed																											X	X	

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Ethylene Glycol: 25,000 ppm
(continued)

Number of Days on Study	7 0 9	7 2 2	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	1 8 4	2 3 3	1 3 1	1 3 2	1 3 4	1 3 5	1 4 3	1 4 4	1 4 5	1 5 1	1 5 4	1 6 1	1 7 5	1 8 3	2 0 2	2 0 3	2 0 4	2 0 5	2 1 4	2 1 5	2 2 1	2 2 4	2 2 5	2 3 4	2 3 5	**Total Tissues/ Tumors**
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Alveolar/bronchiolar adenoma										X									X							3
Fibrosarcoma, metastatic, multiple, skin																										1
Mediastinum, fibrosarcoma, metastatic, skin																										1
Pleura, fibrosarcoma, metastatic, multiple, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Special Senses System																										
Ear																										1
Harderian gland							+					+														3
Adenoma												X														2
Carcinoma							X																			1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Renal tubule, adenoma											X															1
Urethra		+									+				+	+						+			+	18
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic																								X		1
Lymphoma malignant mixed									X			X										X			X	6

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Ethylene Glycol

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Adrenal Cortex: Adenoma				
Overall rates[a]	4/54 (7%)	0/20 (0%)[e]	0/21 (0%)[e]	3/53 (6%)
Adjusted rates[b]	13.8%			13.0%
Terminal rates[c]	4/29 (14%)			3/23 (13%)
First incidence (days)	731 (T)			731 (T)
Life table tests[d]				P=0.628N
Logistic regression tests[d]				P=0.628N
Fisher exact test[d]				P=0.511N
Harderian Gland: Adenoma or Carcinoma				
Overall rates	0/54 (0%)	0/54 (0%)	2/54 (4%)	3/54 (6%)
Adjusted rates	0.0%	0.0%	6.3%	11.7%
Terminal rates	0/29 (0%)	0/32 (0%)	2/32 (6%)	2/23 (9%)
First incidence (days)	–[f]	–	731 (T)	681
Life table tests	P=0.014	–	P=0.260	P=0.095
Logistic regression tests	P=0.016	–	P=0.260	P=0.098
Cochran-Armitage test[d]	P=0.028			
Fisher exact test		–	P=0.248	P=0.121
Liver: Hepatocellular Adenoma				
Overall rates	9/54 (17%)	6/53 (11%)	5/53 (9%)	10/54 (19%)
Adjusted rates	29.3%	17.4%	15.6%	38.7%
Terminal rates	8/29 (28%)	5/32 (16%)	5/32 (16%)	8/23 (35%)
First incidence (days)	469	599	731 (T)	317
Life table tests	P=0.180	P=0.211N	P=0.140N	P=0.292
Logistic regression tests	P=0.229	P=0.192N	P=0.159N	P=0.377
Cochran-Armitage test	P=0.374			
Fisher exact test		P=0.303N	P=0.206N	P=0.500
Liver: Hepatocellular Carcinoma				
Overall rates	10/54 (19%)	17/53 (32%)	12/53 (23%)	9/54 (17%)
Adjusted rates	26.4%	41.0%	30.8%	28.0%
Terminal rates	3/29 (10%)	8/32 (25%)	6/32 (19%)	2/23 (9%)
First incidence (days)	538	601	517	519
Life table tests	P=0.451N	P=0.199	P=0.485	P=0.557
Logistic regression tests	P=0.389N	P=0.143	P=0.417	P=0.599N
Cochran-Armitage test	P=0.250N			
Fisher exact test		P=0.082	P=0.387	P=0.500N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rates	19/54 (35%)	23/53 (43%)	16/53 (30%)	18/54 (33%)
Adjusted rates	50.2%	54.3%	41.5%	56.6%
Terminal rates	11/29 (38%)	13/32 (41%)	10/32 (31%)	10/23 (43%)
First incidence (days)	469	599	517	317
Life table tests	P=0.448	P=0.472	P=0.258N	P=0.397
Logistic regression tests	P=0.526	P=0.457	P=0.303N	P=0.484
Cochran-Armitage test	P=0.309N			
Fisher exact test		P=0.251	P=0.365N	P=0.500N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Ethylene Glycol
(continued)

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Lung: Alveolar/bronchiolar Adenoma				
Overall rates	7/54 (13%)	2/25 (8%)[e]	8/32 (25%)[e]	3/54 (6%)
Adjusted rates	24.1%			11.1%
Terminal rates	7/29 (24%)			2/23 (9%)
First incidence (days)	731 (T)			386
Life table tests				P=0.260N
Logistic regression tests				P=0.211N
Fisher exact test				P=0.160N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rates	1/54 (2%)	2/25 (8%)[e]	2/32 (6%)[e]	0/54 (0%)
Adjusted rates	3.4%			0.0%
Terminal rates	1/29 (3%)			0/23 (0%)
First incidence (days)	731 (T)			–
Life table tests				P=0.546N
Logistic regression tests				P=0.546N
Fisher exact test				P=0.500N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rates	7/54 (13%)	3/25 (12%)[e]	9/32 (28%)[e]	3/54 (6%)
Adjusted rates	24.1%			11.1%
Terminal rates	7/29 (24%)			2/23 (9%)
First incidence (days)	731 (T)			386
Life table tests				P=0.260N
Logistic regression tests				P=0.211N
Fisher exact test				P=0.160N
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rates	3/54 (6%)	8/54 (15%)	6/54 (11%)	1/54 (2%)
Adjusted rates	8.5%	19.8%	15.6%	3.7%
Terminal rates	0/29 (0%)	4/32 (13%)	2/32 (6%)	0/23 (0%)
First incidence (days)	598	514	584	697
Life table tests	P=0.222N	P=0.168	P=0.287	P=0.350N
Logistic regression tests	P=0.175N	P=0.116	P=0.252	P=0.340N
Cochran-Armitage test	P=0.140N			
Fisher exact test		P=0.101	P=0.244	P=0.309N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rates	5/54 (9%)	10/54 (19%)	6/54 (11%)	3/54 (6%)
Adjusted rates	14.8%	25.5%	15.6%	12.1%
Terminal rates	2/29 (7%)	6/32 (19%)	2/32 (6%)	2/23 (9%)
First incidence (days)	598	514	584	697
Life table tests	P=0.263N	P=0.214	P=0.551	P=0.456N
Logistic regression tests	P=0.213N	P=0.176	P=0.518	P=0.426N
Cochran-Armitage test	P=0.153N			
Fisher exact test		P=0.133	P=0.500	P=0.358N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Ethylene Glycol
(continued)

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma				
Overall rates	4/54 (7%)	9/54 (17%)	7/54 (13%)	3/54 (6%)
Adjusted rates	10.5%	21.4%	18.0%	9.7%
Terminal rates	0/29 (0%)	4/32 (13%)	2/32 (6%)	0/23 (0%)
First incidence (days)	350	514	584	595
Life table tests	P=0.393N	P=0.199	P=0.313	P=0.564N
Logistic regression tests	P=0.305N	P=0.107	P=0.267	P=0.527N
Cochran-Armitage test	P=0.263N			
Fisher exact test		P=0.118	P=0.263	P=0.500N
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma				
Overall rates	6/54 (11%)	11/54 (20%)	7/54 (13%)	5/54 (9%)
Adjusted rates	16.6%	27.1%	18.0%	17.5%
Terminal rates	2/29 (7%)	6/32 (19%)	2/32 (6%)	2/23 (9%)
First incidence (days)	350	514	584	595
Life table tests	P=0.417N	P=0.240	P=0.554	P=0.607N
Logistic regression tests	P=0.334N	P=0.161	P=0.511	P=0.566N
Cochran-Armitage test	P=0.261N			
Fisher exact test		P=0.145	P=0.500	P=0.500N
All Organs: Hemangiosarcoma				
Overall rates	3/54 (6%)	0/54 (0%)	1/54 (2%)	0/54 (0%)
Adjusted rates	10.3%	0.0%	3.1%	0.0%
Terminal rates	3/29 (10%)	0/32 (0%)	1/32 (3%)	0/23 (0%)
First incidence (days)	731 (T)	–	731 (T)	–
Life table tests	P=0.102N	P=0.103N	P=0.269N	P=0.163N
Logistic regression tests	P=0.102N	P=0.103N	P=0.269N	P=0.163N
Cochran-Armitage test	P=0.086N			
Fisher exact test		P=0.121N	P=0.309N	P=0.121N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rates	3/54 (6%)	0/54 (0%)	1/54 (2%)	1/54 (2%)
Adjusted rates	10.3%	0.0%	3.1%	4.3%
Terminal rates	3/29 (10%)	0/32 (0%)	1/32 (3%)	1/23 (4%)
First incidence (days)	731 (T)	–	731 (T)	731 (T)
Life table tests	P=0.359N	P=0.103N	P=0.269N	P=0.390N
Logistic regression tests	P=0.359N	P=0.103N	P=0.269N	P=0.390N
Cochran-Armitage test	P=0.296N			
Fisher exact test		P=0.121N	P=0.309N	P=0.309N
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, or Mixed)				
Overall rates	6/54 (11%)	3/54 (6%)	5/54 (9%)	8/54 (15%)
Adjusted rates	19.0%	7.4%	15.6%	29.4%
Terminal rates	4/29 (14%)	1/32 (3%)	5/32 (16%)	5/23 (22%)
First incidence (days)	637	578	731 (T)	597
Life table tests	P=0.100	P=0.195N	P=0.438N	P=0.257
Logistic regression tests	P=0.113	P=0.186N	P=0.448N	P=0.286
Cochran-Armitage test	P=0.201			
Fisher exact test		P=0.244N	P=0.500N	P=0.388

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Ethylene Glycol
(continued)

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
All Organs: Benign Neoplasms				
Overall rates	17/54 (31%)	11/54 (20%)	13/54 (24%)	19/54 (35%)
Adjusted rates	54.3%	32.7%	40.6%	69.2%
Terminal rates	15/29 (52%)	10/32 (31%)	13/32 (41%)	15/23 (65%)
First incidence (days)	469	599	731 (T)	317
Life table tests	P=0.042	P=0.062N	P=0.144N	P=0.135
Logistic regression tests	P=0.075	P=0.049N	P=0.171N	P=0.225
Cochran-Armitage test	P=0.236			
Fisher exact test		P=0.136N	P=0.260N	P=0.419
All Organs: Malignant Neoplasms				
Overall rates	22/54 (41%)	28/54 (52%)	23/54 (43%)	20/54 (37%)
Adjusted rates	52.1%	57.8%	53.5%	54.9%
Terminal rates	9/29 (31%)	12/32 (38%)	12/32 (38%)	7/23 (30%)
First incidence (days)	350	514	517	498
Life table tests	P=0.498	P=0.401	P=0.517N	P=0.477
Logistic regression tests	P=0.459N	P=0.271	P=0.545	P=0.561
Cochran-Armitage test	P=0.236N			
Fisher exact test		P=0.167	P=0.500	P=0.422N
All Organs: Benign or Malignant Neoplasms				
Overall rates	34/54 (63%)	36/54 (67%)	31/54 (57%)	33/54 (61%)
Adjusted rates	79.0%	73.3%	72.1%	86.6%
Terminal rates	20/29 (69%)	19/32 (59%)	20/32 (63%)	18/23 (78%)
First incidence (days)	350	514	517	317
Life table tests	P=0.229	P=0.416N	P=0.226N	P=0.254
Logistic regression tests	P=0.256	P=0.433N	P=0.248N	P=0.293
Cochran-Armitage test	P=0.370N			
Fisher exact test		P=0.420	P=0.347N	P=0.500N

(T)Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.

[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.

[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; therefore, statistical comparisons with the controls are not appropriate.

[f] Not applicable; no neoplasms in animal group

TABLE A4
Historical Incidence of Harderian Gland Neoplasms in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenomas	Carcinomas	Adenomas or Carcinomas
Historical Incidence at Southern Research Institute			
C.I. Pigment Red 3	2/50	0/50	2/50
Ethylene Glycol	0/54	0/54	0/54
Nitrofurantoin	2/50	0/50	2/50
o-Nitroanisole	9/50	1/50	10/50
Polysorbate 80	0/49	0/49	0/49
Rhodamine 6G	7/50	0/50	7/50
Roxarsone	1/50	0/50	1/50
Total	21/353 (5.9%)	1/353 (0.3%)	22/353 (6.2%)
Standard deviation	7.1%	0.8%	7.7%
Range	0%-18%	0%-2%	0%-20%
Overall Historical Incidence			
Total	45/872 (5.2%)	3/872 (0.3%)	48/872 (5.5%)
Standard deviation	4.8%	0.8%	5.3%
Range	0%-18%	0%-2%	0%-20%

[a] Data as of 3 April 1991

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Ethylene Glycol[a]

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-month interim evaluation	6	6	6	6
Early deaths				
Natural deaths	15	8	11	16
Moribund	10	14	11	15
Survivors				
Terminal sacrifice	29	32	32	23
Animals examined microscopically	54	54	54	54
Alimentary System				
Esophagus	(54)	(21)	(22)	(54)
Inflammation, chronic	1 (2%)			
Gallbladder	(51)	(19)	(16)	(48)
Inflammation	1 (2%)		1 (6%)	
Mucosa, hyperplasia			1 (6%)	
Intestine large, cecum	(52)	(18)	(18)	(49)
Parasite metazoan				1 (2%)
Intestine large, rectum	(54)	(19)	(21)	(48)
Inflammation, chronic				1 (2%)
Prolapse		1 (5%)		
Intestine small, duodenum	(53)	(20)	(18)	(46)
Mucosa, inflammation, suppurative				1 (2%)
Intestine small, ileum	(53)	(17)	(17)	(45)
Perforation		1 (6%)		
Lymphoid tissue, hyperplasia, lymphoid				1 (2%)
Intestine small, jejunum	(54)	(18)	(19)	(50)
Hyperplasia, lymphoid				1 (2%)
Inflammation, suppurative				1 (2%)
Liver	(54)	(53)	(53)	(54)
Angiectasis	1 (2%)			
Autolysis				1 (2%)
Bacterium				2 (4%)
Basophilic focus	1 (2%)	2 (4%)		2 (4%)
Congestion			1 (2%)	1 (2%)
Cyst			1 (2%)	
Eosinophilic focus	2 (4%)			1 (2%)
Fatty change		2 (4%)		
Fibrosis				2 (4%)
Focal cellular change	1 (2%)		1 (2%)	
Hematopoietic cell proliferation		3 (6%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid	2 (4%)	3 (6%)	3 (6%)	2 (4%)
Hyperplasia, multifocal	1 (2%)			
Infarct				1 (2%)
Infiltration cellular, mixed cell	4 (7%)	5 (9%)	2 (4%)	8 (15%)
Inflammation, multifocal	6 (11%)	11 (21%)	16 (30%)	13 (24%)
Necrosis, multifocal	10 (19%)	6 (11%)	4 (8%)	8 (15%)
Pigmentation		1 (2%)	1 (2%)	3 (6%)
Artery, media, hypertrophy				1 (2%)
Bile duct, cyst	1 (2%)			
Bile duct, hyperplasia	1 (2%)			

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Ethylene Glycol (continued)

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Alimentary System (continued)				
Liver (continued)				
Centrilobular, necrosis		1 (2%)	2 (4%)	1 (2%)
Centrilobular, vacuolization nuclear				1 (2%)
Hepatocyte, cytomegaly		1 (2%)		
Hepatocyte, centrilobular, degeneration, hyaline			24 (45%)	36 (67%)
Mesentery	(5)	(3)	(1)	(7)
Inflammation, chronic		2 (67%)	1 (100%)	1 (14%)
Inflammation, granulomatous				1 (14%)
Inflammation, suppurative	1 (20%)			3 (43%)
Fat, necrosis				1 (14%)
Pancreas	(54)	(23)	(21)	(54)
Atrophy, focal		1 (4%)		
Fibrosis, focal	1 (2%)			
Focal cellular change	1 (2%)			
Inflammation, focal, chronic				2 (4%)
Necrosis, focal	1 (2%)			
Duct, degeneration, hyaline	1 (2%)			
Duct, dilatation	1 (2%)		1 (5%)	
Duct, hyperplasia, papillary	1 (2%)			
Duct, metaplasia	1 (2%)			
Vein, infiltration cellular, mixed cell	1 (2%)			
Salivary glands	(53)	(21)	(20)	(54)
Atrophy, focal	1 (2%)			
Fibrosis, focal	1 (2%)			
Stomach, forestomach	(54)	(20)	(21)	(54)
Mineralization	1 (2%)			
Mucosa, hyperplasia, papillary	2 (4%)			
Mucosa, ulcer				1 (2%)
Stomach, glandular	(54)	(20)	(21)	(54)
Erosion		1 (5%)		
Inflammation, focal	1 (2%)		1 (5%)	1 (2%)
Mineralization	2 (4%)		2 (10%)	
Epithelium, metaplasia, squamous	1 (2%)			
Epithelium, vacuolization cytoplasmic, multifocal				1 (2%)
Mucosa, cyst	2 (4%)			
Mucosa, erosion				1 (2%)
Tooth	(21)	(4)	(1)	(18)
Incisor, dysplasia	18 (86%)	4 (100%)	1 (100%)	15 (83%)
Incisor, inflammation, chronic	1 (5%)			
Incisor, inflammation, suppurative	2 (10%)			
Cardiovascular System				
Heart	(54)	(21)	(22)	(54)
Inflammation, chronic	3 (6%)			1 (2%)
Inflammation, focal				1 (2%)
Inflammation, suppurative	1 (2%)			3 (6%)
Atrium, thrombus, multiple				1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Ethylene Glycol (continued)

