DRAFT
TOXICOLOGICAL PROFILE FOR
ETHYLENE GLYCOL

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

September 2007

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UPDATE STATEMENT

A Toxicological Profile for Ethylene Glycol and Propylene Glycol was released in 1997. This present edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary. For information regarding the update status of previously released profiles, contact ATSDR at:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine/Applied Toxicology Branch
1600 Clifton Road NE
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Atlanta, Georgia 30333

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FOREWORD

This toxicological profile is prepared in accordance with guidelines developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the hazardous substance described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a hazardous substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health are identified by ATSDR and EPA.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public. We plan to revise these documents in response to public comments and as additional data become available. Therefore, we encourage comments that will make the toxicological profile series of the greatest use.

Comments should be sent to:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine
1600 Clifton Road, N.E.
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The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on December 7, 2005 (70 FR 72840). For prior versions of the list of substances, see *Federal Register* notices dated April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17, 1990 (55 FR 42067); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); February 28, 1994 (59 FR 9486); April 29, 1996 (61 FR 18744); November 17, 1997 (62 FR 61332); October 21, 1999 (64 FR 56792); October 25, 2001 (66 FR 54014); and November 7, 2003 (68 FR 63098). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and is being made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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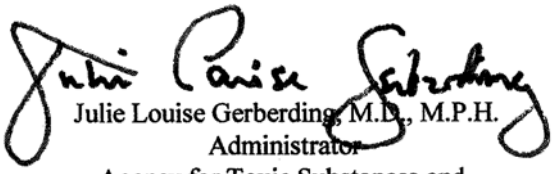

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QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Public Health Statement: The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

Chapter 2: Relevance to Public Health: The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

Chapter 3: Health Effects: Specific health effects of a given hazardous compound are reported by type of health effect (death, systemic, immunologic, reproductive), by route of exposure, and by length of exposure (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

Pediatrics: Four new sections have been added to each Toxicological Profile to address child health issues:

Section 1.6 How Can (Chemical X) Affect Children?
Section 1.7 How Can Families Reduce the Risk of Exposure to (Chemical X)?
Section 3.7 Children's Susceptibility
Section 6.6 Exposures of Children

Other Sections of Interest:

Section 3.8 Biomarkers of Exposure and Effect
Section 3.11 Methods for Reducing Toxic Effects

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY) ***Fax:*** (770) 488-4178
E-mail: cdcinfo@cdc.gov ***Internet***: http://www.atsdr.cdc.gov

The following additional material can be ordered through the ATSDR Information Center:

Case Studies in Environmental Medicine: Taking an Exposure History—The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental Hazards; Skin Lesions and Environmental Exposures; Cholinesterase-Inhibiting Pesticide Toxicity*; and numerous chemical-specific case studies.

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident. Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs) provide answers to frequently asked questions about toxic substances.

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 200 Independence Avenue, SW, Washington, DC 20201 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19, 4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212.

Referrals

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific Minimal Risk Levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

3. Data Needs Review. The Applied Toxicology Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

4. Green Border Review. Green Border review assures the consistency with ATSDR policy.

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PEER REVIEW

A peer review panel was assembled for ethylene glycol. The panel consisted of the following members:

1. Phillip Goad, Ph.D., Center for Toxicology and Environmental Health, LLC, North Little Rock, Arkansas;

2. Jerrold Leiken, M.D., ENH-OMEGA, Glenview, Illinois; and

3. Kenneth McMartin, Ph.D., Louisiana State University, Shreveport, Louisiana.

These experts collectively have knowledge of ethylene glycol's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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CONTENTS

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LIST OF FIGURES

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LIST OF TABLES

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about ethylene glycol and the effects of exposure to it.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites are then placed on the National Priorities List (NPL) and are targeted for long-term federal clean-up activities. Ethylene glycol has been found in at least 37 of the 1,689 current or former NPL sites. Although the total number of NPL sites evaluated for this substance is not known, the possibility exists that the number of sites at which ethylene glycol is found may increase in the future as more sites are evaluated. This information is important because these sites may be sources of exposure and exposure to this substance may harm you.

When a substance is released either from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. Such a release does not always lead to exposure. You can be exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance, or by skin contact.

If you are exposed to ethylene glycol, many factors will determine whether you will be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with it. You must also consider any other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

What is ethylene glycol?

Colorless liquid that is odorless	Ethylene glycol is a synthetic liquid substance that absorbs water. It is odorless, but has a sweet taste.
Used in consumer products	Ethylene glycol is used to make antifreeze and de-icing solutions for cars, airplanes, and boats. Consumer products containing ethylene glycol include: • Antifreeze • Hydraulic brake fluids • Inks used in stamp pads, ballpoint pens, and print shops

For more information on the sources, properties, and uses of ethylene glycol, see Chapters 4 and 5.

What happens to ethylene glycol when it enters the environment?

Released into air, water, and soil	The primary source of ethylene glycol in the environment is from run-off at airports where is used in de-icing agents for runways and airplanes. Ethylene glycol can also enter the environment through the disposal of products that contain it.
Quickly broken down	*Air:* Ethylene glycol in air will break down in about 10 days *Water and soil:* Ethylene glycol in water and in soil will breakdown within several days to a few weeks.

See Chapters 5 and 6 for more information on ethylene glycol in the environment.

How might I be exposed to ethylene glycol?