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Endocrine System				
Adrenal gland	(54)	(22)	(22)	(54)
Infiltration cellular, mixed cell				1 (2%)
Adrenal gland, cortex	(54)	(20)	(21)	(53)
Cyst	1 (2%)			
Focal cellular change				1 (2%)
Hyperplasia, focal		1 (5%)		1 (2%)
Vacuolization cytoplasmic, focal	1 (2%)			
Extra adrenal tissue, fibrosis	1 (2%)			
Extra adrenal tissue, inflammation, suppurative				1 (2%)
Subcapsular, hyperplasia, focal	2 (4%)		2 (10%)	4 (8%)
Unilateral, atrophy			1 (5%)	
Adrenal gland, medulla	(54)	(21)	(21)	(53)
Hyperplasia, focal	1 (2%)		1 (5%)	1 (2%)
Infiltration cellular, mixed cell				1 (2%)
Parathyroid gland	(53)	(18)	(22)	(50)
Cyst			1 (5%)	
Pituitary gland	(52)	(21)	(17)	(49)
Pars distalis, angiectasis			1 (6%)	
Pars distalis, cyst				1 (2%)
Pars distalis, hyperplasia, focal	5 (10%)		1 (6%)	2 (4%)
Thyroid gland	(53)	(53)	(54)	(53)
Cyst	1 (2%)		2 (4%)	
Degeneration, cystic	12 (23%)	13 (25%)	13 (24%)	9 (17%)
Fibrosis			1 (2%)	
Inflammation, focal	1 (2%)			
Follicle, cyst		1 (2%)	4 (7%)	
Follicular cell, hyperplasia	5 (9%)	8 (15%)	4 (7%)	9 (17%)
General Body System				
None				
Genital System				
Coagulating gland			(1)	(1)
Inflammation, suppurative				1 (100%)
Adventitia, hemorrhage			1 (100%)	
Ductus deferens				(1)
Inflammation, chronic				1 (100%)
Epididymis	(54)	(21)	(22)	(54)
Granuloma sperm			1 (5%)	1 (2%)
Hyperplasia, lymphoid	1 (2%)			
Inflammation, chronic				2 (4%)
Inflammation, suppurative	1 (2%)			
Spermatocele	1 (2%)			
Penis	(5)	(1)	(4)	(6)
Fibrosis		1 (100%)		
Hyperplasia				1 (17%)
Inflammation, chronic	1 (20%)	1 (100%)	3 (75%)	1 (17%)
Inflammation, suppurative	1 (20%)			
Necrosis	2 (40%)		1 (25%)	

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Ethylene Glycol (continued)

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Genital System (continued)				
Preputial gland	(12)	(6)	(7)	(12)
Degeneration, cystic	8 (67%)	3 (50%)	3 (43%)	6 (50%)
Inflammation, suppurative	8 (67%)	5 (83%)	2 (29%)	7 (58%)
Prostate	(53)	(20)	(22)	(54)
Dilatation				1 (2%)
Edema				1 (2%)
Inflammation, chronic	6 (11%)	1 (5%)	5 (23%)	10 (19%)
Seminal vesicle	(52)	(23)	(23)	(54)
Dilatation	4 (8%)	3 (13%)	2 (9%)	4 (7%)
Inflammation, chronic	9 (17%)	3 (13%)	1 (4%)	8 (15%)
Epithelium, hyperplasia		1 (4%)		
Testes	(54)	(22)	(22)	(54)
Inflammation, chronic				1 (2%)
Mineralization	1 (2%)	1 (5%)	1 (5%)	3 (6%)
Necrosis, multifocal				1 (2%)
Germinal epithelium, degeneration	3 (6%)	1 (5%)	1 (5%)	5 (9%)
Interstitial cell, hyperplasia				1 (2%)
Tunic, proliferation connective tissue			1 (5%)	
Hematopoietic System				
Blood	(1)	(3)		(1)
Neutrophilia	1 (100%)			
Bone marrow	(54)	(21)	(22)	(54)
Atypical cells, focal				1 (2%)
Hyperplasia, neutrophil	1 (2%)		1 (5%)	
Lymph node	(54)	(33)	(27)	(54)
Iliac, hyperplasia	1 (2%)		1 (4%)	3 (6%)
Inguinal, hyperplasia	2 (4%)	1 (3%)	4 (15%)	1 (2%)
Inguinal, hyperplasia, lymphoid				2 (4%)
Inguinal, inflammation, chronic	1 (2%)			1 (2%)
Mesenteric, angiectasis	19 (35%)	17 (52%)	11 (41%)	22 (41%)
Mesenteric, depletion lymphoid	1 (2%)			
Mesenteric, hematopoietic cell proliferation				1 (2%)
Mesenteric, hyperplasia				3 (6%)
Mesenteric, hyperplasia, lymphoid	1 (2%)	2 (6%)		2 (4%)
Mesenteric, hyperplasia, reticulum cell				1 (2%)
Mesenteric, inflammation, granulomatous, chronic				1 (2%)
Mesenteric, inflammation, suppurative				1 (2%)
Mesenteric, thrombus				1 (2%)
Lymph node, mandibular	(45)	(19)	(17)	(51)
Angiectasis	2 (4%)			
Depletion lymphoid	1 (2%)			
Hyperplasia, lymphoid	1 (2%)			4 (8%)
Spleen	(54)	(53)	(53)	(53)
Angiectasis	1 (2%)			
Congestion				1 (2%)
Depletion	1 (2%)	3 (6%)		1 (2%)
Hematopoietic cell proliferation	17 (31%)	25 (47%)	25 (47%)	28 (53%)
Hyperplasia, lymphoid	1 (2%)		1 (2%)	2 (4%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Ethylene Glycol (continued)

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Hematopoietic System (continued)				
Thymus	(53)	(19)	(19)	(51)
Atypical cells				1 (2%)
Cyst	1 (2%)	1 (5%)		1 (2%)
Degeneration, cystic				1 (2%)
Depletion lymphoid	1 (2%)	2 (11%)	3 (16%)	7 (14%)
Hyperplasia, reticulum cell				1 (2%)
Epithelial cell, hyperplasia, cystic	1 (2%)			
Integumentary System				
Skin	(54)	(45)	(43)	(54)
Erosion				1 (2%)
Exudate		1 (2%)		1 (2%)
Fibrosis			1 (2%)	
Hyperplasia, melanocyte			1 (2%)	
Inflammation, chronic	25 (46%)	24 (53%)	27 (63%)	18 (33%)
Metaplasia, osseous		1 (2%)	1 (2%)	
Ulcer	4 (7%)	5 (11%)	5 (12%)	9 (17%)
Hair follicle, atrophy	1 (2%)	1 (2%)	2 (5%)	
Prepuce, angiectasis	1 (2%)			
Prepuce, fibrosis	1 (2%)			
Prepuce, foreign body	1 (2%)	1 (2%)		
Prepuce, inflammation, chronic	5 (9%)	1 (2%)	2 (5%)	2 (4%)
Prepuce, ulcer	5 (9%)	1 (2%)	3 (7%)	3 (6%)
Subcutaneous tissue, edema	1 (2%)	1 (2%)	1 (2%)	
Subcutaneous tissue, exudate	1 (2%)			
Subcutaneous tissue, fibrosis		1 (2%)	1 (2%)	
Subcutaneous tissue, foreign body	1 (2%)	1 (2%)		
Subcutaneous tissue, inflammation, chronic	1 (2%)			
Subcutaneous tissue, lymphatic, angiectasis				1 (2%)
Tail, subcutaneous tissue, inflammation, granulomatous				1 (2%)
Musculoskeletal System				
Bone	(54)	(21)	(22)	(54)
Distal, joint, femur, arthrosis	1 (2%)	1 (5%)		
Femur, hyperostosis				1 (2%)
Nervous System				
Brain	(54)	(21)	(22)	(54)
Congestion				1 (2%)
Gliosis	1 (2%)			
Infiltration cellular, mixed cell			1 (5%)	
Inflammation, focal			1 (5%)	

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Ethylene Glycol (continued)

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Respiratory System				
Lung	(54)	(25)	(32)	(54)
Bacterium				2 (4%)
Congestion	5 (9%)	2 (8%)	3 (9%)	3 (6%)
Edema		1 (4%)		
Foreign body				1 (2%)
Hemorrhage				2 (4%)
Hyperplasia, lymphoid	4 (7%)		2 (6%)	4 (7%)
Hyperplasia, macrophage	1 (2%)		2 (6%)	1 (2%)
Infiltration cellular, mixed cell	8 (15%)	2 (8%)	7 (22%)	8 (15%)
Inflammation, granulomatous				1 (2%)
Pigmentation				1 (2%)
Alveolar epithelium, hyperplasia	2 (4%)		2 (6%)	
Artery, embolus	1 (2%)			
Interstitium, inflammation		1 (4%)		1 (2%)
Nose	(54)	(21)	(21)	(54)
Lumen, exudate	2 (4%)	2 (10%)		
Lumen, foreign body		1 (5%)		
Nasolacrimal duct, foreign body	1 (2%)			
Nasolacrimal duct, inflammation, suppurative	2 (4%)	1 (5%)		
Special Senses System				
None				
Urinary System				
Kidney	(54)	(53)	(53)	(54)
Angiectasis		1 (2%)		
Atrophy, focal			1 (2%)	1 (2%)
Bacterium	2 (4%)		1 (2%)	4 (7%)
Congestion	3 (6%)		1 (2%)	
Hematopoietic cell proliferation	1 (2%)			
Infiltration cellular, mixed cell				1 (2%)
Inflammation, suppurative	4 (7%)	1 (2%)	1 (2%)	4 (7%)
Metaplasia, osseous	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Mineralization				1 (2%)
Necrosis			1 (2%)	
Necrosis, focal				2 (4%)
Nephropathy	39 (72%)	44 (83%)	41 (77%)	38 (70%)
Papilla, necrosis		1 (2%)	1 (2%)	
Pelvis, crystals				1 (2%)
Pelvis, dilatation				2 (4%)
Pelvis, inflammation, chronic				2 (4%)
Pelvis, mineralization				2 (4%)
Pelvis, transitional epithelium, hyperplasia				1 (2%)
Renal tubule, crystals				8 (15%)
Renal tubule, degeneration, hyaline		1 (2%)		1 (2%)
Renal tubule, dilatation	6 (11%)	2 (4%)	7 (13%)	13 (24%)
Renal tubule, hyperplasia			1 (2%)	
Renal tubule, mineralization		1 (2%)	1 (2%)	
Renal tubule, necrosis	1 (2%)			

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Ethylene Glycol (continued)

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Urinary System (continued)				
Kidney (continued)				
Renal tubule, vacuolization cytoplasmic	51 (94%)	50 (94%)	48 (91%)	52 (96%)
Renal tubule, epithelium, necrosis		2 (4%)		1 (2%)
Urethra	(1)	(4)	(2)	(18)
Fibrosis		1 (25%)		
Hemorrhage				1 (6%)
Inflammation, suppurative	1 (100%)	2 (50%)	2 (100%)	7 (39%)
Perforation	1 (100%)			
Bulbourethral gland, dilatation		2 (50%)		1 (6%)
Bulbourethral gland, fibrosis		1 (25%)		
Lumen, crystals				12 (67%)
Urinary bladder	(53)	(53)	(53)	(54)
Calculus gross observation				2 (4%)
Calculus micro observation only				2 (4%)
Congestion	1 (2%)			
Hemorrhage	1 (2%)		1 (2%)	
Hyperplasia, lymphoid		1 (2%)	2 (4%)	1 (2%)
Inflammation, chronic	1 (2%)	4 (8%)	6 (11%)	8 (15%)
Ulcer				1 (2%)
Artery, serosa, mineralization				1 (2%)
Transitional epithelium, hyperplasia	1 (2%)	1 (2%)		1 (2%)

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

APPENDIX B
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDY OF ETHYLENE GLYCOL

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Ethylene Glycol[a]

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-month interim evaluation	10	10	9	10
Early deaths				
Natural deaths	8	11	9	1
Moribund	9	9	12	12
Survivors				
Terminal sacrifice	33	30	30	37
Animals examined microscopically	50	50	51	50
Alimentary System				
Esophagus	(50)	(20)	(21)	(50)
Gallbladder	(48)	(18)	(21)	(49)
Intestine large, cecum	(48)	(19)	(16)	(50)
Intestine large, colon	(49)	(19)	(19)	(50)
Sarcoma		1 (5%)		
Intestine large, rectum	(49)	(19)	(17)	(50)
Intestine small, duodenum	(47)	(18)	(17)	(50)
Intestine small, ileum	(47)	(19)	(16)	(50)
Intestine small, jejunum	(47)	(19)	(16)	(50)
Liver	(50)	(50)	(51)	(50)
Adenocarcinoma, metastatic, multiple, ovary		1 (2%)		
Hepatocellular carcinoma	3 (6%)	4 (8%)	6 (12%)	3 (6%)
Hepatocellular carcinoma, multiple, two		1 (2%)		
Hepatocellular adenoma	8 (16%)	5 (10%)	8 (16%)	7 (14%)
Mesentery	(17)	(7)	(15)	(7)
Adenocarcinoma, metastatic, multiple, ovary		1 (14%)		
Sarcoma		1 (14%)		1 (14%)
Pancreas	(49)	(21)	(22)	(50)
Adenocarcinoma, metastatic, ovary		1 (5%)		
Sarcoma		1 (5%)		
Salivary glands	(49)	(20)	(21)	(50)
Stomach, forestomach	(50)	(20)	(20)	(50)
Stomach, glandular	(49)	(20)	(20)	(50)
Cardiovascular System				
Heart	(50)	(20)	(21)	(50)
Endocrine System				
Adrenal gland, cortex	(49)	(21)	(21)	(50)
Adrenal gland, medulla	(48)	(21)	(21)	(50)
Pheochromocytoma malignant		1 (5%)		
Pheochromocytoma benign		1 (5%)	1 (5%)	
Islets, pancreatic	(49)	(19)	(21)	(50)
Adenoma		1 (5%)		
Pituitary gland	(47)	(22)	(23)	(47)
Pars distalis, adenoma	17 (36%)	5 (23%)	7 (30%)	16 (34%)
Pars distalis, carcinoma			1 (4%)	
Pars intermedia, adenoma		1 (5%)		1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Ethylene Glycol
(continued)

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Endocrine System (continued)				
Thyroid gland	(49)	(50)	(51)	(50)
Follicular cell, adenoma	1 (2%)	1 (2%)	1 (2%)	3 (6%)
General Body System				
Tissue NOS		(2)	(1)	
Sarcoma		1 (50%)		
Genital System				
Ovary	(49)	(25)	(25)	(48)
Adenocarcinoma		1 (4%)		
Cystadenoma, papillary	2 (4%)	1 (4%)	1 (4%)	4 (8%)
Teratoma NOS				1 (2%)
Oviduct	(3)		(4)	(1)
Uterus	(50)	(45)	(45)	(50)
Adenocarcinoma				1 (2%)
Leiomyosarcoma				1 (2%)
Endometrium, polyp stromal	3 (6%)	3 (7%)	1 (2%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(20)	(21)	(50)
Lymph node	(50)	(24)	(22)	(50)
Mediastinal, adenocarcinoma, metastatic, ovary		1 (4%)		
Lymph node, mandibular	(43)	(21)	(20)	(49)
Spleen	(49)	(29)	(28)	(50)
Hemangiosarcoma			1 (4%)	
Thymus	(47)	(19)	(19)	(47)
Integumentary System				
Mammary gland	(49)	(20)	(21)	(50)
Adenoacanthoma				1 (2%)
Adenocarcinoma	1 (2%)		1 (5%)	
Skin	(49)	(43)	(40)	(50)
Subcutaneous tissue, fibrosarcoma			2 (5%)	
Subcutaneous tissue, hemangiosarcoma			1 (3%)	
Subcutaneous tissue, sarcoma	1 (2%)			
Musculoskeletal System				
Bone	(50)	(20)	(21)	(50)
Osteosarcoma		1 (5%)	1 (5%)	
Skeletal muscle	(2)	(2)	(2)	(1)
Osteosarcoma, metastatic, bone		1 (50%)		
Abdominal, sarcoma				1 (100%)
Neck, sarcoma		1 (50%)		
Thoracic, sarcoma		1 (50%)		
Nervous System				
Brain	(49)	(20)	(21)	(50)

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Ethylene Glycol (continued)

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Respiratory System				
Lung	(50)	(50)	(51)	(50)
Adenocarcinoma, metastatic, multiple, greater than five, ovary		1 (2%)		
Alveolar/bronchiolar adenoma		4 (8%)	4 (8%)	1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)	2 (4%)	2 (4%)	
Carcinoma, metastatic, harderian gland			1 (2%)	1 (2%)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Hepatocellular carcinoma, metastatic, multiple, greater than five, liver	1 (2%)			
Osteosarcoma, metastatic, bone			1 (2%)	
Nose	(50)	(20)	(21)	(50)
Trachea	(49)	(20)	(21)	(50)
Special Senses System				
Harderian gland	(3)	(2)	(2)	(2)
Adenoma	2 (67%)	1 (50%)	1 (50%)	
Carcinoma		1 (50%)	1 (50%)	2 (100%)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Osteosarcoma, metastatic, bone			1 (2%)	
Urinary bladder	(49)	(19)	(21)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(51)	(50)
Lymphoma malignant histiocytic		2 (4%)	2 (4%)	1 (2%)
Lymphoma malignant lymphocytic	3 (6%)	3 (6%)	4 (8%)	
Lymphoma malignant mixed	13 (26%)	8 (16%)	3 (6%)	6 (12%)
Lymphoma malignant undifferentiated cell	1 (2%)			
Neoplasm Summary				
Total animals with primary neoplasms[c]	39	33	31	36
Total primary neoplasms	56	53	49	51
Total animals with benign neoplasms	29	19	19	26
Total benign neoplasms	33	23	24	33
Total animals with malignant neoplasms	21	21	22	16
Total malignant neoplasms	23	30	25	17
Total animals with metastatic neoplasms	2	2	2	2
Total metastatic neoplasms	2	6	3	2
Total animals with malignant neoplasms uncertain primary site				1
Total animals with neoplasms uncertain-benign or malignant				1
Total uncertain neoplasms				1

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm

Number of Days on Study	4 7 2	5 1 8	5 2 0	5 3 6	5 4 6	5 7 4	5 7 8	5 8 1	6 0 5	6 0 6	6 5 5	6 6 5	6 6 5	6 9 6	6 9 6	6 9 7	6 9 8	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 6	7 3 6	7 3 6
Carcass ID Number	5 2 1	5 6 5	5 5 3	5 3 2	5 2 3	5 2 5	5 8 3	5 7 5	5 3 4	5 5 1	5 5 2	4 9 5	5 0 3	5 3 1	5 6 2	5 0 1	5 1 1	4 9 1	4 9 2	4 9 3	4 9 4	5 0 2	5 0 4	5 0 5	5 1 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	A	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma											X		X			X									
Hepatocellular adenoma															X	X				X					
Mesentery	+	+	+	+	+	+	+	+							+					+					+
Pancreas	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth					+																				
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X			X				X				X			X			X	X
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																					X				
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm
(continued)

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	5 1 3	5 1 4	5 1 5	5 2 2	5 2 4	5 3 3	5 3 5	5 4 1	5 4 2	5 4 3	5 4 4	5 4 5	5 5 4	5 5 5	5 6 1	5 6 3	5 6 4	5 7 1	5 7 2	5 7 3	5 7 4	5 8 1	5 8 2	5 8 4	5 8 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																										3
Hepatocellular adenoma				X							X	X		X	X											8
Mesentery							+	+		+							+						+	+		17
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth																				+						2
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	48
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	47
Pars distalis, adenoma					X	X					X				X		X	X	X			X		X	X	17
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell, adenoma																										1
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm
(continued)