Antifreeze	The general public can be exposed to ethylene glycol through skin contact when using automobile antifreeze. Accidental or intentional ingestion can occur because antifreeze is a sweet tasting, brightly colored liquid.
Air, water, soil	Background concentrations of ethylene glycol in air, surface water, groundwater, drinking water, soil, and sediment have not been reported. Exposure to ethylene glycol in air, drinking water, or soil is not expected.
Workplace air	People who work in industries that use ethylene glycol may be exposed by touching products such as solvents, antifreeze, and feedstocks that contain this substance Workers can also be exposed to low levels from ethylene glycol-containing products such as airplane de-icing solutions that have been sprayed into the air.

See Chapter 6 for more information on exposure to ethylene glycol.

How can ethylene glycol enter and leave my body?

Enters your body after ingestion, inhalation, or dermal contact	Ingested ethylene glycol is quickly absorbed in large amounts. There is some information suggesting that inhaled ethylene glycol is also absorbed. Ethylene glycol can also slowly enter your bloodstream through your skin if you come in direct contact with it and do not wash it off.
Typically leaves your body within 1–2 days	Once in your body, most of the ethylene glycol is broken down (into other more toxic chemicals) and some of it remains unchanged. Ethylene glycol and its break down products are removed from your body through the excretion of urine.

How can ethylene glycol affect my health?

Scientists use many tests to protect the public from harmful effects of toxic chemicals and to find ways for treating persons who have been harmed.

The effect of ethylene glycol on human health depends on how much ethylene glycol is present.

Very small amounts will not affect your health	Your health is not likely to be seriously affected by the very small amounts of ethylene glycol that could be tasted or otherwise accidentally eaten (for example, by putting your fingers in your mouth after getting them wet with antifreeze). Accidental or intentional ingestion of larger amounts of ethylene glycol can cause serious illness or death.
Large amounts can damage the kidneys, nervous system, and heart	When ethylene glycol breaks down in the body, it forms chemicals that crystallize, and the crystals can collect in your kidneys and affect kidney function. Ethylene glycol also forms acidic chemicals in the body, which can change the body's acid/base balance and affect your nervous system, lungs, and heart.
Early treatment can prevent damage	Treatment after early diagnosis has been very successful in people drinking large amounts of ethylene glycol.

Further information on the health effects of ethylene glycol in humans and animals can be found in Chapters 2 and 3.

How can ethylene glycol affect children?

This section discusses potential health effects in humans from exposures during the period from conception to maturity at 18 years of age.

Children are likely to have similar effects as adults	Clinical findings in children who were poisoned by accidentally or intentionally drinking ethylene glycol indicate that it is likely that children would show the same health effects as adults. We do not know whether children differ in their susceptibility to the effects of ethylene glycol.
Birth defects	We do not know whether ethylene glycol causes birth defects in people. Skeletal defects and low birth weights have occurred in newborn animals whose mothers ingested large amounts of ethylene glycol during pregnancy.
Lactation exposure	We do not know whether ethylene glycol can accumulate in breast milk.

How can families reduce the risk of exposure to ethylene glycol?

Avoid ingestion of antifreeze by careful handling and storage	Antifreeze products should be used with caution and kept out of the reach of children. Open bottles of antifreeze should not be left on or near the ground where children can reach them. Antifreeze should not be stored in anything other than the original container, such as in a cup or soft drink bottle, to avoid someone mistaking it for a beverage. Antifreeze containers should have a child-proof cap, be stored away from food, and be properly marked.
Get medical advice if antifreeze is ingested	Ethylene glycol poisoning can be effectively treated, but early diagnosis is needed to prevent serious injury. Medical attention should be sought as soon as possible in cases of known or suspected antifreeze ingestion.
Limit dermal exposure to products containing ethylene glycol	Minimize skin contact when using antifreeze and other consumer products containing ethylene glycol. Avoid spilling or draining antifreeze on the ground to prevent children from playing in a puddle of ethylene glycol.

Is there a medical test to determine whether I have been exposed to ethylene glycol?

Analysis of blood and urine	Ethylene glycol and its effects can be measured in blood and urine. The metabolites cause characteristic chemical changes in the blood and urine that help to diagnose ethylene glycol poisoning. You should have these tests done within a few hours after exposure occurs because ethylene glycol leaves the body very quickly and early diagnosis is necessary for effective treatment. The presence of crystals in the urine may indicate kidney damage.

Refer to Chapters 3 and 7 for more information on these tests.

What recommendations has the federal government made to protect human health?

The federal government develops regulations and recommendations to protect public health. Regulations *can* be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. Recommendations provide valuable guidelines to protect public health, but *cannot* be enforced by law. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Regulations and recommendations can be expressed as "not-to-exceed" levels, that is, levels of a toxic substance in air, water, soil, or food that do not exceed a critical value that is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans. Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that provides it.

Some regulations and recommendations for ethylene glycol include the following:

Levels in drinking water set by EPA	The EPA has determined that exposure to ethylene glycol in drinking water at concentrations of 20 milligrams per liter (mg/L) for 1 day or 6 mg/L for 10 days is not expected to cause any adverse effects in a child. The EPA has determined that lifetime exposure to 14 mg/L ethylene glycol in drinking water is not expected to cause any adverse effects.

For more information on the regulations and guidelines that apply to ethylene glycol, see Chapter 8.

Where can I get more information?

If you have any more questions or concerns, please contact your regional poison control center (1-800-222-1222), community or state health or environmental quality department, or contact ATSDR at the address and phone number below.

ATSDR can also tell you the location of occupational and environmental health clinics. These clinics specialize in recognizing, evaluating, and treating illnesses that result from exposure to hazardous substances.

Toxicological profiles are also available on-line at www.atsdr.cdc.gov and on CD-ROM. You may request a copy of the ATSDR ToxProfiles™ CD-ROM by calling the toll-free information and technical assistance number at 1-800-CDCINFO (1-800-232-4636), by e-mail at cdcinfo@cdc.gov, or by writing to:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine
1600 Clifton Road NE
Mailstop F-32
Atlanta, GA 30333
Fax: 1-770-488-4178

Organizations for-profit may request copies of final Toxicological Profiles from the following:

National Technical Information Service (NTIS)
5285 Port Royal Road
Springfield, VA 22161
Phone: 1-800-553-6847 or 1-703-605-6000
Web site: http://www.ntis.gov/

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2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO ETHYLENE GLYCOL IN THE UNITED STATES

Ethylene glycol is a colorless, odorless liquid that mixes completely with water. It is released into the environment primarily through industrial emissions and through the use and disposal of ethylene glycol-based automobile antifreeze and airport de-icing formulations. Ethylene glycol that is released into the environment does not persist since it is degraded within days to a few weeks in air, water, and soil. Available monitoring data indicate that ethylene glycol is only found near areas of release. Ethylene glycol vapor concentrations measured in the air at airports during de-icing spray operations ranged from 0.05 to 22 mg/m^3. Ethylene glycol has also been detected in airport stormwater. Background concentrations of ethylene glycol in the environment are not available.

Since ethylene glycol is not expected to be present away from areas where it is released, background exposure of the general population to this substance is not expected to be important. The most common route of exposure to ethylene glycol for the general population is through dermal contact with ethylene glycol-containing automobile antifreeze. However, accidental or intentional ingestion of antifreeze is the most serious route of exposure, resulting in thousands of poisonings reported each year in the United States. Ethylene glycol concentrations in blood, urine, tissue, or breast milk are not available for the general population.

Individuals who live near hazardous waste sites, industrial facilities where ethylene glycol is produced or used, or areas where ethylene glycol-based de-icing formulations are used may be exposed to ethylene glycol through dermal contact with contaminated soil or water, inhalation of ethylene glycol vapor or mist, or ingestion of contaminated groundwater. Occupational exposure through dermal contact and inhalation of ethylene glycol vapor or mist is expected for individuals involved in airport de-icing spray operations. Ethylene glycol has been detected in urine samples collected from airport de-icing workers.

Ingestion of ethylene glycol containing antifreeze is a potential route of exposure for children since they are attracted to the bright colors of antifreeze formulations and the sweet taste of ethylene glycol. Exposure through ingestion is more likely to occur when adults leave opened antifreeze containers within reach or store antifreeze in other types of containers such as beverage bottles. A bittering agent has been added to some ethylene glycol antifreeze formulations in order to deter ingestion; however, caution should still be used since ingestion poisoning has occurred even when a bittering agent was present.

2.2 SUMMARY OF HEALTH EFFECTS

Ethylene glycol is quickly and extensively absorbed through the gastrointestinal tract of many species, but dermal absorption is slow in rodents and is expected to be slow in humans. Limited information is available on absorption of inhaled ethylene glycol, but the existing toxicity studies suggest absorption via the respiratory tract by both humans and rodents. Following absorption, ethylene glycol is distributed in aqueous compartments throughout the body. Ethylene glycol is initially metabolized to glycolaldehyde by alcohol dehydrogenase (with possible contribution from cytochrome P-450 enzymes). Glycolaldehyde is rapidly converted to glycolate and glyoxal by aldehyde oxidase and aldehyde dehydrogenase. Metabolism of glycolate by glycolate oxidase or lactate dehydrogenase results in the formation of glyoxylate, which may be further metabolized to formate, oxalate, glycine, and carbon dioxide. Elimination of ethylene glycol occurs via exhaled carbon dioxide and urinary elimination of both ethylene glycol and glycolic acid. The half-life for elimination in humans has been estimated to be in the range of 2.5–8.4 hours.

The vast majority of information relating to the toxicity of ethylene glycol is from studies of oral exposure. Information on the health effects of oral exposure in humans is largely limited to case reports of acute accidental or intentional ingestion of ethylene glycol. These case reports have identified three stages of acute oral ethylene glycol toxicity in humans. These stages are well documented and occur within 72 hours after ingestion. The first stage involves central nervous system depression, metabolic changes (hyperosmolality), and gastrointestinal upset, and spans the period from 30 minutes to 12 hours. During the second stage (12–24 hours after ingestion), metabolic acidosis and associated cardio-pulmonary symptoms (tachypnea, hyperpnea, tachycardia, cyanosis, pulmonary edema, and/or cardiac failure) become evident. During stage three, which covers the period 24–72 hours after ethylene glycol ingestion, renal involvement becomes evident. The third stage is characterized by flank pain and oliguria/anuria. Histopathological findings show renal tubular necrosis and deposition of calcium oxalate crystals. Often, the cardiopulmonary effects in the second stage are not evident, so the distinguishing symptoms of ethylene glycol intoxication are central nervous system depression, acidosis, and nephrotoxicity. Limited information suggests that a fourth stage involving cranial nerves may occur 6 or more days after exposure. This stage is characterized by neurological symptoms including deafness, facial paralysis, and other sequelae.