Number of Days on Study	4 7 2	5 1 8	5 2 0	5 3 6	5 4 6	5 7 4	5 7 8	5 8 1	6 0 5	6 0 6	6 5 5	6 6 5	6 6 5	6 9 6	6 9 6	6 9 7	6 9 8	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 6	7 3 6	7 3 6
Carcass ID Number	5 2 1	5 6 5	5 5 3	5 3 2	5 2 3	5 2 5	5 8 3	5 7 5	5 3 4	5 5 1	5 5 2	4 9 5	5 0 3	5 3 1	5 6 2	5 0 1	5 1 1	4 9 1	4 9 2	4 9 3	4 9 4	5 0 2	5 0 4	5 0 5	5 1 2
Genital System																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma, papillary										X							X								
Oviduct			+																						
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal											X								X						
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	X	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma															X										
Skin	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, sarcoma														X											
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle					+			+																	
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																									
Hepatocellular carcinoma, metastatic, liver											X														
Hepatocellular carcinoma, metastatic, multiple, greater than five, liver													X												
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm
(continued)

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	5 1 3	5 1 4	5 1 5	5 2 2	5 2 4	5 3 3	5 3 5	5 4 1	5 4 2	5 4 3	5 4 4	5 4 5	5 5 4	5 5 5	5 6 1	5 6 3	5 6 4	5 7 1	5 7 2	5 7 3	5 7 4	5 8 1	5 8 2	5 8 4	5 8 5	Total Tissues/ Tumors
Genital System																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	49
Cystadenoma, papillary							+																			2
Oviduct	+						+																			3
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal																				X						3
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	M	M	+	+	+	+	43
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																			X							1
Hepatocellular carcinoma, metastatic, liver																										1
Hepatocellular carcinoma, metastatic, multiple, greater than five, liver																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm
(continued)

Number of Days on Study	4	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	
	7	1	2	3	4	7	7	8	0	0	5	6	6	9	9	9	9	3	3	3	3	3	3	3	3	
	2	8	0	6	6	4	8	1	5	6	5	5	5	6	6	7	8	1	1	1	1	1	6	6	6	
Carcass ID Number	5	5	5	5	5	5	5	5	5	5	5	4	5	5	5	5	5	4	4	4	4	5	5	5	5	
	2	6	5	3	2	2	8	7	3	5	5	9	0	3	6	0	1	9	9	9	9	0	0	0	1	
	1	5	3	2	3	5	3	5	4	1	2	5	3	1	2	1	1	1	2	3	4	2	4	5	2	
Special Senses System																										
Harderian gland														+											+	
Adenoma																									X	
Urinary System																										
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymphoma malignant lymphocytic			X					X							X											
Lymphoma malignant mixed												X		X										X		
Lymphoma malignant undifferentiated cell type					X																					

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 0 ppm
(continued)

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	5 1 3	5 1 4	5 1 5	5 2 2	5 2 4	5 3 3	5 3 5	5 4 1	5 4 2	5 4 3	5 4 4	5 4 5	5 5 4	5 5 5	5 5 1	5 6 3	5 6 4	5 7 1	5 7 2	5 7 3	5 7 4	5 8 1	5 8 2	5 8 4	5 8 5	Total Tissues/ Tumors
Special Senses System																										
Harderian gland							+																			3
Adenoma							X																			2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										3
Lymphoma malignant mixed						X	X	X		X					X	X	X	X		X	X					13
Lymphoma malignant undifferentiated cell type																										1

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 12,500 ppm

Number of Days on Study	4 7 2	4 7 6	5 3 3	5 3 5	5 3 9	5 4 3	5 5 4	5 6 1	5 7 7	5 9 4	6 1 2	6 3 3	6 4 4	6 6 3	6 7 9	6 8 8	6 9 6	6 9 6	7 1 8	7 2 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6
Carcass ID Number	8 8 1	9 2 1	9 4 4	8 7 4	8 6 5	8 7 3	8 9 2	8 8 3	9 2 5	9 1 2	9 1 3	9 1 4	8 9 1	8 6 2	8 7 2	8 5 1	8 5 4	8 9 5	8 8 4	9 0 2	8 5 2	8 5 3	8 5 5	8 6 1	8 6 3
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Gallbladder	+	M	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+					
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+					
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+					
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+					
Sarcoma																X									
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+					
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+					
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	M	+	+	+	+	A	+	+	+	+	+					
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+					
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+					
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, multiple, ovary															X										
Hepatocellular carcinoma															X					X					
Hepatocellular carcinoma, multiple, two																									
Hepatocellular adenoma															X										
Mesentery				+			+					+			+	+			+						
Adenocarcinoma, metastatic, multiple, ovary															X										
Sarcoma																X									
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Adenocarcinoma, metastatic, ovary															X										
Sarcoma																X									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Tooth								+		+	+								+						
Cardiovascular System																									
Blood vessel									+										+						
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 12,500 ppm
(continued)

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	8 6 4	8 7 1	8 7 5	8 8 2	8 8 5	8 9 3	8 9 4	9 0 1	9 0 3	9 0 4	9 0 5	9 1 1	9 1 5	9 2 2	9 2 3	9 2 4	9 3 1	9 3 2	9 3 3	9 3 4	9 3 5	9 4 1	9 4 2	9 4 3	9 4 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus																										20
Gallbladder																										18
Intestine large																										19
Intestine large, cecum																										19
Intestine large, colon																										19
Sarcoma																										1
Intestine large, rectum																										19
Intestine small																										19
Intestine small, duodenum																										18
Intestine small, ileum																										19
Intestine small, jejunum																										19
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, multiple, ovary																										1
Hepatocellular carcinoma							X																		X	4
Hepatocellular carcinoma, multiple, two																							X			1
Hepatocellular adenoma		X													X			X		X						5
Mesentery																								+		7
Adenocarcinoma, metastatic, multiple, ovary																										1
Sarcoma																										1
Pancreas											+				+											21
Adenocarcinoma, metastatic, ovary																										1
Sarcoma																										1
Salivary glands																										20
Stomach																										20
Stomach, forestomach																										20
Stomach, glandular																										20
Tooth																										4
Cardiovascular System																										
Blood vessel																										2
Heart																										20

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 12,500 ppm
(continued)

Number of Days on Study	4 7 2	4 7 6	5 3 3	5 3 5	5 3 9	5 4 3	5 5 4	5 6 1	5 7 7	5 9 4	6 1 2	6 3 3	6 4 4	6 6 3	6 7 9	6 8 8	6 9 6	7 1 6	7 2 8	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6
Carcass ID Number	8 8 1	9 2 1	9 4 4	8 7 4	8 6 5	8 7 3	8 9 2	8 8 3	9 2 5	9 1 2	9 1 3	9 1 4	8 9 1	8 6 2	8 7 2	8 5 1	8 5 4	8 9 5	8 8 4	9 0 2	8 5 2	8 5 3	8 5 5	8 6 1	8 6 3
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
Pheochromocytoma malignant																									
Pheochromocytoma benign												X													
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
Adenoma										X															
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+						
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
Pars distalis, adenoma													X				X		X						
Pars intermedia, adenoma																		X							
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma													X												
General Body System																									
Tissue NOS																+			+						
Sarcoma																			X						
Genital System																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Adenocarcinoma															X										
Cystadenoma, papillary																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal					X															X					
Hematopoietic System																									
Blood												+													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Mediastinal, adenocarcinoma, metastatic, ovary															X										
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+		
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+						
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 12,500 ppm
(continued)

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	8 6 4	8 7 1	8 7 5	8 8 2	8 8 5	8 9 3	8 9 4	9 0 1	9 0 3	9 0 4	9 0 5	9 1 1	9 1 5	9 2 2	9 2 3	9 2 4	9 3 1	9 3 2	9 3 3	9 3 4	9 3 5	9 4 1	9 4 2	9 4 3	9 4 5	Total Tissues/ Tumors
Endocrine System																										
Adrenal gland																									+	21
Adrenal gland, cortex																									+	21
Adrenal gland, medulla																									+	21
Pheochromocytoma malignant																									X	1
Pheochromocytoma benign																										1
Islets, pancreatic																										19
Adenoma																										1
Parathyroid gland																										18
Pituitary gland																				+	+					22
Pars distalis, adenoma																				X	X					5
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
General Body System																										
Tissue NOS																										2
Sarcoma																										1
Genital System																										
Ovary			+											+				+						+		25
Adenocarcinoma																										1
Cystadenoma, papillary			X																							1
Uterus	+		+	+	+	+	+		+		+		+	+	+	+	+	+	+		+	+	+	+	+	45
Endometrium, polyp stromal																							X			3
Hematopoietic System																										
Blood																										1
Bone marrow																										20
Lymph node										+				+	+											24
Mediastinal, adenocarcinoma, metastatic, ovary																										1
Lymph node, mandibular														+												21
Spleen	+											+		+	+				+	+				+		29
Thymus																										19
Integumentary System																										
Mammary gland																										20
Skin	+	+	+		+		+	+	+	+	+	+	+	+	+		+		+	+		+		+	+	43

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 12,500 ppm
(continued)

Number of Days on Study	4 7 2	4 7 6	5 3 3	5 3 5	5 3 9	5 4 3	5 5 4	5 6 1	5 7 7	5 9 4	6 1 2	6 3 3	6 4 4	6 6 3	6 7 9	6 8 8	6 9 6	6 9 6	7 1 8	7 2 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6
Carcass ID Number	8 8 1	9 2 1	9 4 4	8 7 4	8 6 5	8 7 3	8 9 2	8 8 3	9 2 5	9 1 2	9 1 3	9 1 4	8 9 1	8 6 2	8 7 2	8 5 1	8 5 4	8 9 5	8 8 4	8 0 2	8 5 2	8 5 3	8 5 5	8 6 1	8 6 3
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Osteosarcoma			X																						
Skeletal muscle			+													+									
Osteosarcoma, metastatic, bone			X																						
Neck, sarcoma																X									
Thoracic, sarcoma																X									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, multiple, greater than five, ovary															X										
Alveolar/bronchiolar adenoma	X												X												X
Alveolar/bronchiolar carcinoma																	X								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Special Senses System																									
Harderian gland																									
Adenoma																									
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic		X																X							
Lymphoma malignant lymphocytic																					X				
Lymphoma malignant mixed			X							X				X											

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 12,500 ppm
(continued)

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	8 6 4	8 7 1	8 7 5	8 8 2	8 8 5	8 9 3	8 9 4	9 0 1	9 0 3	9 0 4	9 0 5	9 1 1	9 1 5	9 2 2	9 2 3	9 2 4	9 3 1	9 3 2	9 3 3	9 3 4	9 3 5	9 4 1	9 4 2	9 4 3	9 4 5	**Total Tissues/ Tumors**
Musculoskeletal System																										
Bone																										20
Osteosarcoma																										1
Skeletal muscle																										2
Osteosarcoma, metastatic, bone																										1
Neck, sarcoma																										1
Thoracic, sarcoma																										1
Nervous System																										
Brain																										20
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, multiple, greater than five, ovary																										1
Alveolar/bronchiolar adenoma																						X				4
Alveolar/bronchiolar carcinoma												X														2
Nose																										20
Trachea																										20
Special Senses System																										
Harderian gland			+																					+		2
Adenoma			X																							1
Carcinoma																								X		1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder																										19
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																										2
Lymphoma malignant lymphocytic																X			X							3
Lymphoma malignant mixed	X											X	X	X						X						8

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 25,000 ppm

Number of Days on Study	0 9 8	3 6 7	4 9 0	5 0 0	5 1 1	5 3 5	5 3 8	5 4 8	5 7 9	6 1 2	6 3 4	6 3 8	6 3 9	6 4 4	6 4 5	6 6 5	6 6 1	6 8 1	6 9 0	7 0 8	7 3 0	7 3 6	7 3 6	7 3 6	7 3 6
Carcass ID Number	8 3 1	7 6 1	8 0 5	7 6 4	7 9 1	8 0 4	8 0 3	8 2 5	7 8 2	8 1 4	8 1 2	7 6 2	7 6 3	7 5 1	7 9 4	8 2 3	7 4 2	7 3 3	7 8 4	7 9 5	8 1 5	7 3 1	7 3 2	7 3 4	7 3 5
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Intestine large	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+				
Intestine large, cecum	M	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	A	A				
Intestine large, colon	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+				
Intestine large, rectum	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	A	A				
Intestine small	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	A	A				
Intestine small, duodenum	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	A	A				
Intestine small, ileum	M	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	A	A				
Intestine small, jejunum	M	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	A	A				
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma										X								X						X	
Hepatocellular adenoma											X	X			X										
Mesentery			+			+	+	+	+	+		+	+	+	+		+			+	+				
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+				
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+				
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+				
Tooth																+									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Pheochromocytoma benign				X																					
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Parathyroid gland	M	+	+	+	+	+	+	M	+	+	+	M	M	M	M	+	+	M	+	+	+				
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+				
Pars distalis, adenoma										X					X		X		X						
Pars distalis, carcinoma				X																					
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 25,000 ppm
(continued)

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	7 4 1	7 4 3	7 4 4	7 4 5	7 5 2	7 5 3	7 5 4	7 5 5	7 6 5	7 7 1	7 7 2	7 7 3	7 7 4	7 7 5	7 7 8	7 8 1	7 8 3	7 9 2	7 9 3	8 0 1	8 0 2	8 1 1	8 1 5	8 1 3	8 2 1	8 2 2	8 2 4	**Total Tissues/ Tumors**
Alimentary System																												
Esophagus																												21
Gallbladder																												21
Intestine large																												19
Intestine large, cecum																												16
Intestine large, colon																												19
Intestine large, rectum																												17
Intestine small																												17
Intestine small, duodenum																												17
Intestine small, ileum																												16
Intestine small, jejunum																												16
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Hepatocellular carcinoma													X	X											X			6
Hepatocellular adenoma				X		X													X			X		X				8
Mesentery			+											+														15
Pancreas																												22
Salivary glands																												21
Stomach																												20
Stomach, forestomach																												20
Stomach, glandular																												20
Tooth																												1
Cardiovascular System																												
Heart																												21
Endocrine System																												
Adrenal gland																												21
Adrenal gland, cortex																												21
Adrenal gland, medulla																												21
Pheochromocytoma benign																												1
Islets, pancreatic																												21
Parathyroid gland																												14
Pituitary gland													+						+						+			23
Pars distalis, adenoma													X						X						X			7
Pars distalis, carcinoma																												1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Follicular cell, adenoma																X												1

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 25,000 ppm
(continued)

Number of Days on Study	0 9 8	3 6 7	4 9 0	5 0 0	5 1 1	5 3 5	5 3 8	5 4 8	5 7 9	6 1 2	6 3 4	6 3 8	6 3 9	6 4 4	6 6 5	6 6 5	6 8 1	6 9 1	7 0 0	7 0 8	7 3 0	7 3 6	7 3 6	7 3 6	7 3 6
Carcass ID Number	8 3 1	7 6 1	8 0 5	7 6 4	7 9 1	8 0 4	8 0 3	8 2 5	7 8 2	8 1 4	8 1 2	7 6 2	7 6 3	7 5 1	7 9 4	7 2 3	8 4 3	7 3 2	7 8 3	7 9 4	8 1 5	7 3 5	7 3 1	7 3 2	7 3 4	7 3 5
General Body System																									
Tissue NOS																									
Genital System																									
Ovary	+		+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+				
Cystadenoma, papillary																									
Oviduct			+			+	+	+																	
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
Endometrium, polyp stromal																				X					
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+				
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+		+	
Hemangiosarcoma																									
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+				
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Adenocarcinoma											X														
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Subcutaneous tissue, fibrosarcoma																						X			
Subcutaneous tissue, hemangiosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Osteosarcoma		X																							
Skeletal muscle			+											+											
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 25,000 ppm
(continued)

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	7 4 1	7 4 3	7 4 4	7 4 5	7 5 2	7 5 3	7 5 4	7 5 5	7 6 5	7 7 1	7 7 2	7 7 3	7 7 4	7 7 5	7 8 1	7 8 3	7 9 2	7 9 3	8 0 1	8 0 2	8 1 1	7 8 5	8 1 3	8 2 1	8 2 2	8 2 4	**Total Tissues/ Tumors**
General Body System																										
Tissue NOS																									+	1
Genital System																										
Ovary										+							+		+		+		+		+	25
Cystadenoma, papillary										X																1
Oviduct																										4
Uterus	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+		+	+	+	+		+	+	45
Endometrium, polyp stromal																										1
Hematopoietic System																										
Bone marrow																										21
Lymph node								+																		22
Lymph node, mandibular								+																		20
Spleen			+											+				+			+	+		+		28
Hemangiosarcoma																		X								1
Thymus																										19
Integumentary System																										
Mammary gland																										21
Adenocarcinoma																										1
Skin	+	+	+	+	+	+			+	+	+					+		+	+		+	+	+		+	40
Subcutaneous tissue, fibrosarcoma				X																						2
Subcutaneous tissue, hemangiosarcoma																		X								1
Musculoskeletal System																										
Bone																										21
Osteosarcoma																										1
Skeletal muscle																										2
Nervous System																										
Brain																										21

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 25,000 ppm
(continued)

Number of Days on Study	0 9 8	3 6 7	4 9 0	5 0 0	5 1 1	5 3 5	5 3 8	5 4 8	5 7 9	6 1 2	6 3 4	6 3 8	6 3 9	6 4 4	6 6 5	6 6 5	6 8 1	6 9 1	7 0 0	7 0 8	7 3 0	7 3 6	7 3 6	7 3 6	7 3 6
Carcass ID Number	8 3 1	7 6 1	8 0 5	7 6 4	7 9 1	8 0 4	8 0 3	8 2 5	7 8 2	8 1 4	8 1 2	7 6 2	7 6 3	7 5 1	7 9 4	7 2 3	8 4 2	7 3 3	7 8 4	7 9 5	8 1 5	7 3 1	7 3 2	7 3 4	7 3 5
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X																	
Alveolar/bronchiolar carcinoma																					X				
Carcinoma, metastatic, harderian gland																			X						
Osteosarcoma, metastatic, bone		X																							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Special Senses System																									
Eye																									
Harderian gland																			+						
Adenoma																									
Carcinoma																			X						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
Osteosarcoma, metastatic, bone		X																							
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic															X										
Lymphoma malignant lymphocytic										X				X											
Lymphoma malignant mixed												X	X												

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 25,000 ppm
(continued)

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	7 4 1	7 4 3	7 4 4	7 4 5	7 5 2	7 5 3	7 5 4	7 5 5	7 6 5	7 7 1	7 7 2	7 7 3	7 7 4	7 7 5	7 7 1	7 8 3	7 8 2	7 9 3	8 0 1	8 0 2	8 1 1	7 8 5	8 1 3	8 2 1	8 2 2	8 2 4	**Total Tissues/ Tumors**
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Alveolar/bronchiolar adenoma																		X					X		X		4
Alveolar/bronchiolar carcinoma			X																								2
Carcinoma, metastatic, harderian gland																											1
Osteosarcoma, metastatic, bone																											1
Nose																											21
Trachea																											21
Special Senses System																											
Eye																									+		1
Harderian gland																									+		2
Adenoma																									X		1
Carcinoma																											1
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, bone																											1
Urinary bladder																											21
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymphoma malignant histiocytic																						X					2
Lymphoma malignant lymphocytic			X					X																			4
Lymphoma malignant mixed					X																						3

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 50,000 ppm

Number of Days on Study	3 9 1	4 5 1	4 6 2	5 0 2	5 4 2	6 3 2	6 3 4	6 5 5	6 6 5	6 6 5	6 6 4	6 8 3	7 0 1	7 3 1	7 3 2	7 3 3	7 3 5	7 3 4	7 3 1	7 3 2	7 3 3	7 3 4	7 3 5	7 3 1	7 3 3
Carcass ID Number	6 2 1	6 8 1	6 2 2	6 4 2	6 6 2	6 7 5	6 1 4	6 2 5	6 6 4	6 6 5	6 7 4	6 2 3	6 5 1	6 1 1	6 1 2	6 1 3	6 1 5	6 2 4	6 3 1	6 3 2	6 3 3	6 3 4	6 3 5	6 4 1	6 4 3
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma								X		X															
Hepatocellular adenoma							X							X				X				X		X	
Mesentery				+	+	+					+		+				+								
Sarcoma																	X								
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																	+								
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	M	M	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Pars distalis, adenoma																	X		X		X	X			X
Pars intermedia, adenoma			X																						
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																				X	X				
General Body System																									
None																									

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 50,000 ppm
(continued)

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	6 4 4	6 4 5	6 5 2	6 5 3	6 5 4	6 5 5	6 6 1	6 6 3	6 7 1	6 7 2	6 7 3	6 8 2	6 8 3	6 8 4	6 8 5	6 9 1	6 9 2	6 9 3	6 9 4	6 9 5	7 0 1	7 0 2	7 0 3	7 0 4	7 0 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																							X			3
Hepatocellular adenoma	X											X														7
Mesentery	+																									7
Sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																	+			+						3
Cardiovascular System																										
Blood vessel																								+		1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	M	+	+	M	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	42
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	47
Pars distalis, adenoma		X	X	X	X		X		X						X		X	X		X				X		16
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma			X																							3
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 50,000 ppm
(continued)

Number of Days on Study	3 9 1	4 5 7	4 6 9	5 0 0	5 4 1	6 3 4	6 3 9	6 5 9	6 6 5	6 6 5	6 6 5	6 8 2	7 0 8	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6
Carcass ID Number	6 2 1	6 8 1	6 2 2	6 4 2	6 6 2	6 7 5	6 1 4	6 2 5	6 6 4	6 6 5	6 7 4	6 2 3	6 5 1	6 1 1	6 1 2	6 1 3	6 1 5	6 2 4	6 3 1	6 3 2	6 3 3	6 3 4	6 3 5	6 4 1	6 4 3
Genital System																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma, papillary			X									X			X					X					
Teratoma NOS		X																							
Oviduct																					+				
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma									X																
Leiomyosarcoma															X										
Endometrium, polyp stromal																				X					
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoacanthoma						X																			
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																	+								
Abdominal, sarcoma																	X								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma														X											
Carcinoma, metastatic, harderian gland																									
Carcinoma, metastatic, uncertain primary site																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 50,000 ppm
(continued)

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	6 4 4	6 4 5	6 5 2	6 5 3	6 5 4	6 5 5	6 6 1	6 6 3	6 7 1	6 7 2	6 7 3	6 8 2	6 8 3	6 8 4	6 8 5	6 9 1	6 9 2	6 9 3	6 9 4	6 9 5	7 0 1	7 0 2	7 0 3	7 0 4	7 0 5	Total Tissues/ Tumors
Genital System																										
Ovary	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	48
Cystadenoma, papillary																										4
Teratoma NOS																										1
Oviduct																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma																										1
Leiomyosarcoma																										1
Endometrium, polyp stromal																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoacanthoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Abdominal, sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Carcinoma, metastatic, harderian gland																			X							1
Carcinoma, metastatic, uncertain primary site	X																									1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 50,000 ppm
(continued)

Number of Days on Study	3	4	4	5	5	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7
	9	5	6	0	4	3	3	5	6	6	6	8	0	3	3	3	3	3	3	3	3	3	3	3	3
	1	7	9	0	1	4	9	9	5	5	5	2	8	6	6	6	6	6	6	6	6	6	6	6	6
Carcass ID Number	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	2	8	2	4	6	7	1	2	6	6	7	2	5	1	1	1	1	2	3	3	3	3	3	4	4
	1	1	2	2	2	5	4	5	4	5	4	3	1	1	2	3	5	4	1	2	3	4	5	1	3
Special Senses System																									
Eye																		+							
Harderian gland																		+							
Carcinoma																		X							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic				X																					
Lymphoma malignant mixed							X				X		X												X

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Ethylene Glycol: 50,000 ppm
(continued)

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	6 4 4	6 4 5	6 5 2	6 5 3	6 5 4	6 5 5	6 6 1	6 6 3	6 7 1	6 7 2	6 7 3	6 8 2	6 8 3	6 8 4	6 8 5	6 9 1	6 9 2	6 9 3	6 9 4	6 9 5	7 0 1	7 0 2	7 0 3	7 0 4	7 0 5	Total Tissues/ Tumors
Special Senses System																										
Eye																										1
Harderian gland																			+							2
Carcinoma																			X							2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																										1
Lymphoma malignant mixed					X												X									6

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Ethylene Glycol

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Liver: Hepatocellular Adenoma				
Overall rates[a]	8/50 (16%)	5/50 (10%)	8/51 (16%)	7/50 (14%)
Adjusted rates[b]	22.7%	15.7%	22.9%	18.1%
Terminal rates[c]	6/33 (18%)	4/30 (13%)	5/30 (17%)	6/37 (16%)
First incidence (days)	696	679	634	639
Life table tests[d]	P=0.444N	P=0.338N	P=0.540	P=0.414N
Logistic regression tests[d]	P=0.505N	P=0.303N	P=0.583	P=0.449N
Cochran-Armitage test[d]	P=0.553N			
Fisher exact test[d]		P=0.277N	P=0.590N	P=0.500N
Liver: Hepatocellular Carcinoma				
Overall rates	3/50 (6%)	5/50 (10%)	6/51 (12%)	3/50 (6%)
Adjusted rates	7.7%	15.3%	17.9%	7.2%
Terminal rates	0/33 (0%)	3/30 (10%)	4/30 (13%)	1/37 (3%)
First incidence (days)	655	679	612	659
Life table tests	P=0.443N	P=0.320	P=0.219	P=0.619N
Logistic regression tests	P=0.502N	P=0.344	P=0.238	P=0.659
Cochran-Armitage test	P=0.516N			
Fisher exact test		P=0.357	P=0.254	P=0.661N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rates	10/50 (20%)	9/50 (18%)	14/51 (27%)	10/50 (20%)
Adjusted rates	26.5%	27.9%	38.6%	24.4%
Terminal rates	6/33 (18%)	7/30 (23%)	9/30 (30%)	7/37 (19%)
First incidence (days)	655	679	612	639
Life table tests	P=0.484N	P=0.581N	P=0.195	P=0.500N
Logistic regression tests	P=0.515	P=0.539N	P=0.218	P=0.573N
Cochran-Armitage test	P=0.468			
Fisher exact test		P=0.500N	P=0.260	P=0.598N
Lung: Alveolar/bronchiolar Adenoma				
Overall rates	0/50 (0%)	4/50 (8%)	4/51 (8%)	1/50 (2%)
Adjusted rates	0.0%	10.9%	12.0%	2.7%
Terminal rates	0/33 (0%)	2/30 (7%)	3/30 (10%)	1/37 (3%)
First incidence (days)	–[e]	472	548	731 (T)
Life table tests	P=0.566N	P=0.058	P=0.055	P=0.523
Logistic regression tests	P=0.568	P=0.068	P=0.063	P=0.523
Cochran-Armitage test	P=0.570			
Fisher exact test		P=0.059	P=0.061	P=0.500
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rates	1/50 (2%)	6/50 (12%)	6/51 (12%)	1/50 (2%)
Adjusted rates	3.0%	16.6%	18.0%	2.7%
Terminal rates	1/33 (3%)	3/30 (10%)	4/30 (13%)	1/37 (3%)
First incidence (days)	731 (T)	472	548	731 (T)
Life table tests	P=0.337N	P=0.051	P=0.049	P=0.736N
Logistic regression tests	P=0.384N	P=0.060	P=0.054	P=0.736N
Cochran-Armitage test	P=0.388N			
Fisher exact test		P=0.056	P=0.059	P=0.753N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Ethylene Glycol
(continued)

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Ovary: Cystadenoma				
Overall rates	2/49 (4%)	1/25 (4%)[f]	1/25 (4%)[f]	4/48 (8%)
Adjusted rates	5.3%			10.0%
Terminal rates	0/32 (0%)			2/35 (6%)
First incidence (days)	606			469
Life table tests				P=0.371
Logistic regression tests				P=0.325
Fisher exact test				P=0.329
Pituitary Gland (Pars Distalis): Adenoma				
Overall rates	17/47 (36%)	5/22 (23%)[f]	7/23 (30%)[f]	16/47 (34%)
Adjusted rates	47.8%			45.7%
Terminal rates	14/32 (44%)			16/35 (46%)
First incidence (days)	578			731 (T)
Life table tests				P=0.375N
Logistic regression tests				P=0.409N
Fisher exact test				P=0.500N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rates	17/47 (36%)	5/22 (23%)[f]	8/23 (35%)[f]	16/47 (34%)
Adjusted rates	47.8%			45.7%
Terminal rates	14/32 (44%)			16/35 (46%)
First incidence (days)	578			731 (T)
Life table tests				P=0.375N
Logistic regression tests				P=0.409N
Fisher exact test				P=0.500N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rates	1/49 (2%)	1/50 (2%)	1/51 (2%)	3/50 (6%)
Adjusted rates	3.0%	2.6%	3.3%	8.1%
Terminal rates	1/33 (3%)	0/30 (0%)	1/30 (3%)	3/37 (8%)
First incidence (days)	731 (T)	644	731 (T)	731 (T)
Life table tests	P=0.205	P=0.744	P=0.741	P=0.346
Logistic regression tests	P=0.182	P=0.759N	P=0.741	P=0.346
Cochran-Armitage test	P=0.167			
Fisher exact test		P=0.747N	P=0.742N	P=0.316
Uterus: Stromal Polyp				
Overall rates	3/50 (6%)	3/50 (6%)	1/51 (2%)	1/50 (2%)
Adjusted rates	8.4%	8.7%	3.2%	2.7%
Terminal rates	2/33 (6%)	2/30 (7%)	0/30 (0%)	1/37 (3%)
First incidence (days)	655	539	730	731 (T)
Life table tests	P=0.144N	P=0.626	P=0.337N	P=0.270N
Logistic regression tests	P=0.160N	P=0.662	P=0.316N	P=0.292N
Cochran-Armitage test	P=0.163N			
Fisher exact test		P=0.661N	P=0.301N	P=0.309N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Ethylene Glycol
(continued)

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)				
Overall rates	17/50 (34%)	13/50 (26%)	9/51 (18%)	7/50 (14%)
Adjusted rates	42.5%	35.2%	23.9%	16.5%
Terminal rates	11/33 (33%)	8/30 (27%)	4/30 (13%)	3/37 (8%)
First incidence (days)	520	476	612	500
Life table tests	P=0.008N	P=0.354N	P=0.101N	P=0.015N
Logistic regression tests	P=0.010N	P=0.256N	P=0.054N	P=0.018N
Cochran-Armitage test	P=0.010N			
Fisher exact test		P=0.257N	P=0.049N	P=0.017N
All Organs: Benign Neoplasms				
Overall rates	29/50 (58%)	19/50 (38%)	19/51 (37%)	26/50 (52%)
Adjusted rates	72.4%	47.7%	47.5%	64.7%
Terminal rates	22/33 (67%)	10/30 (33%)	10/30 (33%)	23/37 (62%)
First incidence (days)	578	472	500	469
Life table tests	P=0.272N	P=0.105N	P=0.101N	P=0.172N
Logistic regression tests	P=0.420N	P=0.041N	P=0.041N	P=0.254N
Cochran-Armitage test	P=0.467N			
Fisher exact test		P=0.036N	P=0.029N	P=0.344N
All Organs: Malignant Neoplasms				
Overall rates	21/50 (42%)	21/50 (42%)	22/51 (43%)	17/50 (34%)
Adjusted rates	49.4%	53.0%	52.6%	37.5%
Terminal rates	12/33 (36%)	12/30 (40%)	11/30 (37%)	9/37 (24%)
First incidence (days)	520	476	367	500
Life table tests	P=0.150N	P=0.457	P=0.400	P=0.202N
Logistic regression tests	P=0.219N	P=0.568	P=0.520	P=0.271N
Cochran-Armitage test	P=0.222N			
Fisher exact test		P=0.580N	P=0.534	P=0.268N
All Organs: Benign or Malignant Neoplasms				
Overall rates	39/50 (78%)	33/50 (66%)	31/51 (61%)	36/50 (72%)
Adjusted rates	86.6%	74.7%	71.6%	75.0%
Terminal rates	27/33 (82%)	19/30 (63%)	18/30 (60%)	25/37 (68%)
First incidence (days)	520	472	367	457
Life table tests	P=0.179N	P=0.361N	P=0.237N	P=0.178N
Logistic regression tests	P=0.342N	P=0.149N	P=0.070N	P=0.304N
Cochran-Armitage test	P=0.362N			
Fisher exact test		P=0.133N	P=0.048N	P=0.322N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no neoplasms in animal group
[f] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; therefore, statistical comparisons with the controls are not appropriate.

TABLE B4a
Historical Incidence of Alveolar/bronchiolar Neoplasms in Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Southern Research Institute			
C.I. Pigment Red 3	3/50	1/50	4/50
Ethylene Glycol	0/50	1/50	1/50
Nitrofurantoin	2/50	1/50	3/50
o-Nitroanisole	4/50	2/50	6/50
Polysorbate 80	3/50	0/50	3/50
Rhodamine 6G	3/50	1/50	4/50
Roxarsone	1/50	2/50	3/50
Total	16/350 (4.6%)	8/350 (2.3%)	24/350 (6.9%)
Standard deviation	2.8%	1.4%	3.0%
Range	0%-8%	0%-4%	2%-12%
Overall Historical Incidence			
Total	51/870 (5.9%)	20/870 (2.3%)	70/870 (8.0%)[b]
Standard deviation	5.8%	1.5%	5.8%
Range	0%-24%	0%-6%	2%-26%

[a] Data as of 3 April 1991
[b] Includes one animal with both an adenoma and a carcinoma

TABLE B4b
Historical Incidence of Malignant Lymphomas in Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls
Historical Incidence at Southern Research Institute	
C.I. Pigment Red 3	15/50
Ethylene Glycol	17/50
Nitrofurantoin	12/50
o-Nitroanisole	5/50
Polysorbate 80	18/50
Rhodamine 6G	16/50
Roxarsone	13/50
Total	96/350 (27.4%)
Standard deviation	8.8%
Range	0%-36%
Overall Historical Incidence	
Total	259/870 (29.8%)
Standard deviation	10.5%
Range	10%-44%

[a] Data as of 3 April 1991. Includes data for histiocytic, lymphocytic, mixed, NOS, and undifferentiated cell type malignant lymphomas.

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Ethylene Glycol[a]

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-month interim evaluation	10	10	9	10
Early deaths				
Natural deaths	8	11	9	1
Moribund	9	9	12	12
Survivors				
Terminal sacrifice	33	30	30	37
Animals examined microscopically	50	50	51	50
Alimentary System				
Gallbladder	(48)	(18)	(21)	(49)
Inflammation				1 (2%)
Intestine large, cecum	(48)	(19)	(16)	(50)
Edema				1 (2%)
Lymphoid tissue, hyperplasia, lymphoid				1 (2%)
Intestine large, colon	(49)	(19)	(19)	(50)
Inflammation, suppurative	1 (2%)			
Intestine large, rectum	(49)	(19)	(17)	(50)
Edema				1 (2%)
Intestine small, ileum	(47)	(19)	(16)	(50)
Amyloid deposition				2 (4%)
Dilatation			1 (6%)	
Inflammation, chronic			1 (6%)	
Lymphoid tissue, hyperplasia, lymphoid				2 (4%)
Lymphoid tissue, inflammation, granulomatous				1 (2%)
Intestine small, jejunum	(47)	(19)	(16)	(50)
Amyloid deposition		1 (5%)		1 (2%)
Dilatation			1 (6%)	
Liver	(50)	(50)	(51)	(50)
Angiectasis		1 (2%)		
Basophilic focus		1 (2%)		1 (2%)
Congestion		1 (2%)		1 (2%)
Cyst		1 (2%)		
Fatty change			2 (4%)	3 (6%)
Fibrosis			1 (2%)	
Hematopoietic cell proliferation	2 (4%)	6 (12%)	7 (14%)	2 (4%)
Hyperplasia, lymphoid	14 (28%)	14 (28%)	16 (31%)	12 (24%)
Infarct	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, mixed cell	4 (8%)	10 (20%)	11 (22%)	4 (8%)
Inflammation, multifocal	17 (34%)	10 (20%)	19 (37%)	24 (48%)
Mineralization			1 (2%)	
Necrosis, multifocal	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Pigmentation				1 (2%)
Bile duct, hyperplasia		1 (2%)		
Hepatocyte, erythrophagocytosis				2 (4%)
Hepatocyte, centrilobular, degeneration, hyaline			1 (2%)	26 (52%)
Portal vein, media, hyperplasia				1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Ethylene Glycol (continued)