Reports of fatalities following ingestion of ethylene glycol indicate that a volume of 150–1,500 mL consumed at one time may cause death. In humans, the lethal dose of ethylene glycol is estimated to be in the range of 1,400–1,600 mg/kg. Based on these estimates, it appears that humans may be more susceptible to the acute lethality of ingested ethylene glycol than other species. In laboratory animals (rats, mice, monkeys), oral doses of ≥4,000 mg/kg were needed to cause death. However, difficulties in quantifying the amounts consumed by persons who have succumbed to the toxic effects lead to uncertainty in the human lethal dose estimates.

A study with human subjects found that inhalation exposure to ethylene glycol vapor at an average concentration of 30 mg/m^3 for 20–22 hours/day for 30 days was well tolerated, with effects that were essentially limited to occasional complaints of mild upper respiratory tract irritation. There were no indications of renal or other systemic effects as shown by urinalysis, hematology and clinical chemistry evaluations, and neurobehavioral tests throughout the exposure period. Short-term, high-exposure sessions found that respiratory tract irritation became common at approximately 140 mg/m^3, and was tolerated for only 15 minutes at 188 mg/m^3, 2 minutes at 244 mg/m^3, and one or two breaths at 308 mg/m^3. This study was used as the basis for an acute-duration inhalation MRL for ethylene glycol (see Section 2.3).

Animal studies indicate that oral exposure to ethylene glycol can cause effects in a number of different organ systems, although the developing fetus and kidneys are particularly sensitive and well-documented targets of toxicity. Oral effects have also been observed in the central and peripheral nervous systems, heart, liver, hematopoietic system, and immunological and lymphoreticular systems. Available information suggests that the neurological and cardiopulmonary effects stem from metabolic acidosis associated with acute, high-dose exposures. Reported effects on the immunological and lymphoreticular systems are limited to suppressed immune responses in mice given a single near-lethal oral dose, and neutrophilia and lymph node hemosiderosis in rats orally exposed for 2 years. Effects on hematological parameters have largely been observed at high doses in longer-term studies, and are not consistently reported across studies or across species.

Oral studies in animals have identified the developing fetus as the most sensitive target for acute-duration exposure to ethylene glycol. Gavage exposure of laboratory rodents to ethylene glycol during gestation results in a consistent pattern of developmental effects including reduced fetal body weight and increases in malformations, particularly axial skeletal malformations. Developmental toxicity has also been assessed by the inhalation and dermal routes. Results of the inhalation developmental studies are

generally consistent with the oral findings, but are confounded by concurrent oral exposure via ingestion of aerosolized ethylene glycol on the fur of exposed animals. A single study of dermal exposure to ethylene glycol in pregnant mice did not indicate developmental effects.

The kidney is clearly identified as the most sensitive target organ in rats and mice after intermediate-duration oral exposure. Typical renal effects included oxalate crystal deposition and renal tubular dilation, vacuolation, and degeneration. Oxalate, a metabolite of glycolic acid, forms a precipitate in the presence of calcium, and the deposition of these crystals in the renal tubules are hallmarks of ethylene glycol toxicity. Glycolic acid accumulation and metabolic acidosis do not contribute to renal toxicity, which is solely caused by oxalate crystal accumulation. Males were more sensitive than females, and rats were more sensitive than mice. Chronic oral studies confirm that the kidney is a main target organ in male rats, although a minor liver effect (slight fatty metamorphosis) occurred in female rats at doses lower than those inducing kidney effects. No hepatic effects were observed in intermediate-duration studies.

There is no indication that ethylene glycol is carcinogenic based on results of a limited renal cancer mortality study in chemical plant workers and well-designed chronic oral bioassays in rats (one study) and mice (two studies).

A more detailed discussion of the developmental and renal effects associated with ethylene glycol exposure follows. The reader is referred to Section 3.2, Discussion of Health Effects by Route of Exposure, for additional information on these and other health effects.

Developmental Effects. No studies have addressed the developmental toxicity of ethylene glycol in humans. The developmental toxicity of ethylene glycol in animals has been assessed by inhalation, oral, and dermal exposure in acute-duration studies and by oral exposure in intermediate-duration studies. The acute oral studies indicate that developmental effects (a skeletal variation and total malformations) occur at doses of ≥500 mg/kg/day when administered by gavage during gestation days (Gd) 6–15 to CD-1 mice. Dose-response data for these developmental effects in mice were used to derive an acute-duration oral MRL for ethylene glycol (see Section 2.3). Reduced fetal body weight occurred in mice given gavage doses of ≥750 mg/kg/day. In CD rats, doses of ≥1,000 mg/kg/day by gavage on Gd 6–15 have resulted in increased incidences of skeletal malformations. In F344 rats dosed on Gd 6–15 with 1,000 mg/kg/day in feed, skeletal malformations were not observed, suggesting the possible importance of dose-rate in producing developmental effects; however, strain differences in response cannot be ruled out. No

teratogenic effects were observed in rabbits exposed to maternally lethal oral doses of 2,000 mg/kg/day during gestation. In the only dermal exposure study, no developmental toxicity occurred in pregnant CD-1 mice that were treated with 6-hour daily exposures to ethylene glycol (estimated doses up to 3,549 mg/kg/day) by occluded cutaneous application on Gd 6–15.