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Alimentary System (continued)				
Mesentery	(17)	(7)	(15)	(7)
Cyst			1 (7%)	
Edema				1 (14%)
Hemorrhage	1 (6%)			
Inflammation, suppurative	6 (35%)	5 (71%)	9 (60%)	2 (29%)
Fat, necrosis	8 (47%)			1 (14%)
Pancreas	(49)	(21)	(22)	(50)
Atrophy, diffuse		2 (10%)		
Atrophy, focal		1 (5%)		3 (6%)
Ectopic tissue				1 (2%)
Hyperplasia, lymphoid				2 (4%)
Inflammation, suppurative		1 (5%)	1 (5%)	
Acinar cell, hypoplasia, focal				1 (2%)
Duct, dilatation	1 (2%)	1 (5%)	1 (5%)	3 (6%)
Duct, dilatation, multiple				1 (2%)
Salivary glands	(49)	(20)	(21)	(50)
Hyperplasia, focal	1 (2%)			
Stomach, forestomach	(50)	(20)	(20)	(50)
Inflammation, focal			1 (5%)	2 (4%)
Mineralization	1 (2%)			1 (2%)
Mucosa, hyperplasia, papillary				1 (2%)
Stomach, glandular	(49)	(20)	(20)	(50)
Hyperplasia, lymphoid	2 (4%)			1 (2%)
Inflammation, focal	1 (2%)			4 (8%)
Mineralization	1 (2%)			3 (6%)
Epithelium, degeneration, hyaline				2 (4%)
Epithelium, metaplasia, squamous				1 (2%)
Mucosa, cyst				2 (4%)
Tooth	(2)	(4)	(1)	(3)
Incisor, dysplasia	2 (100%)	4 (100%)	1 (100%)	3 (100%)
Incisor, inflammation, suppurative		1 (25%)		
Cardiovascular System				
Blood vessel		(2)		(1)
Aorta, thrombus		1 (50%)		
Mesenteric artery, inflammation, chronic		1 (50%)		1 (100%)
Heart	(50)	(20)	(21)	(50)
Infiltration cellular, mixed cell			1 (5%)	
Inflammation, suppurative	1 (2%)			
Artery, media, hyperplasia	1 (2%)			
Endocrine System				
Adrenal gland, cortex	(49)	(21)	(21)	(50)
Accessory adrenal cortical nodule				1 (2%)
Amyloid deposition				1 (2%)
Angiectasis				1 (2%)
Focal cellular change		1 (5%)		

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Ethylene Glycol (continued)

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Endocrine System (continued)				
Adrenal gland, cortex (continued)				
Hematopoietic cell proliferation			1 (5%)	
Hemorrhage, focal	1 (2%)			
Hyperplasia, focal				1 (2%)
Infiltration cellular, mixed cell		1 (5%)		
Inflammation, suppurative				1 (2%)
Vacuolization cytoplasmic, focal				2 (4%)
Subcapsular, hyperplasia, focal				1 (2%)
Adrenal gland, medulla	(48)	(21)	(21)	(50)
Ectopic tissue				2 (4%)
Hyperplasia, focal	1 (2%)	1 (5%)		1 (2%)
Parathyroid gland	(49)	(18)	(14)	(42)
Cyst	1 (2%)			
Pituitary gland	(47)	(22)	(23)	(47)
Pars distalis, angiectasis	1 (2%)			
Pars distalis, bacterium	1 (2%)			
Pars distalis, cyst	2 (4%)			
Pars distalis, focal cellular change		2 (9%)	2 (9%)	
Pars distalis, hyperplasia, focal	13 (28%)		2 (9%)	14 (30%)
Pars distalis, necrosis, focal		1 (5%)		
Pars nervosa, inflammation, focal			1 (4%)	
Thyroid gland	(49)	(50)	(51)	(50)
Degeneration, cystic	4 (8%)	6 (12%)	4 (8%)	4 (8%)
Hyperplasia, lymphoid				1 (2%)
Inflammation, focal	3 (6%)		3 (6%)	3 (6%)
C-cell, hyperplasia			1 (2%)	1 (2%)
Follicular cell, hyperplasia	8 (16%)	16 (32%)	22 (43%)	14 (28%)
General Body System				
Tissue NOS		(2)	(1)	
Abdominal, foreign body		1 (50%)		
Abdominal, infiltration cellular, lymphocyte			1 (100%)	
Genital System				
Ovary	(49)	(25)	(25)	(48)
Angiectasis	1 (2%)	2 (8%)		3 (6%)
Atrophy				2 (4%)
Fibrosis, focal				1 (2%)
Granuloma			1 (4%)	
Hemorrhage		1 (4%)		2 (4%)
Hyperplasia, lymphoid			1 (4%)	
Inflammation, chronic			1 (4%)	
Inflammation, suppurative	8 (16%)	10 (40%)	9 (36%)	3 (6%)
Mineralization				2 (4%)
Thrombus		1 (4%)		
Follicle, cyst	11 (22%)	5 (20%)	8 (32%)	9 (19%)
Follicle, cyst, multiple		1 (4%)		
Oviduct	(3)		(4)	(1)
Hyperplasia, cystic				1 (100%)
Inflammation, suppurative	2 (67%)		4 (100%)	

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Ethylene Glycol (continued)

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Genital System (continued)				
Uterus	(50)	(45)	(45)	(50)
Angiectasis		4 (9%)	1 (2%)	2 (4%)
Hemorrhage		1 (2%)	1 (2%)	2 (4%)
Hydrometra	3 (6%)	4 (9%)	2 (4%)	4 (8%)
Inflammation, suppurative	6 (12%)	7 (16%)	5 (11%)	3 (6%)
Thrombus		1 (2%)		
Endometrium, cyst	1 (2%)			2 (4%)
Endometrium, hyperplasia, cystic	44 (88%)	40 (89%)	40 (89%)	47 (94%)
Hematopoietic System				
Bone marrow	(50)	(20)	(21)	(50)
Hyperplasia, neutrophil	1 (2%)	1 (5%)	1 (5%)	2 (4%)
Lymph node	(50)	(24)	(22)	(50)
Iliac, hyperplasia		1 (4%)	4 (18%)	1 (2%)
Inguinal, hyperplasia				1 (2%)
Inguinal, hyperplasia, lymphoid			1 (5%)	
Mediastinal, angiectasis		1 (4%)		
Mediastinal, hyperplasia	3 (6%)	3 (13%)	3 (14%)	1 (2%)
Mediastinal, hyperplasia, lymphoid	1 (2%)	2 (8%)		2 (4%)
Mediastinal, inflammation, suppurative	5 (10%)	1 (4%)	1 (5%)	
Mesenteric, angiectasis	4 (8%)	2 (8%)		6 (12%)
Mesenteric, hyperplasia	2 (4%)	1 (4%)	2 (9%)	2 (4%)
Mesenteric, hyperplasia, lymphoid	5 (10%)		2 (9%)	7 (14%)
Mesenteric, inflammation, suppurative	2 (4%)			
Pancreatic, hyperplasia			1 (5%)	
Renal, hyperplasia	2 (4%)	1 (4%)	5 (23%)	1 (2%)
Renal, inflammation, suppurative	1 (2%)	1 (4%)		
Lymph node, *mandibular*	(43)	(21)	(20)	(49)
Hyperplasia	1 (2%)			2 (4%)
Hyperplasia, lymphoid	2 (5%)	1 (5%)	1 (5%)	6 (12%)
Spleen	(49)	(29)	(28)	(50)
Depletion	1 (2%)			
Fibrosis, focal	1 (2%)			
Hematopoietic cell proliferation	14 (29%)	12 (41%)	20 (71%)	13 (26%)
Hyperplasia, lymphoid	11 (22%)	2 (7%)	6 (21%)	13 (26%)
Thymus	(47)	(19)	(19)	(47)
Cyst		1 (5%)		1 (2%)
Hyperplasia				1 (2%)
Hyperplasia, lymphoid	7 (15%)	1 (5%)		6 (13%)
Integumentary System				
Mammary gland	(49)	(20)	(21)	(50)
Dilatation	4 (8%)		1 (5%)	6 (12%)
Hyperplasia	2 (4%)			
Inflammation, suppurative	1 (2%)			
Duct, metaplasia, squamous	1 (2%)			

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Ethylene Glycol (continued)

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Integumentary System (continued)				
Skin	(49)	(43)	(40)	(50)
Inflammation, chronic	16 (33%)	5 (12%)	6 (15%)	11 (22%)
Subcutaneous tissue, edema				1 (2%)
Subcutaneous tissue, hyperplasia, lymphoid				1 (2%)
Subcutaneous tissue, inflammation, chronic				2 (4%)
Musculoskeletal System				
Bone	(50)	(20)	(21)	(50)
Cranium, hyperostosis				1 (2%)
Distal, joint, femur, arthrosis	1 (2%)			
Femur, hyperostosis		1 (5%)		
Skeletal muscle	(2)	(2)	(2)	(1)
Inflammation, suppurative			1 (50%)	
Nervous System				
Brain	(49)	(20)	(21)	(50)
Compression	1 (2%)	1 (5%)	1 (5%)	3 (6%)
Gliosis		1 (5%)		
Hemorrhage, multifocal	2 (4%)			
Infiltration cellular, lymphocyte		1 (5%)		
Necrosis, focal		1 (5%)		
Artery, inflammation, chronic			1 (5%)	
Artery, meninges, inflammation, chronic		1 (5%)		
Meninges, hyperplasia, lymphoid				1 (2%)
Meninges, infiltration cellular, mixed cell			1 (5%)	
Respiratory System				
Lung	(50)	(50)	(51)	(50)
Bacterium	1 (2%)			
Congestion		2 (4%)	2 (4%)	
Hemorrhage	2 (4%)		4 (8%)	
Hyperplasia, lymphoid	17 (34%)	16 (32%)	15 (29%)	21 (42%)
Hyperplasia, macrophage		2 (4%)	2 (4%)	1 (2%)
Infiltration cellular, mixed cell	3 (6%)	3 (6%)	3 (6%)	1 (2%)
Inflammation, granulomatous			2 (4%)	
Inflammation, suppurative	1 (2%)		1 (2%)	
Mineralization				1 (2%)
Pigmentation				1 (2%)
Alveolar epithelium, hyperplasia	1 (2%)			
Artery, inflammation				1 (2%)
Artery, media, hyperplasia	3 (6%)	10 (20%)	10 (20%)	23 (46%)
Bronchiole, bronchiectasis			1 (2%)	
Bronchiole, epithelium, hyperplasia			1 (2%)	
Mediastinum, inflammation, suppurative	1 (2%)		3 (6%)	
Vein, intima, mineralization	1 (2%)			

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Ethylene Glycol (continued)

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Respiratory System (continued)				
Nose	(50)	(20)	(21)	(50)
Lumen, exudate	2 (4%)			1 (2%)
Lumen, foreign body	1 (2%)			1 (2%)
Mucosa, angiectasis	1 (2%)			
Mucosa, hemorrhage	1 (2%)			
Nasolacrimal duct, inflammation, suppurative	1 (2%)			
Trachea	(49)	(20)	(21)	(50)
Artery, peritracheal tissue, inflammation, chronic		1 (5%)		
Special Senses System				
Eye			(1)	(1)
Atrophy			1 (100%)	
Cataract				1 (100%)
Fibrosis			1 (100%)	
Retinal detachment				1 (100%)
Cornea, inflammation				1 (100%)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Bacterium	1 (2%)	1 (2%)		
Cyst				1 (2%)
Infiltration cellular, mixed cell	1 (2%)	2 (4%)		1 (2%)
Inflammation, chronic	1 (2%)			
Inflammation, suppurative		2 (4%)	2 (4%)	
Metaplasia, osseous	1 (2%)	1 (2%)	3 (6%)	
Necrosis, multifocal		1 (2%)		
Nephropathy	25 (51%)	20 (40%)	33 (66%)	28 (56%)
Capsule, inflammation, suppurative		1 (2%)		
Pelvis, dilatation				1 (2%)
Renal tubule, degeneration, hyaline		3 (6%)		2 (4%)
Renal tubule, dilatation			1 (2%)	1 (2%)
Renal tubule, hyperplasia		1 (2%)		
Renal tubule, mineralization	1 (2%)			
Renal tubule, pigmentation				2 (4%)
Renal tubule, vacuolization cytoplasmic			1 (2%)	
Urinary bladder	(49)	(19)	(21)	(50)
Hyperplasia, lymphoid	2 (4%)			5 (10%)
Inflammation, chronic			1 (5%)	1 (2%)
Ulcer				1 (2%)
Transitional epithelium, hyperplasia		1 (5%)		
Transitional epithelium, metaplasia, squamous				1 (2%)

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

APPENDIX C
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA PROTOCOL

Testing was performed as reported by Haworth *et al.* (1983) and Zeiger *et al.* (1987). Ethylene glycol was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strain (TA100, TA1535, TA1537, and TA98) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C prior to the addition of soft agar supplemented with *l*-histidine and *d*-biotin, and subsequent plating on minimal glucose agar plates. Incubation continued for an additional 48 hours.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of ethylene glycol. High dose did not exceed 10,000 μg/plate. All assays were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants which is not dose-related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies was observed following chemical treatment.

MOUSE LYMPHOMA CELL PROTOCOL

The experimental protocol is presented by McGregor *et al.* (1991). Ethylene glycol was supplied as a coded aliquot by Radian Corporation (Austin, TX). The highest dose of ethylene glycol did not exceed 5,000 μg/mL. Mouse lymphoma L5178Y/TK cells were maintained at 37° C as suspension cultures in Fischer's medium supplemented with 2 mM *l*-glutamine, 110 μg/mL sodium pyruvate, 0.05% pluronic F68, antibiotics, and heat-inactivated horse serum; normal cycling time was about 10 hours. To reduce the number of spontaneously occurring trifluorothymidine (TFT) resistant cells, subcultures were exposed once to medium containing THMG (thymidine, hypoxanthine, methotrexate, glycine) for 1 day, to 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. Freshly prepared S9 metabolic activation factors were obtained from the livers of either Aroclor 1254-induced or noninduced Fischer 344/N male rats.

All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in a 10 mL volume of medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with ethylene glycol continued for 4 hours, at which time the medium plus chemical was removed and the cells were resuspended in 20 mL of 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 TFT for selection of TFT-resistant cells ($TK^{-/-}$), and 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. All data were evaluated statistically for both trend and peak response. 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.

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in McGregor *et al.* (1991). This assay is initially performed without S9; if a clearly positive response is not obtained, the experiment is repeated with induced S9.

Chinese Hamster Ovary Cell Cytogenetics Assays

Testing was performed as reported by Galloway *et al.* (1985, 1987) and is presented briefly below. Ethylene glycol was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was 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 (BrdU)-substituted DNA. Each test consisted of concurrent solvent and positive controls, and of at least three doses of ethylene glycol; the high dose did not exceed 5,000 μg/mL.

In the SCE test without S9, CHO cells were incubated for 26 hours with ethylene glycol in McCoy's 5A medium supplemented with 10% fetal bovine serum, *l*-glutamine (2mM), and antibiotics. BrdU was added 2 hours after culture initiation. After 26 hours, the medium containing ethylene glycol was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2.5 to 3.5 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 ethylene glycol, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and no ethylene glycol, and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining was the same as for cells treated without S9.

In the Abs test without S9, cells were incubated in McCoy's 5A medium with the ethylene glycol for 10 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 ethylene glycol and S9 for 2 hours, after which the treatment medium was removed and the cells incubated for 10 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.

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. For the SCE test, 50 second-division metaphase cells were scored for frequency of SCEs per cell from each dose level; 100 first-division metaphase cells were scored at each dose level for the Abs test. Classes of Abs included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more Abs).

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. 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. Chromosomal Ab data are presented as percentage of cells with Abs. As with the test for SCEs, both the dose-response curve and individual dose points were statistically analyzed. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) were considered weak evidence for a positive response (+w); significant differences for two or more doses indicated the trial was positive (+) (Galloway *et al.*, 1987).

RESULTS

Negative results were obtained in all *in vitro* genotoxicity assays with ethylene glycol. Ethylene glycol was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with a preincubation protocol at concentrations up to 10,000 μg/plate in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 (Table C1; Zeiger *et al.*, 1987). In the mouse lymphoma assay, ethylene glycol was tested in the presence and absence of Aroclor 1254-induced male Fischer rat liver S9 activation enzymes, and did not induce TFT resistance in L5178Y cells (Table C2; McGregor *et al.*, 1991). In the absence of S9, inconsistent responses were seen among the three trials; a positive response was obtained in Trial 2, but this was not reproduced in the other two trials performed without S9. The results of two trials with S9 were negative. Results of *in vitro* cytogenetic tests for induction of SCEs and chromosomal Abs with ethylene glycol in CHO cells were negative, with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9 (Tables C3 and C4). In the SCE and the Abs tests, doses up to 5,000 μg/mL ethylene glycol were tested.