Developmental toxicity studies of inhaled ethylene glycol in mice and rats found effects consistent with the oral findings, but all of the studies are confounded by concurrent ingestion of ethylene glycol deposited on the fur. In inhalation studies using whole-body exposure, significant effects on implant viability, weight of live fetuses, and incidence of external, visceral, and skeletal malformations were observed in mice exposed to ≥1,000 mg/m^3 for 6 hours/day on Gd 6–15. In rats exposed similarly, reduced ossification at some sites in the axial skeleton occurred at ≥1,000 mg/m^3; however, in an Expert Panel Review, the National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction concluded that the relationship of this effect to treatment was uncertain due to the lack of a dose-response relationship. In a follow-up study aimed at reducing the confounding oral exposure, pregnant CD-1 mice were exposed nose-only to 500–2,500 mg/m^3 aerosolized ethylene glycol. At 2,500 mg/m^3, live fetal body weight was significantly reduced, and there was a significant increase in the one type of skeletal malformation (fused ribs). Increases in some skeletal variations were also observed at 2,500 mg/m^3, and one type (extra ossification sites in the sagittal suture) was significantly increased at ≥500 mg/m^3. The authors observed that the animals in the nose-only experiment were also exposed by ingestion of ethylene glycol deposited on the face. Furthermore, one study noted that stress from restraint in the nose-only exposure study may have contributed to the developmental effects observed with ethylene glycol, which were similar in nature to effects observed in a study of restrained nose-only exposure to water vapor. Because of the confounding oral exposure in both the whole-body and nose-only experiments, a study concluded that the data from these studies were not suitable for evaluation of effect levels from inhalation exposure to ethylene glycol.

Developmental effects of intermediate-duration oral exposure to ethylene glycol include kidney effects in offspring and decreased pup body weights. In mice tested in a continuous breeding assay, pup body weights were reduced in both F_1 and F_2 generations at drinking water doses of ≥897 mg/kg/day. In a 15-day gestational exposure study (Gd 6–20), postnatal effects on kidney weights were observed in pups of CD rats exposed to gavage doses of ≥1,250 mg/kg/day *in utero*. In a three-generation study of rats, no effects on gestation survival or pup body weight through postpartum day (ppd) 21 were observed in F_1 or F_2 pups after parental exposure to dietary doses up to 1,000 mg/kg/day.

Recent reviews of mechanistic studies on ethylene glycol developmental toxicity have concluded that glycolic acid, alone or in combination with its downstream metabolites and resultant metabolic acidosis, was likely the proximate toxicant responsible for the developmental effects of ethylene glycol. Using a physiologically based pharmacokinetic (PBPK) model developed for humans, a study estimated that the glycolic acid blood threshold concentration for developmental effects in rodents would only be reached in human females ingesting doses of 350 mg/kg (assuming a 58-kg female). While the model has been validated against data from acute human oral and inhalation exposures to ethylene glycol, the model was not calibrated to pregnancy, and it is not clear whether physiological and/or biochemical changes that could affect ethylene glycol toxicokinetics might occur during pregnancy. Further, one study noted that additional data were needed to fully delineate the rate of glycolic acid metabolism in humans; such additional data may alter the model predictions of peak glycolic acid concentrations in humans exposed to ethylene glycol.

Renal Effects. The renal toxicity of ethylene glycol in humans is well documented in numerous case reports of accidental or intentional ingestion. Adverse renal effects occur in the third stage of human ethylene glycol poisoning, which occurs 24–72 hours after acute exposure. The hallmark of renal toxicity is the presence of calcium oxalate monohydrate crystals in the renal tubules and urine following ingestion of large amounts of ethylene glycol. Characteristic histopathological changes include renal tubular focal degeneration, atrophy, and interstitial inflammation. Renal damage, if untreated, can lead to renal failure. With therapy, normal or near-normal renal function can be restored.

Humans who inhaled ethylene glycol showed no indications of impaired renal function. No significant alterations in renal end points were found in volunteers exposed to ethylene glycol aerosol at an average concentration of 30 mg/m^3 for 20–22 hours/day for 30 days. Evaluations were performed throughout the study and included examination of urine for presence of oxalate crystals and erythrocytes; determinations of urine volume, specific gravity, color, clarity, pH, amino acid nitrogen, and creatinine; and determination of blood urea nitrogen. There also was no indication of renal impairment in aviation workers who were intermittently exposed to ethylene glycol during airplane de-icing operations over a 2-month winter period. Ethylene glycol concentrations as high as 22 mg/m^3 for vapor and 190 mg/m^3 for mist were measured, although the vast majority of samples were below the limit of quantification (2.5 mg/m^3 for vapor and 17 mg/m^3 for mist); the frequency and average levels and durations of exposure were not reported. Measurements of urinary albumin, β-*N*-acetyl-glucosaminidase, β-2-microglobulin, and retinol-binding protein were used to assess kidney function.

Renal effects in orally exposed animals are consistent with those observed in humans. In acute-duration studies, effects occurred in the kidneys of rats exposed to 1,250–2,500 mg/kg/day by gavage or 2,615–5,270 mg/kg/day in drinking water for 9–29 days, and rabbits exposed to 2,000 mg/kg/day by gavage for 13 days. Evaluation of these animals showed effects that generally included increased kidney weight and renal tubular calcium oxalate deposits, dilation, degeneration, and/or necrosis.