TABLE C1
Mutagenicity of Ethylene Glycol in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	160 ± 3.4	105 ± 7.0	247 ± 2.1	222 ± 17.5	252 ± 6.4	189 ± 19.9
	100	144 ± 6.2	101 ± 3.2	194 ± 11.0	205 ± 15.9	226 ± 12.7	185 ± 7.8
	333	132 ± 2.2	113 ± 13.1	207 ± 21.0	179 ± 29.3	220 ± 9.9	164 ± 1.2
	1,000	137 ± 3.2	94 ± 2.3	240 ± 15.0	208 ± 4.9	220 ± 7.1	174 ± 12.9
	3,333	150 ± 2.5	112 ± 7.4	246 ± 13.7	241 ± 23.1	196 ± 4.1	202 ± 44.0
	10,000	140 ± 14.0	107 ± 10.4	238 ± 16.2	202 ± 14.8	223 ± 1.2	165 ± 24.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		559 ± 41.3	371 ± 38.6	886 ± 18.9	852 ± 49.7	494 ± 19.7	397 ± 38.1
TA1535	0	6 ± 2.9	9 ± 0.9	11 ± 0.3	11 ± 1.2	14 ± 2.6	13 ± 2.7
	100	13 ± 1.5	10 ± 2.1	11 ± 0.9	9 ± 1.0	11 ± 0.3	13 ± 1.8
	333	13 ± 1.7	10 ± 2.1	12 ± 2.2	7 ± 2.7	14 ± 1.2	13 ± 1.3
	1,000	9 ± 1.0	9 ± 0.9	16 ± 1.3	6 ± 1.5	22 ± 0.3	16 ± 4.2
	3,333	8 ± 2.5	8 ± 0.3	17 ± 2.3	9 ± 1.2	24 ± 1.2	15 ± 2.3
	10,000	7 ± 0.3	8 ± 1.2	16 ± 0.3	8 ± 1.2	24 ± 2.7	15 ± 1.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		466 ± 10.7	449 ± 71.8	47 ± 11.3	48 ± 9.4	42 ± 6.9	33 ± 3.1
TA1537	0	15 ± 0.9	3 ± 0.3	14 ± 1.7	6 ± 0.9	20 ± 1.0	6 ± 0.9
	100	12 ± 0.3	4 ± 1.2	12 ± 2.6	5 ± 0.3	16 ± 2.6	8 ± 2.6
	333	16 ± 3.5	4 ± 1.9	14 ± 3.5	8 ± 2.0	22 ± 1.5	7 ± 1.3
	1,000	15 ± 1.9	3 ± 1.2	15 ± 2.6	7 ± 0.6	15 ± 1.8	10 ± 3.5
	3,333	13 ± 1.0	2 ± 0.3	19 ± 2.9	8 ± 0.9	19 ± 2.1	5 ± 0.3
	10,000	12 ± 3.0	2 ± 0.9	16 ± 1.2	5 ± 1.3	16 ± 2.6	4 ± 0.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		186 ± 38.1	154 ± 34.8	66 ± 7.8	61 ± 7.2	51 ± 6.3	33 ± 5.0
TA98	0	19 ± 2.8	16 ± 1.7	33 ± 4.7	31 ± 2.7	32 ± 0.7	28 ± 2.3
	100	24 ± 0.9	18 ± 1.9	38 ± 2.7	28 ± 4.3	26 ± 9.9	24 ± 2.9
	333	15 ± 2.6	14 ± 0.7	40 ± 7.9	29 ± 4.5	29 ± 1.2	34 ± 4.7
	1,000	21 ± 4.8	18 ± 0.0	37 ± 6.4	29 ± 3.2	32 ± 2.0	28 ± 0.6
	3,333	24 ± 1.5	16 ± 3.1	29 ± 2.1	32 ± 2.9	32 ± 5.8	28 ± 1.2
	10,000	20 ± 2.0	15 ± 1.2	29 ± 1.9	29 ± 2.3	32 ± 4.3	32 ± 2.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		414 ± 5.8	193 ± 44.0	704 ± 107.6	800 ± 18.3	310 ± 31.7	394 ± 23.7

[a] Study performed at Case Western Reserve University. The detailed protocol and these data are presented in Zeiger *et al.* (1987).
[b] Revertants are presented as mean ± standard error from three plates.
[c] 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE C2
Induction of Trifluorothymidine Resistance in Mouse L5178Y Lymphoma Cells by Ethylene Glycol[a]

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction
-S9						
Trial 1						
Distilled water		91	102	114	42	
		88	91	101	38	
		98	102	129	44	
		78	105	111	48	43
Methyl methanesulfonate	15	27	15	241	296	
		28	21	264	312	304*
Ethylene glycol	1,000	76	92	127	56	
		96	119	144	50	
		79	73	100	42	49
	2,000	70	75	115	55	
		62	94	76	41	
		89	97	103	39	45
	3,000	100	103	147	49	
		75	72	118	52	
		69	89	119	58	53
	4,000	72	93	143	66	
		68	93	138	68	
		75	106	125	55	63*
	5,000	81	103	122	50	
		101	96	130	43	
		83	72	134	54	49
Trial 2						
Distilled water		56	112	75	45	
		66	102	102	52	
		55	85	91	55	51
Methyl methanesulfonate	15	27	25	171	211	
		20	19	163	269	240*
Ethylene glycol	2,000	52	91	103	65	
		42	81	92	73	69
	3,000	44	92	94	71	
		37	107	100	90	81
	4,000	41	100	132	108	
		50	95	141	95	101*
	5,000	46	101	141	103	
		39	79	115	98	100*

TABLE C2
Induction of Trifluorothymidine Resistance in Mouse L5178Y Lymphoma Cells by Ethylene Glycol
(continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9 (continued)						
Trial 3						
Distilled water		99	101	134	45	
		84	102	142	56	
		79	99	112	47	
		79	98	86	36	46
Ethyl methanesulfonate	250	49	62	487	334	
		56	64	481	288	311*
Methyl methanesulfonate	15	43	23	254	198	
		38	27	204	179	188*
Ethylene glycol	1,000	96	107	124	43	
		105	116	127	40	42
	2,000	73	89	120	55	
		76	94	105	46	51
	3,000	65	85	124	63	
		76	86	138	61	62
	4,000	57	65	96	56	
		71	79	161	76	66
	5,000	63	79	97	52	
		66	79	82	42	47
+S9						
Trial 1						
Distilled water		83	104	91	37	
		88	103	78	30	
		89	88	70	26	
		91	104	79	29	30
Methylcholanthrene	2.5	52	34	403	259	
		49	30	418	283	271*
Ethylene glycol	1,000	84	113	71	28	
		82	93	71	29	29
	2,000	86	92	63	24	
		93	97	79	28	26
	3,000	81	90	80	33	
		68	93	72	35	34
	4,000	70	98	65	31	
		77	93	78	34	32
	5,000	77	111	47	20	
		81	109	79	33	27

TABLE C2
Induction of Trifluorothymidine Resistance in Mouse L5178Y Lymphoma Cells by Ethylene Glycol
(continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9 (continued)						
Trial 2						
Distilled water		66	112	86	43	
		71	84	118	56	
		71	107	146	69	
		64	97	96	50	54
Methylcholanthrene	2.5	44	29	558	424	
		39	22	455	391	407*
Ethylene glycol	1,000	69	98	161	78	
		69	91	146	71	74
	2,000	62	73	143	76	
		66	95	142	72	74
	3,000	55	93	101	61	
		67	100	133	66	64
	4,000	74	99	174	78	
		71	88	147	69	73
	5,000	78	94	198	85	
		73	111	140	64	75

* Significant positive response ($P \leq 0.05$)
[a] Study performed at Inveresk Research International. The experimental protocol and these data are presented by McGregor *et al.* (1991).
[b] Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by three (to arrive at MF/1 × 10^6 cells treated).

TABLE C3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Ethylene Glycol[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)[b]
-S9								
Trial 1								
Summary: Negative								
Medium		50	1,046	404	0.38	8.1	27.5	
Mitomycin-C	0.0050	50	1,045	539	0.51	10.8	27.5	33.54
	0.0100	10	208	164	0.78	16.4	27.5	104.14
Ethylene glycol	160	50	1,041	400	0.38	8.0	27.5	–0.52
	500	50	1,041	388	0.37	7.8	27.5	–3.50
	1,600	50	1,047	419	0.40	8.4	27.5	3.61
	5,000	50	1,040	436	0.41	8.7	27.5	8.54
								P=0.091[c]
Trial 2								
Summary: Negative								
Medium		50	1,048	425	0.40	8.5	26.5	
Mitomycin-C	0.0008	50	1,052	644	0.61	12.9	26.5	50.95
	0.0100	10	210	580	2.76	58.0	26.5	581.06
Ethylene glycol	1,600	50	1,050	420	0.40	8.4	26.5	–1.36
	3,000	50	1,048	395	0.37	7.9	26.5	–7.06
	4,000	50	1,049	431	0.41	8.6	26.5	1.31
	5,000	50	1,048	423	0.40	8.5	26.5	–0.47
								P=0.518
+S9								
Trial 1								
Summary: Negative								
Medium		50	1,047	418	0.39	8.4	26.0	
Cyclophosphamide	0.3	50	1,044	607	0.58	12.1	26.0	45.63
	2.0	10	210	381	1.81	38.1	26.0	354.44
Ethylene glycol	160	50	1,046	450	0.43	9.0	26.0	7.76
	500	50	1,045	430	0.41	8.6	26.0	3.07
	1,600	50	1,048	435	0.41	8.7	26.0	3.97
	5,000	50	1,048	478	0.45	9.6	26.0	14.24
								P=0.060

TABLE C3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Ethylene Glycol
(continued)

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)
+S9 (continued)								
Trial 2								
Summary: Negative								
Medium		50	1,046	416	0.39	8.3	26.0	
Cyclophosphamide	0.3	50	1,050	605	0.57	12.1	26.0	44.88
	2.0	10	209	414	1.98	41.4	26.0	398.08
Ethylene glycol	1,600	50	1,048	430	0.41	8.6	26.0	3.17
	3,000	50	1,048	409	0.39	8.2	26.0	-1.87
	4,000	50	1,050	427	0.40	8.5	26.0	2.25
	5,000	50	1,050	435	0.41	8.7	26.0	4.17
								P=0.370

[a] Study performed at Environmental Health Research and Testing, Inc. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway *et al.* (1985, 1987).
[b] Percent increase in SCEs/chromosome of culture exposed to ethylene glycol relative to those of culture exposed to solvent. Values at least 20% above control levels are considered positive.
[c] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose

TABLE C4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Ethylene Glycol[a]

-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: 12.0 hours Summary: Negative					**Trial 1** – Harvest time: 12.0 hours Summary: Negative				
Medium					Medium				
	100	0	0.00	0.0		100	2	0.02	2.0
Mitomycin-C					Cyclophosphamide				
0.250	100	23	0.23	17.0	15.0	100	42	0.42	29.0
1.000	50	35	0.70	40.0	50.0	50	52	1.04	54.0
Ethylene glycol					Ethylene glycol				
160	100	0	0.00	0.0	160	100	2	0.02	2.0
500	100	0	0.00	0.0	500	100	2	0.02	2.0
1,600	100	1	0.01	1.0	1,600	100	1	0.01	1.0
5,000	100	2	0.02	2.0	5,000	100	1	0.01	1.0
				P=0.020[b]					P=0.776

[a] Study performed at Environmental Health Research and Testing, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1985, 1987).
[b] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose

APPENDIX D
ORGAN WEIGHTS AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE D1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Feed Studies of Ethylene Glycol[a]

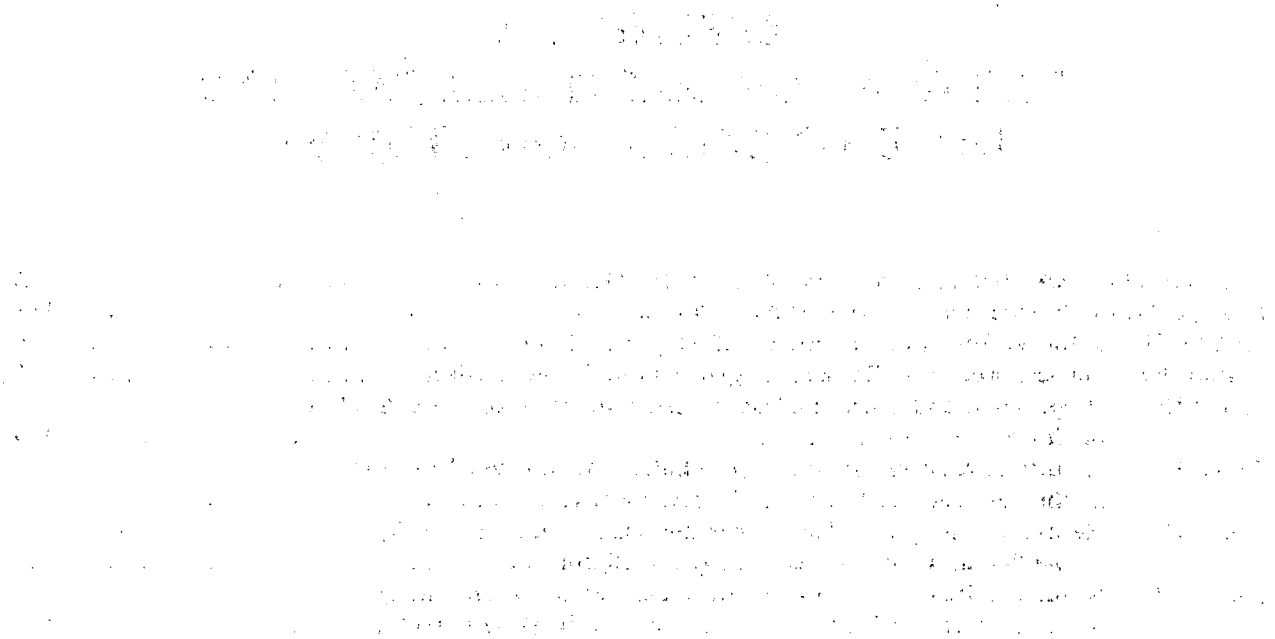
	0 ppm	3,200 ppm	6,300 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	32.4 ± 0.6	31.8 ± 0.7	32.0 ± 1.0	29.4 ± 0.9*	32.0 ± 0.6	30.3 ± 0.6*
Brain						
Absolute	0.437 ± 0.012	0.455 ± 0.003	0.445 ± 0.007	0.442 ± 0.004	0.449 ± 0.003	0.441 ± 0.006
Relative	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.15 ± 0.00*	0.14 ± 0.00	0.15 ± 0.00
Heart						
Absolute	0.156 ± 0.008	0.158 ± 0.006	0.139 ± 0.006	0.134 ± 0.005*	0.142 ± 0.004*	0.140 ± 0.006*
Relative	4.80 ± 0.22	4.96 ± 0.08	4.37 ± 0.19	4.56 ± 0.11	4.44 ± 0.09	4.63 ± 0.21
R. Kidney						
Absolute	0.254 ± 0.012	0.265 ± 0.013	0.232 ± 0.014	0.226 ± 0.007	0.281 ± 0.011	0.260 ± 0.011
Relative	7.84 ± 0.32	8.30 ± 0.25	7.25 ± 0.43	7.70 ± 0.19	8.77 ± 0.25	8.58 ± 0.31
Liver						
Absolute	1.49 ± 0.05	1.70 ± 0.10	1.50 ± 0.06	1.26 ± 0.04*	1.46 ± 0.04	1.37 ± 0.05
Relative	46.0 ± 1.43	53.2 ± 2.50*	46.8 ± 1.60	42.9 ± 1.27	45.8 ± 1.05	45.2 ± 1.51
Lungs						
Absolute	0.166 ± 0.003	0.173 ± 0.007	0.181 ± 0.012	0.168 ± 0.006	0.162 ± 0.003	0.166 ± 0.009
Relative	5.14 ± 0.15	5.44 ± 0.16	5.65 ± 0.30	5.74 ± 0.20	5.08 ± 0.13	5.50 ± 0.31
R. Testis						
Absolute	0.107 ± 0.006	0.116 ± 0.010	0.106 ± 0.004	0.105 ± 0.004	0.106 ± 0.003	0.108 ± 0.005
Relative	3.30 ± 0.18	3.67 ± 0.34	3.32 ± 0.13	3.58 ± 0.10	3.32 ± 0.09	3.57 ± 0.17
Thymus						
Absolute	0.048 ± 0.006	0.045 ± 0.004	0.046 ± 0.004	0.042 ± 0.003	0.040 ± 0.004	0.041 ± 0.005
Relative	1.46 ± 0.18	1.42 ± 0.13	1.40 ± 0.10	1.43 ± 0.10	1.26 ± 0.12	1.35 ± 0.17
Female						
n	10	10	10	10	10	10
Necropsy body wt	25.5 ± 0.6	24.7 ± 0.6	24.8 ± 0.6	25.6 ± 0.7	26.1 ± 0.7	24.8 ± 0.6
Brain						
Absolute	0.468 ± 0.005	0.475 ± 0.006	0.465 ± 0.010	0.458 ± 0.009	0.459 ± 0.008	0.456 ± 0.011
Relative	0.18 ± 0.00	0.19 ± 0.00	0.19 ± 0.00	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.00
Heart						
Absolute	0.131 ± 0.007	0.114 ± 0.003	0.129 ± 0.004	0.114 ± 0.003[b]	0.125 ± 0.005	0.120 ± 0.005
Relative	5.13 ± 0.19	4.63 ± 0.13	5.20 ± 0.11	4.53 ± 0.11[b]	4.82 ± 0.26	4.85 ± 0.22
R. Kidney						
Absolute	0.186 ± 0.009	0.170 ± 0.007	0.183 ± 0.007	0.167 ± 0.005	0.176 ± 0.008	0.185 ± 0.005
Relative	7.28 ± 0.25	6.90 ± 0.29	7.37 ± 0.17	6.55 ± 0.20	6.74 ± 0.19	7.47 ± 0.17
Liver						
Absolute	1.23 ± 0.06	1.07 ± 0.03*	1.12 ± 0.04	1.13 ± 0.03[b]	1.24 ± 0.05	1.14 ± 0.04
Relative	48.4 ± 2.01	43.3 ± 1.37*	45.1 ± 0.75	45.1 ± 1.07[b]	47.6 ± 1.20	45.8 ± 0.71
Lungs						
Absolute	0.157 ± 0.007	0.171 ± 0.007	0.162 ± 0.004	0.166 ± 0.006	0.160 ± 0.007[b]	0.167 ± 0.010
Relative	6.15 ± 0.20	6.95 ± 0.33	6.56 ± 0.21	6.50 ± 0.24	6.14 ± 0.22[b]	6.70 ± 0.29
Thymus						
Absolute	0.049 ± 0.003	0.050 ± 0.004	0.052 ± 0.004	0.052 ± 0.006	0.050 ± 0.003	0.045 ± 0.005
Relative	1.93 ± 0.13	2.04 ± 0.17	2.10 ± 0.14	2.02 ± 0.22	1.92 ± 0.12	1.79 ± 0.16

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

TABLE D2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 15-Month Interim Evaluations in the 2-Year Feed Studies of Ethylene Glycol[a]

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm
Male				
n	6	6	6	6
Necropsy body wt	38.2 ± 1.2	38.8 ± 2.0	39.0 ± 1.9	37.5 ± 1.3
Brain				
Absolute	0.487 ± 0.006	0.472 ± 0.004	0.482 ± 0.010	0.478 ± 0.008
Relative	12.8 ± 0.43	12.3 ± 0.55	12.5 ± 0.54	12.8 ± 0.43
R. Kidney				
Absolute	0.408 ± 0.031	0.380 ± 0.017	0.415 ± 0.024	0.405 ± 0.009
Relative	10.6 ± 0.55	9.8 ± 0.25	10.7 ± 0.55	10.9 ± 0.45
Liver				
Absolute	1.83 ± 0.04	2.09 ± 0.32	1.81 ± 0.11	2.38 ± 0.37
Relative	48.0 ± 0.94	53.3 ± 6.88	46.5 ± 2.66	62.9 ± 8.31
	0 ppm	**12,500 ppm**	**25,000 ppm**	**50,000 ppm**
Female				
n	10	10	9	10
Necropsy body wt	44.6 ± 2.3	43.6 ± 1.9	46.0 ± 1.3	38.0 ± 1.1*
Brain				
Absolute	0.483 ± 0.007	0.505 ± 0.009	0.479 ± 0.024	0.504 ± 0.011
Relative	11.1 ± 0.49	11.8 ± 0.57	10.4 ± 0.47	13.3 ± 0.43**
R. Kidney				
Absolute	0.221 ± 0.008	0.250 ± 0.008*	0.261 ± 0.024*	0.269 ± 0.007**
Relative	5.02 ± 0.19	5.81 ± 0.25*	5.66 ± 0.49*	7.11 ± 0.21**
Liver				
Absolute	1.60 ± 0.07	1.69 ± 0.03	1.72 ± 0.06	1.51 ± 0.05
Relative	36.2 ± 1.07	39.3 ± 1.45	37.4 ± 0.72	39.8 ± 1.39*

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).



APPENDIX E
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF ETHYLENE GLYCOL

Ethylene glycol was obtained from Ashland Chemical Company (Columbus, OH; lot A021180). Reports from the analytical chemistry laboratory, Midwest Research Institute (MRI; Kansas City, MO), on analyses performed in support of the ethylene glycol studies are on file at the National Institute of Environmental Health Sciences.