The renal effects of intermediate-duration oral exposure to ethylene glycol are well characterized in a number of studies in rats and mice. These studies indicate that renal toxicity varies with sex, species, and strain, with males more sensitive than females, rats more sensitive than mice, and Wistar rats more sensitive than other strains of rats. Renal effects in Sprague-Dawley rats that were exposed to ethylene glycol in drinking water for 90 days included renal tubular oxalate crystal deposition, dilation, and degeneration in males at ≥947 mg/kg/day and females at 3,087 mg/kg/day. Findings in F344 rats exposed for 13 weeks via diet included renal tubular dilation, necrosis, fibrosis, and oxalate crystal deposition in males at ≥2,500 mg/kg/day, and mild renal lesions (e.g., inflammation and vacuolation) with no crystal deposition in females at 10,000 mg/kg/day. Results of 16-week dietary studies showed that male Wistar rats are approximately twice as sensitive as male F344 rats to ethylene glycol nephrotoxicity, and that kidney lesions in male Wistar rats occurred at average doses as low as 180 mg/kg/day. In a 13-week dietary study in B6C3F1 mice, effects were observed in the kidneys (minimal to mild tubule dilation, cytoplasmic vacuolation, and regenerative hyperplasia, without tubular oxalate crystal deposition) of males at ≥6,450 mg/kg/day, with no renal effects in females at doses ≤16,000 mg/kg/day.

Chronic toxicity studies provide information on renal effects in rats and mice exposed to ethylene glycol in the diet for up to 2 years. Males were more sensitive than females and rats were more sensitive than mice. Renal effects in rats included oxalate nephrosis in Wistar males at ≥300 mg/kg/day, oxalate crystal deposition and apparent tubular degenerative changes in Sprague-Dawley males at ≥375 mg/kg/day and females at ≥750 mg/kg/day, and oxalate nephrosis (and consequent mortality) in F344 males at 1,000 mg/kg/day, with changes in F344 females at this dose limited to increased kidney weight and crystalluria without histopathology. No kidney histopathology occurred in male or female CD-1 mice exposed to 1,000 mg/kg/day or female B6C3F1 mice exposed to ≤12,000 mg/kg/day, and effects in male B6C3F1 mice were limited to small numbers of oxalate-like crystals and/or calculi in the renal tubules, urethrae, and urinary bladder in a few animals at 6,000 mg/kg/day.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for ethylene glycol. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990a), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

- An MRL of 2 mg/m^3 has been derived for acute-duration inhalation exposure (14 days or less) to ethylene glycol.

Information on the toxicity of acute-duration inhalation exposure to ethylene glycol is available from an experimental study in humans (Wills et al. 1974) and three developmental toxicity studies in rats and mice (Tyl 1988a; Tyl et al. 1995a, 1995b). In the human study, exposure to ethylene glycol aerosol at an average concentration of 23 mg/m^3 for 20–22 hours/day for 14 days was well tolerated, with effects that were essentially limited to occasional complaints of mild upper respiratory tract irritation (Wills et al. 1974). There were no indications of renal or other systemic effects as shown by urinalysis and hematology, clinical chemistry, and neurobehavioral evaluations. Short-term high-exposure sessions showed that respiratory tract irritation became common at approximately 140 mg/m^3 and intolerable for more than a few minutes at approximately 200 mg/m^3.

Developmental toxicity studies were conducted in rats and mice using whole-body exposure to 150, 1,000, or 2,500 mg/m^3 of ethylene glycol aerosol for 6 hours/day on Gd 6–15 (Tyl 1988a; Tyl et al. 1995a). Reduced ossification at some sites in the axial skeleton occurred in rats at ≥1,000 mg/m^3, although an Expert Panel Review (NTP-CERHR 2004) concluded that the relationship of this effect to treatment was uncertain due to the lack of a dose-response relationship. In mice, significant effects on implant viability, weight of live fetuses, and incidence of external, visceral, and skeletal malformations were observed at ≥1,000 mg/m^3. Maternal toxicity (e.g., increased liver weight in rats and reduced body weight gain in mice) was evident at 2,500 and 1,000 mg/m^3 in rats and mice, respectively (Tyl et al. 1995a). Both of these whole-body exposure studies were confounded by concurrent ingestion exposure to ethylene glycol deposited on the fur. In a follow-up study aimed at reducing concurrent exposure from ingestion, mice were exposed nose-only to 500, 1,000, or 2,500 mg/m^3 aerosolized ethylene glycol for 6 hours/day on Gd 6–15 (Tyl 1988a; Tyl et al. 1995b). In maternal animals, there were no effects other than changes in kidney weights. Absolute kidney weight was significantly increased at 1,000 and 2,500 mg/mg^3, and relative kidney weight was increased at 2,500 mg/m^3; however, the increases were small (6.6–9.5% higher than controls) and microscopic examination of kidneys showed no histopathological changes. At 2,500 mg/m^3, live fetal body weight was significantly reduced, and there was a significant increase in the one type of skeletal malformation (fused ribs). Increases in some skeletal variations were observed at 2,500 mg/m^3, and one type (extra ossification sites in the sagittal suture) was significantly increased at concentrations of ≥500 mg/m^3. The authors observed that the animals in the nose-only experiment were also exposed by ingestion of ethylene glycol deposited on the face (Tyl 1988a; Tyl et al. 1995a). Furthermore, stress from restraint in the nose-only exposure study may have contributed to the developmental effects observed with ethylene glycol (NTP-CERHR 2004; Tyl et al. 1995a), which were similar in nature to effects observed in a study of restrained nose-only exposure to water vapor (Tyl et al. 1994).

Because of the confounding oral exposures in both the whole-body and nose-only developmental toxicity studies, NTP-CERHR (2004) concluded that the data from these studies were not suitable for evaluation of effect levels from inhalation exposure to ethylene glycol. The available data do, however, provide a conservative estimate of the inhalation no-observed-adverse-effect level (NOAEL), with the caveat that total exposure to ethylene glycol in these studies included intake via ingestion. Collectively, these studies suggest that inhalation exposure to ethylene glycol at a nominal concentration of about 150 mg/m^3 is not associated with developmental toxicity in mice or rats, or renal toxicity in mice (kidney histopathology not assessed in rats). The next highest concentration (500 mg/m^3 in the nose-only study) was associated

with developmental effects (increased incidence of skeletal variations), but it is not possible to conclusively relate these effects to inhalation of ethylene glycol.

As indicated above, the developmental studies (Tyl 1988a; Tyl et al. 1995a, 1995b) collectively suggest that 150 mg/m^3 is a conservative NOAEL for developmental toxicity in rats and kidney toxicity in mice. This concentration is similar to the 140 mg/m^3 lowest-observed-adverse-effect level (LOAEL) for respiratory tract irritation in humans (Wills et al. 1974). The human NOAEL of 23 mg/m^3 is a suitable basis for MRL derivation because it is based on evaluations for renal and other systemic effects as well as local irritation, and is well within the NOAEL range for developmental toxicity in animals. As summarized below, the human study (Wills et al. 1974) was conducted in prisoners. ATSDR recognizes that there is some ethical concern about using a study in prisoners for MRL derivation, but the protocol was acceptable at the time the study was conducted.

In the human study, health effects were assessed in 19 male prisoners who voluntarily were exposed to ethylene glycol aerosol for 20–22 hours/day for 30 days (Wills et al. 1974). The diameter of the aerosol droplets ranged from 1 to 5 μm. Mean daily and mean weekly concentrations during the first 14 days of the study were 0.8–44.8 and 17–29 mg/m^3, respectively. Mean daily and mean weekly concentrations during the entire 30-day exposure period were 0.8–67 and 17–49 mg/m^3, respectively. The average mean weekly exposure was 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30. Calculations of the average exposure levels did not include brief periods in which the concentration was intentionally raised to higher levels to assess acute responses. A control group consisted of 14 male prisoners; 10 of these men were never exposed to ethylene glycol, whereas the remaining 4 men had been exposed to a mean concentration of 37 mg/m^3 for 20–22 hours/day for 7 days during the week that preceded the start of the study. Subjective responses (symptoms) were monitored throughout the study. During the last 10 days of the study, the concentration of ethylene glycol was occasionally intentionally increased to various high levels (up to 308 mg/m^3) when the volunteers left the exposure chamber during meals; subjective responses to short exposures to the high concentrations were assessed when they reentered the chamber. Complete physical examinations that included slit-lamp, electrocardiographic, and electroencephalographic studies, and a battery of psychological tests designed to reveal effects on simple reaction time, reaction time with discrimination, visual-motor coordination, depth perception, and mental ability (encoding and subtraction accuracy), were conducted on all subjects pre-exposure and after 14 and 30 days of exposure. Blood samples were collected on days 0, 1, 3, 5, 8, 12, 19, 22, 26, and 29 for evaluation of hematology, clinical chemistry (including blood urea nitrogen, serum creatinine, and liver enzymes), and ethylene glycol concentration. Urine was evaluated daily for oxalate crystals,

erythrocytes, and ethylene glycol, and twice weekly for volume, specific gravity, color, clarity, pH, amino acid nitrogen, and creatinine. Concentrations of ethylene glycol in the blood and urine were similar in the exposed and control groups. The near-continuous exposure levels (average 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30) were tolerated with effects that were limited to occasional complaints of upper respiratory tract irritation, slight headache, and low backache (incidences and other information not reported). The short-term, high-exposure sessions showed that the irritation became common at approximately 140 mg/m^3, and tolerated for only 15 minutes at 188 mg/m^3, 2 minutes at 244 mg/m^3, and one or two breaths at 308 mg/m^3. Based on these results and those of other trials, the investigators concluded that concentrations of about ≥200 mg/m^3 were intolerable due to strong irritation of the upper respiratory tract that included a burning sensation in the trachea and a burning cough. Because the near-continuous exposures were tolerated with respiratory irritation that was infrequent and not serious, and not accompanied by neurological, hematology, clinical chemistry, or urinalysis findings indicative of renal or other systemic effects, the interim (12–14-day) findings in this study identified a NOAEL of 23 mg/m^3 for acute-duration exposure in humans. The LOAEL in humans was 140 mg/m^3 because brief exposures to this concentration commonly caused respiratory irritation.

The NOAEL of 23 mg/m^3 for respiratory tract irritation and systemic toxicity in humans (Wills et al. 1974) was divided by an uncertainty factor of 10 (for human variability) to derive an MRL of 2 mg/m^3 for acute inhalation exposure to ethylene glycol. The NOAEL was not adjusted for discontinuous daily exposure (20 hours/24 hours) because the critical effect is concentration dependent and not duration dependent.