Lot A021180, a colorless liquid, was identified as ethylene glycol by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra (Figures E1 and E2) were consistent with those expected for the structure and with the literature description for the spectra of ethylene glycol (*Sadtler Standard Spectra*).

The purity of the lot was found to be greater than 99% by elemental analyses, Karl Fischer water analysis, thin-layer chromatography (TLC), and gas chromatography. Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for ethylene glycol. Karl Fischer water analysis indicated less than 0.25% water. Periodate oxidation followed by iodometric titration indicated a purity of greater than 99%. Titration of acidic components with sodium hydroxide indicated 0.7 ppm acetic acid.

TLC was performed on silica gel plates with two solvent systems: 1) acetone:acetic acid (99:1) and 2) acetonitrile:acetic acid (99:1). After drying, plates were sprayed with 0.5 g potassium permanganate dissolved in 100 mL *N* sodium hydroxide and examined under ultraviolet light (254 nm). Each system indicated one major spot.

Gas chromatographic analysis was performed with a Varian 3700 gas chromatograph with a nitrogen carrier gas at a flow rate of 70 mL/minute. Two 1.8 m × 4 mm ID glass columns were used: 1) a 60/80 Tenax-GC column and 2) a 10% Carbowax 20M-TPA on 80/100 Chromosorb W(AW) column. Gas chromatography using the first column indicated a major peak and two impurities with a total area of 0.30% relative to the major peak. With the second column a major peak and one impurity with a total area of 0.12% relative to the major peak was observed.

Stability studies were performed by the analytical chemistry laboratory using gas chromatography and column 1) described above, and with a solution of 0.5% (w/v) ethylene glycol in methanol containing 0.5% (v/v) hexyl alcohol as an internal standard. These studies indicated that ethylene glycol was stable as a bulk chemical for 2 weeks at temperatures up to 60° C when protected from light. The identity and stability of the bulk chemical were confirmed periodically at the study laboratory with infrared spectroscopy and gas chromatography methods similar to those described above. Identity was confirmed and no change in purity was observed.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing ethylene glycol and feed in a blender (Patterson-Kelly Twin Shell with intensifier bar) for 5 minutes with the intensifier bar on and 10 minutes with the intensifier bar off (Table E1). Studies were conducted by the analytical chemistry laboratory to determine homogeneity and stability of the dosed feed preparations. For homogeneity and stability analyses, the formulations were extracted with a solvent of methanol:water:concentrated hydrochloric acid (500:495:5) and centrifuged. These aliquots were then mixed with a methanolic sodium hydroxide solution (1.25 g sodium hydroxide in 25 mL methanol) and the internal standard solution of *n*-amyl

alcohol in methanol. The precipitate was separated by centrifugation and the clear supernatant solution was analyzed using a Varian 3700 gas chromatograph with a glass 1.8 m × 2 mm ID column packed with 100-120 mesh Chromosorb 101, and a gas carrier of nitrogen at 30 mL/minute. Homogeneity was confirmed; stability of the formulations was established for 2 weeks when stored in the dark at temperatures up to 25° C. During the studies, the dose formulations were stored at 5° C for up to 14 days before use and at room temperature during use.

Periodic analyses of the dose formulations of ethylene glycol were conducted at the study laboratory and the analytical chemistry laboratory with gas chromatography. During the 13-week studies, the dose formulations were analyzed at the initiation and the midpoint of the studies (Table E2). During the 2-year studies, the dose formulations were analyzed at least once every 8 weeks (Table E3) with 98% (97/99) of the dose formulations within 10% of the target concentrations. Results of the periodic referee analysis performed by the analytical chemistry laboratory indicated good agreement with the results obtained by the study laboratory (Table E4).

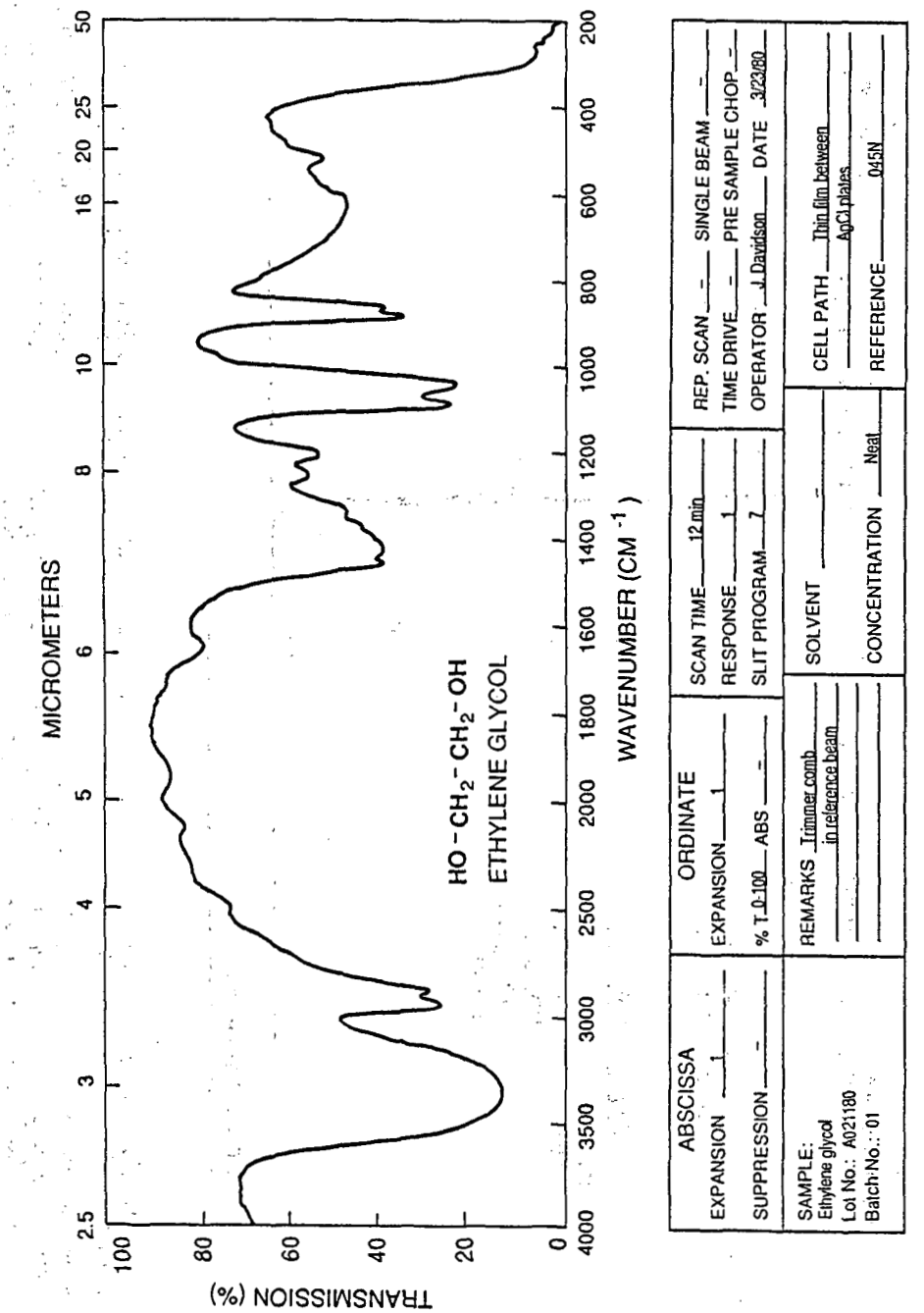


FIGURE E1
Infrared Absorption Spectrum of Ethylene Glycol

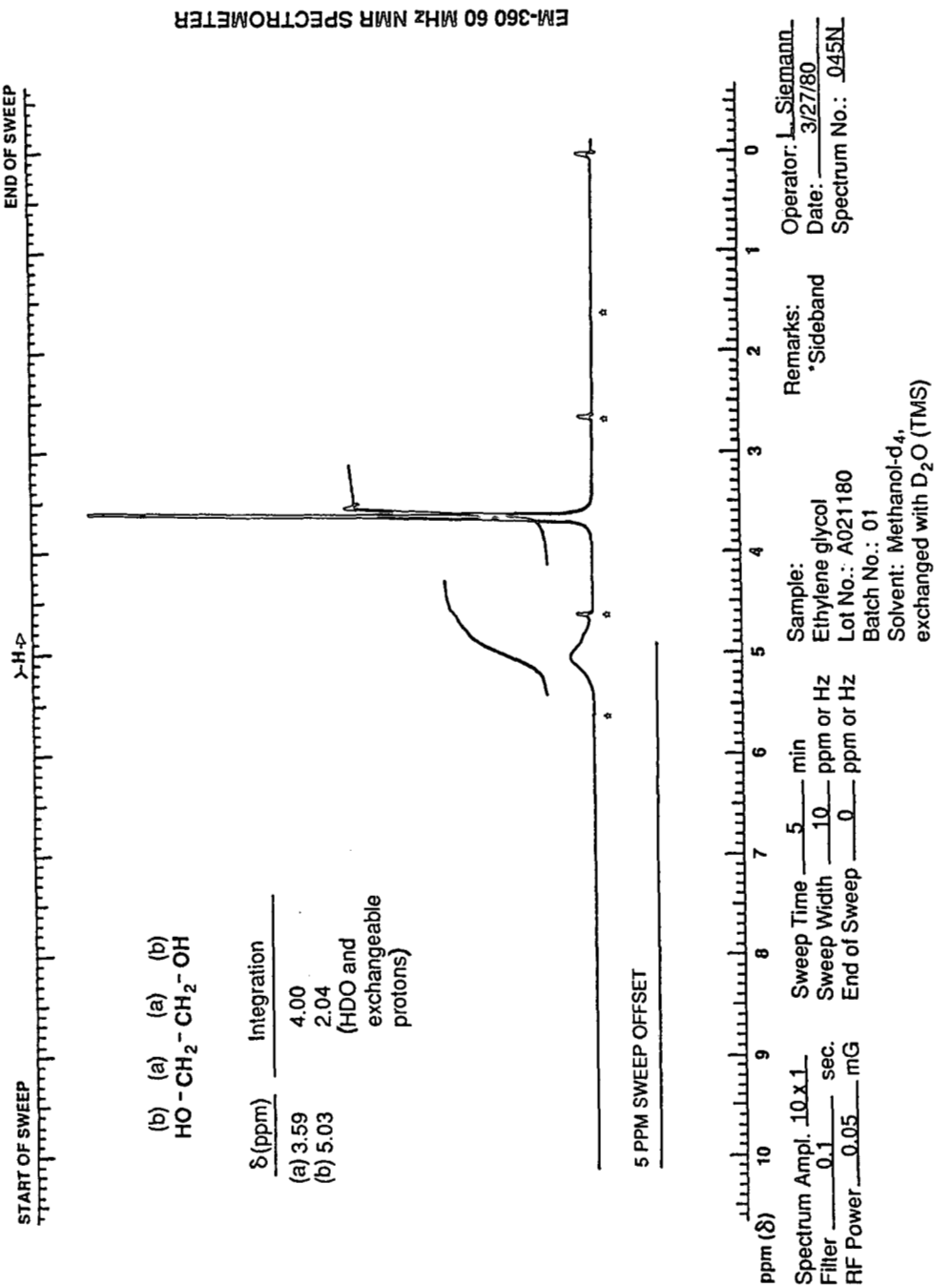


FIGURE E2
Nuclear Magnetic Resonance Spectrum of Ethylene Glycol

TABLE E1
Preparation and Storage of Dose Formulations in the Feed Studies of Ethylene Glycol

13-Week Studies	2-Year Studies
Preparation	
Premix was prepared by mixing ethylene glycol and feed (v/w); premix and remaining feed added to blender with intensifier bar and mixed for 5 minutes with the intensifier bar on and 10 minutes with the intensifier bar off.	Same as 13-week studies
Lot Number	
A021180	Same as 13-week studies
Maximum Storage Time	
14 days	Same as 13-week studies
Storage	
Stored at 5° C prior to use and at room temperature during use	Same as 13-week studies

TABLE E2
Results of Analysis of Dose Formulations Administered to Mice in the 13-Week Feed Studies of Ethylene Glycol

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
18 May 1981	21 May 1981	6,300	6,200	–2
18 May 1981	4 June 1981	3,200	2,800	–12
		12,500	13,400	+7
		25,000	26,900	+8
		50,000	53,200	+6
6 July 1981	8 July 1981	3,200	3,090	–3
		6,300	6,130	–3
		12,500	13,700	+10
		25,000	24,000	–4
		50,000	47,400	–5

TABLE E3
Results of Analysis of Dose Formulations Administered to Mice in the 2-Year Feed Studies of Ethylene Glycol

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
16 September 1982	22 September 1982	6,250[a]	6,210	–1
		6,250[a]	6,150	–2
		6,250[a]	5,880	–6
		12,500	1,140	–9
		25,000	2,260	–10
		50,000[a]	4,930	–1
		50,000[a]	4,310	–14
		50,000[a]	4,630	–7
11 November 1982	12 November 1982	6,250	6,190	–1
		12,500	11,500	–8
		25,000	23,900	–4
		50,0000	48,200	–4
6 January 1983	7 January 1983	6,250	5,707	–8
		12,500	11,900	–5
		12,500	12,000	–4
		25,000	25,100	0
		50,000	48,800	–2
3 March 1983	4 March 1983	6,250	6,200	–1
		12,500	12,900	+3
		12,500	12,400	–1
		25,000	25,700	+3
		25,000	26,600	+6
		50,000	52,400	+5
28 April 1983	30 April 1983	6,250	5,800	–7
		12,500	11,200	–10
		12,500	11,900	–5
		25,000[b]	21,200	–15
		25,000	23,600	–6
		50,000	48,000	–4
3 May 1983	4 May 1983	25,000[c]	22,400	–10
23 June 1983	23-27 June 1983	6,250	6,490	+4
		12,500	11,500	–8
		12,500	12,000	–4
		25,000	22,700	–9
		25,000	24,800	–1
		50,000	46,500	–7
18 August 1983	19 August 1983	6,250	6,180	–1
		12,500	12,000	–4
		12,500	12,300	–2
		25,000	24,800	–1
		25,000	24,200	–3
		50,000	49,800	0

TABLE E3
Results of Analysis of Dose Formulations Administered to Mice in the 2-Year Feed Studies of Ethylene Glycol (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
13 October 1983	13, 14 October 1983	6,250	6,140	–2
		12,500	12,000	–4
		12,500	11,900	–5
		25,000	24,100	–4
		25,000	25,100	0
		50,000	50,100	0
8 December 1983	9 December 1983	6,250	6,520	+4
		6,250	6,100	–2
		12,500	11,700	–6
		12,500	11,500	–8
		12,500	12,200	–2
		25,000	26,200	+5
		25,000	25,400	+2
		50,000	50,000	0
16 February 1984	16, 17 February 1984	6,250	6,300	+1
		12,500	12,600	+1
		12,500	13,300	+6
		12,500	12,600	+1
		25,000	25,200	+1
		25,000	25,200	+1
		25,000	25,100	0
		50,000	51,000	+2
		50,000	51,300	+3
12 April 1984	12, 13 April 1984	6,250	6,060	–3
		12,500	12,400	–1
		12,500	11,900	–5
		25,000	25,300	+1
		25,000	25,000	0
		50,000	51,600	+3
24 May 1984	24 May 1984	6,250	6,540	+5
		12,500	12,800	+2
		12,500	12,100	–3
		25,000	24,400	–2
		25,000	25,300	+1
		50,000	51,700	+3
24 May 1984	29 May 1984[d]	6,250	5,960	–5
		12,500	12,100	–3
		12,500	12,600	+1
		25,000	25,200	+1
		25,000	25,300	+1
		50,000	48,800	–2
5 July 1984	5 July 1984[e]	6,250	5,640	–10
		12,500	12,800	+2
		12,500	12,700	+2
		25,000	24,400	–2
		25,000	24,700	–1
		50,000	49,900	0

TABLE E3
Results of Analysis of Dose Formulations Administered to Mice in the 2-Year Feed Studies of Ethylene Glycol (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
23 August 1984	26 August 1984[e]	6,250[a]	6,100	–2
		6,250[a]	6,000	–4
		6,250[a]	5,920	–5
		12,500	12,600	+1
		12,500	12,100	–3
		25,000	24,600	–2
		25,000	23,000	–8
		50,000[a]	48,700	–3
		50,000[a]	49,400	–1
		50,000[a]	46,200	–8

[a] Homogeneity analysis (top left, top right, bottom ports, respectively)
[b] Preparation out of specification; not used in study
[c] Remix
[d] Comparative analysis of Chromosorb 101 column and a new chromatographic column [0.8% THEED (tetrahydroxy ethylethylene diamine) on 80/100 mesh Carbopak C; temperatures for injector, oven, and detector were 160, 115, and 180° C, respectively]
[e] 0.8% THEED column used

TABLE E4
Results of Referee Analysis of Dose Formulations Administered to Mice in the 13-Week and 2-Year Feed Studies of Ethylene Glycol

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
13-Week Studies				
6 July 1981	8 July 1981	25,000	24,100	–4
2-Year Studies				
16 September 1982	22 September 1982	6,250	6,170	–1
3 March 1983	4 March 1983	12,500	11,900	–5
18 August 1983	19 August 1983	50,000	46,800	–6
16 February 1984	16, 17 February 1984	25,000	25,900	+4
5 July 1984	5 July 1984	12,500	11,800	–6

APPENDIX F
FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR FEED STUDIES

TABLE F1
Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of Ethylene Glycol

Weeks on Study	0 ppm		6,250 ppm			12,500 ppm			25,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
2	7.2	24.8	9.5	25.6	2,321	8.7	25.4	4,295	8.6	25.8	8,320
3	11.8	25.3	9.5	27.4	2,162	8.8	27.0	4,094	9.7	26.9	9,022
6	6.7	29.2	7.4	29.7	1,561	7.4	29.5	3,136	8.8	30.0	7,317
7	7.8	29.6	8.0	30.8	1,621	7.6	29.9	3,175	7.9	29.5	6,708
10	3.5	30.1	3.4	32.5	651	3.9	31.4	1,539	3.2	31.5	2,515
11	8.3	31.1	8.7	33.1	1,649	9.1	32.0	3,539	7.8	31.7	6,153
13	8.3	32.4	9.9	33.7	1,828	10.3	33.5	3,845	9.2	33.3	6,942
14	7.4	32.4	8.5	34.2	1,561	8.7	32.7	3,335	8.0	33.2	6,047
17	6.9	33.3	7.4	35.0	1,323	8.3	35.6	2,927	7.7	34.3	5,586
21	7.8	33.2	8.3	35.5	1,457	8.6	35.5	3,037	9.0	34.5	6,529
25	8.7	34.2	9.2	36.4	1,578	9.6	36.9	3,266	11.2	36.6	7,683
29	9.8	35.5	8.4	38.0	1,382	10.2	38.0	3,362	10.0	37.8	6,581
33	10.6	36.3	9.1	38.9	1,459	10.4	39.1	3,328	10.4	38.6	6,742
37	12.0	37.3	10.7	39.6	1,688	12.9	39.6	4,087	12.0	40.0	7,508
41	10.9	37.9	9.9	40.6	1,524	10.5	40.0	3,284	10.9	40.1	6,773
45	11.3	38.4	10.3	41.0	1,577	12.7	40.2	3,950	11.2	41.6	6,715
49	4.7	38.7	5.0	40.7	769	5.2	41.3	1,575	5.1	41.6	3,057
54	5.7	38.7	6.0	40.0	944	6.2	39.1	1,986	5.9	40.3	3,667
58	6.6	39.0	7.1	39.6	1,124	6.9	39.8	2,179	10.8	38.5	6,998
62	9.1	38.9	9.1	39.3	1,448	9.6	40.4	2,968	12.4	38.9	7,984
66	9.0	39.2	8.3	39.6	1,306	8.1	40.7	2,489	10.7	38.6	6,941
70	5.4	40.1	5.6	40.2	866	5.5	40.9	1,667	7.2	39.3	4,580
74	5.0	39.8	5.3	41.5	791	5.4	41.6	1,610	5.4	39.2	3,473
78	5.6	40.0	5.6	41.1	856	5.7	41.4	1,719	5.6	39.0	3,562
82	5.1	39.5	5.2	39.9	821	5.2	41.2	1,567	5.0	37.7	3,313
86	5.5	39.3	4.9	39.2	787	5.3	42.1	1,583	5.3	37.4	3,572
90	5.2	38.6	5.1	38.6	829	5.0	40.2	1,547	5.0	36.9	3,419
94	4.8	38.7	5.1	38.6	824	5.0	39.7	1,571	4.9	35.6	3,423
98	5.4	37.4	5.3	39.2	849	5.2	40.0	1,640	5.1	36.6	3,507
102	4.8	39.0	4.9	39.6	767	4.8	40.7	1,467	4.7	36.3	3,271
Mean for weeks											
1-13	7.7	28.9	8.1	30.4	1,685	8.0	29.8	3,375	7.9	29.8	6,711
14-52	9.0	35.7	8.7	38.0	1,432	9.7	37.9	3,215	9.5	37.8	6,322
52-102	5.9	39.1	6.0	39.7	939	6.0	40.6	1,846	6.8	38.0	4439

[a] Grams of feed consumed per animal per day; not corrected for spillage or scatter
[b] Milligrams of ethylene glycol consumed per day per kilogram body weight

TABLE F2
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Ethylene Glycol

Weeks on Study	0 ppm		12,500 ppm			25,000 ppm			50,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
2	7.6	19.6	8.7	18.6	5,850	9.0	19.0	11,847	8.5	19.6	21,780
3	7.8	20.0	8.5	20.8	5,094	8.1	21.1	9,654	9.6	20.7	23,300
6	6.7	22.8	7.8	23.2	4,222	8.3	23.3	8,958	8.3	23.1	17,940
7	7.5	23.5	6.8	24.0	3,532	8.5	23.8	8,946	9.0	23.4	19,219
10	3.5	24.4	4.1	24.1	2,109	4.0	24.5	4,116	4.1	24.9	8,288
11	8.9	25.4	9.0	25.2	4,450	9.1	25.8	8,854	10.7	26.2	20,434
13	8.1	25.8	9.1	26.1	4,347	9.5	26.2	9,049	7.6	26.0	14,594
14	6.7	25.8	8.0	26.3	3,781	7.8	26.4	7,429	8.1	26.0	15,605
17	6.2	27.0	7.5	27.4	3,417	7.8	27.2	7,204	7.5	27.4	13,682
21	6.1	28.8	7.2	28.3	3,174	7.3	29.3	6,240	7.4	29.1	12,733
25	7.6	29.9	8.5	30.5	3,479	8.8	30.1	7,272	8.2	30.5	13,451
29	6.8	31.5	7.5	32.2	2,917	6.8	31.9	5,303	7.3	32.1	11,383
33	7.7	32.8	8.6	32.9	3,268	8.6	32.0	6,680	9.4	32.7	14,408
37	7.4	33.0	9.0	34.4	3,279	9.1	34.6	6,600	8.9	33.9	13,158
41	6.0	34.2	6.8	35.0	2,414	7.7	35.8	5,357	7.7	34.6	11,066
45	7.7	35.1	8.2	36.7	2,776	8.7	36.8	5,894	9.3	35.8	12,951
49	5.3	36.1	4.9	37.7	1,639	5.4	37.2	3,619	4.9	36.9	6,602
54	5.5	37.2	5.4	38.0	1,778	5.4	38.2	3,527	5.6	37.7	7,486
58	5.3	40.8	5.6	40.0	1,738	5.6	40.7	3,425	6.1	39.8	7,657
62	4.6	42.5	4.8	42.1	1,438	4.8	43.1	2,776	4.8	41.0	5,866
66	5.5	43.7	5.1	42.5	1,486	6.5	42.7	3,782	7.4	41.8	8,805
70	9.5	44.6	8.9	43.3	2,583	11.4	43.4	6,554	14.4	42.6	16,922
73	8.9	45.5	8.4	44.5	2,367	10.9	45.7	5,976	15.2	44.3	17,197
77	6.3	45.1	5.8	43.2	1,684	5.6	46.0	3,049	6.2	42.8	7,250
81	5.5	45.2	5.5	43.7	1,581	5.6	45.7	3,062	5.8	43.6	6,610
85	6.9	46.9	6.8	43.4	1,964	7.3	45.4	4,020	9.0	42.9	10,509
89	6.0	46.2	6.2	43.0	1,807	6.0	43.8	3,436	6.0	42.5	7,100
93	5.5	45.2	4.8	43.4	1,388	5.3	44.8	2,950	5.1	43.2	5,955
97	7.1	45.7	6.4	44.4	1,812	6.6	44.3	3,741	6.2	44.3	6,989
102	6.6	46.6	6.5	44.6	1,820	5.9	44.3	3,339	5.9	44.0	6,726
Mean for weeks											
1-13	7.2	23.1	7.7	23.1	4,229	8.1	23.4	8,775	8.3	23.4	17,936
14-52	6.8	31.4	7.6	32.1	3,014	7.8	32.1	6,160	7.9	31.9	12,504
52-102	6.4	44.2	6.2	42.8	1,804	6.7	43.7	3,818	7.5	42.3	8,852

[a] Grams of feed consumed per day; not corrected for spillage or scatter
[b] Milligrams of ethylene glycol consumed per day per kilogram body weight

APPENDIX G
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-07 RAT AND MOUSE RATION

TABLE G1
Ingredients of NIH-07 Rat and Mouse Ration[a]

Ingredients[b]	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground whole wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

[a] NCI, 1976; NIH, 1978
[b] Ingredients ground to pass through a U.S. Standard Screen No. 16 before being mixed

TABLE G2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
d-α-Tocopherol acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 μg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product

TABLE G3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.76 ± 1.16	21.2–25.9	26
Crude Fat (% by weight)	5.16 ± 0.58	4.2–6.3	26
Crude Fiber (% by weight)	3.52 ± 0.37	2.8–4.5	26
Ash (% by weight)	6.68 ± 0.25	6.3–7.3	26
Amino Acids (% of total diet)			
Arginine	1.308 ± 0.606	1.210–1.390	8
Cystine	0.306 ± 0.084	0.181–0.400	8
Glycine	1.150 ± 0.047	1.060–1.210	8
Histidine	0.576 ± 0.024	0.531–0.607	8
Isoleucine	0.917 ± 0.029	0.881–0.944	8
Leucine	1.946 ± 0.055	1.850–2.040	8
Lysine	1.270 ± 0.058	1.200–1.370	8
Methionine	0.448 ± 0.128	0.306–0.699	8
Phenylalanine	0.987 ± 0.140	0.665–1.110	8
Threonine	0.877 ± 0.042	0.824–0.940	8
Tryptophan	0.236 ± 0.176	0.107–0.671	8
Tyrosine	0.676 ± 0.105	0.564–0.794	8
Valine	1.103 ± 0.040	1.050–1.170	8
Essential Fatty Acids (% of total diet)			
Linoleic	2.393 ± 0.258	1.830–2.570	7
Linolenic	0.280 ± 0.040	0.210–0.320	7
Vitamins			
Vitamin A (IU/kg)	11,404 ± 4,343	4,200–22,000	26
Vitamin D (IU/kg)	4,450 ± 1,382	3,000–6,300	4
α-Tocopherol (ppm)	37.95 ± 9.41	22.50–48.90	8
Thiamine (ppm)	18.50 ± 3.82	12.0–31.0	26
Riboflavin (ppm)	7.92 ± 0.87	6.10–9.00	8
Niacin (ppm)	103.38 ± 26.59	65.0–150.0	8
Pantothenic acid (ppm)	29.54 ± 3.60	23.0–34.0	8
Pyridoxine (ppm)	9.55 ± 3.48	5.60–14.0	8
Folic Acid (ppm)	2.25 ± 0.73	1.80–3.70	8
Biotin (ppm)	0.254 ± 0.042	0.19–0.32	8
Vitamin B_{12} (ppb)	38.45 ± 22.01	10.6–65.0	8
Choline (ppm)	3,089 ± 328.69	2,400–3,430	8
Minerals			
Calcium (%)	1.23 ± 0.12	0.97–1.43	26
Phosphorus (%)	0.95 ± 0.05	0.86–1.10	26
Potassium (%)	0.883 ± 0.078	0.772–0.971	6
Chloride (%)	0.526 ± 0.092	0.380–0.635	8
Sodium (%)	0.313 ± 0.390	0.258–0.371	8
Magnesium (%)	0.168 ± 0.010	0.151–0.181	8
Sulfur (%)	0.280 ± 0.064	0.208–0.420	8
Iron (ppm)	360.54 ± 100	255.0–523.0	8
Manganese (ppm)	91.97 ± 6.01	81.70–99.40	8
Zinc (ppm)	54.72 ± 5.67	46.10–64.50	8
Copper (ppm)	11.06 ± 2.50	8.090–15.39	8
Iodine (ppm)	3.37 ± 0.92	1.52–4.13	6
Chromium (ppm)	1.79 ± 0.36	1.04–2.09	8
Cobalt (ppm)	0.681 ± 0.14	0.490–0.780	4

TABLE G4
Contaminant Levels in NIH-07 Rat and Mouse Ration

Contaminants	Mean ± Standard Deviation[a]	Range	Number of Samples
Arsenic (ppm)	0.55 ± 0.15	0.18–0.77	26
Cadmium (ppm)	0.12 ± 0.04	<0.10–0.20	26
Lead (ppm)	0.52 ± 0.20	0.24–1.00	26
Mercury (ppm)	<0.05		26
Selenium (ppm)	0.31 ± 0.06	0.21–0.45	26
Aflatoxins (ppb)	<5.0		26
Nitrate nitrogen (ppm)	9.21 ± 3.89	2.50–19.0	26
Nitrite nitrogen (ppm)	1.07 ± 1.37	<0.10–6.10	26
BHA (ppm)[b]	4.00 ± 4.99	<2.00–20.00	26
BHT (ppm)[b]	3.04 ± 2.58	<1.00–13.00	26
Aerobic plate count (CFU/g)[c]	146,296 ± 143,824	6,600–420,000	26
Coliform (MPN/g)[d]	496 ± 780	3.00–2,400	26
E. coli (MPN/g)[e]	3.80 ± 2.36	3.00–15.0	25
E. coli (MPN/g)[f]	9.42 ± 28.79	3.00–150	26
Total nitrosoamines (ppb)[g]	5.85 ± 5.89	0.80–30.30	26
N-Nitrosodimethylamine (ppb)[g]	5.01 ± 5.88	0.50–30.00	26
N-Nitrosopyrrolidine (ppb)[g]	0.84 ± 0.71	0.30–2.70	26
Pesticides			
α-BHC[h]	<0.01		26
ß-BHC	<0.02		26
ß-BHC	<0.01		26
δ-BHC	<0.01		26
Heptachlor	<0.01		26
Aldrin	<0.01		26
Heptachlor epoxide	<0.01		26
DDE	<0.01		26
DDD	<0.01		26
DDT	<0.01		26
HCB	<0.01		26
Mirex	<0.01		26
Methoxychlor	<0.05		26
Dieldrin	<0.01		26
Endrin	<0.01		26
Telodrin	<0.01		26
Chlordane	<0.05		26
Toxaphene	<0.1		26
Estimated PCBs	<0.2		26
Ronnel	<0.01		26
Ethion	<0.02		26
Trithion	<0.05		26
Diazinon	<0.1		26
Methyl parathion	<0.02		26
Ethyl parathion	<0.02		26
Malathion[i]	0.15 ± 0.15	0.05–0.81	26
Endosulfan I	<0.01		26
Endosulfan II	<0.01		26
Endosulfan sulfate	<0.03		26

[a] For values less than the limit of detection, the detection limit is given for the mean.
[b] Sources of contamination: soy oil and fish meal
[c] CFU = colony-forming units
[d] MPN = most probable number
[e] Mean, standard deviation, and range exclude one large value of 150 MPN/g obtained on 26 August 1983.
[f] Mean, standard deviation, and range include the value of 150 MPN/g obtained on 26 August 1983.
[g] All values were corrected for % recovery.
[h] BHC is hexachlorocyclohexane or benzene hexachloride.
[i] Fourteen lots contained more than 0.05 ppm.

APPENDIX H
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. The sentinel animals come from the same production source and weanling groups as animals used for the studies of chemical compounds, and these animals and the study animals are subject to identical environmental conditions.

During the 13-week studies, three male and two female B6C3F$_1$ mice were maintained with the study animals to serve as sentinel animals. At termination of the 13-week studies, blood samples were taken from the sentinel mice. The blood was allowed to clot, and the serum was separated. The serum was cooled and sent to Microbiological Associates, Incorporated (Bethesda, MD), for determination of antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM (pneumonia virus of mice)	Study termination
Reovirus 3	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
Polyoma virus	Study termination
Sendai	Study termination
MVM (minute virus of mice)	Study termination
Ectromelia virus (mouse pox)	Study termination
Complement Fixation	
LCM (lymphocytic choriomeningitis virus)	Study termination
ELISA	
MHV (mouse hepatitis virus)	Study termination

During the 2-year studies, 15 B6C3F$_1$ mice of each sex were maintained with the study animals to serve as sentinel animals. Blood was drawn from four or five mice of each sex at 6, 12, and 18 months following study initiation. Five randomly selected control animals of each sex were bled at study termination (24 months). Blood collected from each animal was allowed to clot and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Incorporated (Bethesda, MD), for determination of antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM	6, 12, and 18 months
Reovirus 3	6, 12, and 18 months
GDVII	6, 12, and 18 months
Polyoma virus	6, 12, 18, and 24 months
Sendai	6, 12, and 18 months
MVM	6, 12, 18, and 24 months
Ectromelia virus	6, 12, and 18 months

Method of Analysis (continued)	Time of Analysis (continued)
Complement Fixation	
Mouse adenoma virus	6, 12, and 18 months
LCM	6, 12, 18, and 24 months
ELISA	
PVM	24 months
Reovirus 3	24 months
GDVII	24 months
MHV	12, 18, and 24 months
Mouse adenoma virus	24 months
Ectromelia virus	24 months
Sendai	24 months
Mycoplasma pulmonis	12, 18, and 24 months
Immunofluorescence Assay	
EDIM (epizootic diarrhea of infant mice)	24 months
Mycoplasma arthritidis	24 months

All test results were negative for presence of the above pathogens.

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TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	1,2-Dibromo-3-chloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butyl Benzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-Ethylhexyl)phthalate
219	2,6-Dichloro-p-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	*D*-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-Methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
243	Trichlorethylene (Rats and Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
246	Chrysotile Asbestos (Hamsters)
247	L-Ascorbic Acid
248	4,4′-Methylenedianiline Dihydrochloride
249	Amosite Asbestos (Hamsters)
250	Benzyl Acetate
251	2,4- & 2,6-Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
254	Dichloromethane (Methylene Chloride)
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	1,2-Propylene Oxide
269	Telone II® (1,3-Dichloropropene)
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four Rat Strains)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
277	Tremolite
278	2,6-Xylidine
279	Amosite Asbestos
280	Crocidolite Asbestos
281	HC Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl) phosphonium Sulfate & Tetrakis(hydroxymethyl) phosponium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane (Methylene Chloride)
307	Ephedrine Sulfate
308	Chlorinated Pariffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
313	Mirex
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	2-Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS PRINTED AS OF FEBRUARY 1993 (CONT.)

TR No.	CHEMICAL
336	Penicillin VK
337	Nitrofurazone
338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
340	Iodinated Glycerol
341	Nitrofurantoin
342	Dichlorvos
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Roxarsone
346	Chloroethane
347	D-Limonene
348	α-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
351	*p*-Chloroaniline Hydrochloride
352	N-Methylolacrylamide
353	2,4-Dichlorophenol
354	Dimethoxane
355	Diphenhydramine Hydrochloride
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
360	N,N-Dimethylaniline
361	Hexachloroethane
362	4-Vinyl-1-Cyclohexene Diepoxide
363	Bromoethane (Ethyl Bromide)
364	Rhodamine 6G (C.I. Basic Red 1)
365	Pentaerythritol Tetranitrate
366	Hydroquinone
367	Phenylbutazone
368	Nalidixic Acid
369	Alpha-Methylbenzyl Alcohol
370	Benzofuran
371	Toluene
372	3,3-Dimethoxybenzidine Dihydrochloride
373	Succinic Anhydride
374	Glycidol
375	Vinyl Toluene
376	Allyl Glycidyl Ether
377	*o*-Chlorobenzalmalononitrile
378	Benzaldehyde
379	2-Chloroacetophenone
380	Epinephrine Hydrochloride
381	*d*-Carvone
382	Furfural
385	Methyl Bromide
386	Tetranitromethane
387	Amphetamine Sulfate
388	Ethylene Thiourea
389	Sodium Azide
390	3,3′-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
392	Chlorinated Water and Chloraminated Water
393	Sodium Fluoride
394	Acetaminophen
395	Probenecid
396	Monochloroacetic Acid
397	C.I. Direct Blue 15
399	Titanocene Dichloride
401	2,4-Diaminophenol Dihydrochloride
402	Furan
403	Resorcinol
405	C.I. Acid Red 114
406	γ-Butyrolactone
407	C.I. Pigment Red 3
409	Quercetin
410	Naphthalene
411	C.I. Pigment Red 23
412	4,4-Diamino-2,2-Stilbenedisulfonic Acid
415	Polysorbate 80
419	HC Hellow 4

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