An MRL has not been derived for intermediate-duration inhalation exposure (15–364 days) to ethylene glycol. Information on the toxicity of intermediate-duration inhalation exposure to ethylene glycol is available from two studies in humans (Gérin et al. 1997; Wills et al. 1974) and one multiple species study in animals (Coon et al. 1970).

In one of the human studies, health effects were assessed in 19 male prisoners who voluntarily were exposed to ethylene glycol aerosol at an average concentration of 30 mg/m^3 for 20–22 hours/day for 30 days (Wills et al. 1974). The exposure was well tolerated, with effects that were essentially limited to occasional complaints of mild upper respiratory tract irritation; there were no indications of renal or other systemic effects as shown by urinalysis and hematology, clinical chemistry, and neurobehavioral evaluations. This study is summarized in detail in the acute inhalation MRL section. The other human study assessed kidney function in 33 male aviation workers who were intermittently exposed to ethylene

glycol-based de-icing fluid during airplane de-icing operations during a 2-month winter period (Gérin et al. 1997). Personal exposures to ethylene glycol ranged up to 22 mg/m^3 for vapor and 190 mg/m^3 for mist, although the vast majority of samples were below the limits of quantification (2.5 mg/m^3 for vapor and 17 mg/m^3 for mist). The frequency and average levels and durations of exposure were not reported. Measurements of urinary albumin, β-*N*-acetyl-glucosaminidase, β-2-microglobulin, and retinol-binding protein indicated no impairment of renal function.

In the animal study, Sprague-Dawley and Long-Evans rats (15/concentration, mixed strains and sexes), Princeton-derived guinea pigs (15/concentration, mixed sexes), New Zealand rabbits (3 males/concentration), Beagle dogs (2 males/concentration), and Squirrel monkeys (2–3 males/concentration) were exposed to apparently aerosolized ethylene glycol in concentrations of 0, 10, or 57 mg/m^3 for 8 hours/day, 5 days/week for 6 weeks, or to 0 or 12 mg/m^3 continuously for 90 days (Coon et al. 1970). Evaluations included clinical signs, limited hematology, serum liver enzymes, gross pathology, and limited histology (mainly kidney, liver, spleen, heart, and lung). No clear treatment-related effects were observed in the intermittent exposure 6-week study. In the 90-day study, continuous exposure to 12 mg/m^3 caused moderate to severe eye irritation in 3/3 rabbits and corneal opacity with possible blindness in 2/15 rats, and mortality in 1/3 rabbits, 1/15 rats, and 3/15 guinea pigs.

The experimental study in humans (Wills et al. 1974) identified a NOAEL of 30 mg/m^3 for respiratory irritation and systemic effects for near-continuous exposure to ethylene glycol for 30 days. Urinalysis in this study showed no indications of renal effects (e.g., presence of oxalate crystals in urine), and are consistent with the negative results of kidney function evaluations in the aviation workers who were intermittently exposed to lower levels of ethylene glycol for 2 months (Gérin et al. 1997). The 6-week intermittent exposure study in rats, guinea pigs, and small numbers of rabbits, dogs, and monkeys (Coon et al. 1970) identified a NOAEL of 57 mg/m^3 for kidney histopathology and other systemic effects in all species. Continuous exposure to 12 mg/m^3 for 90 days caused ocular irritation in rats and rabbits (Coon et al. 1970), but confidence in this LOAEL is low due to small numbers of affected animals, and its relevance is unclear because there was no eye irritation in the humans near-continuously exposed to 30 mg/m^3 for 30 days (Wills et al. 1974). Continuous exposure to 12 mg/m^3 for 90 days also caused mortality in rats, rabbits, and guinea pigs, although the reliability of this LOAEL is low due to low incidences, small numbers of animals, and likely confounding by oral exposure from ingestion of aerosol deposited on the fur. Documentation for this probable confounder is provided by the developmental toxicity studies (Tyl et al. 1995a, 1995b) discussed in the acute inhalation MRL derivation. The human NOAEL of 30 mg/m^3 is not a suitable basis for intermediate-duration MRL derivation due to insufficient

information on renal and other possible systemic effects of exposures longer than 30 days. Although exposures as long as 90 days were conducted in the animal study, it was limited in scope (e.g., lacked sufficient numbers of animals and urinalysis) and likely confounded by oral exposure.

An MRL has not been derived for chronic-duration inhalation exposure (365 days or more) to ethylene glycol. Information on the health effects of chronic exposure to ethylene glycol is essentially limited to the negative results of an epidemiologic study on renal cancer mortality in humans (Bond et al. 1985). This study does not provide a basis for MRL consideration because it lacks noncancer end points, measured exposure concentrations, and other relevant information.

Oral MRLs

- An MRL of 0.8 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to ethylene glycol.

Information on effects of acute-duration oral exposure to ethylene glycol is available from human case reports, a 10-day drinking water study in rats (Robinson et al. 1990), a 4-day gavage study examining effects on hematology and reproductive organs (Hong et al. 1988), and developmental toxicity studies in mice, rats, and rabbits (Maronpot et al. 1983; Marr et al. 1992; Neeper-Bradley et al. 1995, Price et al. 1985; Schuler et al. 1984; Tyl 1989; Tyl et al. 1993).

The available human studies consist of clinical case reports of high-dose intentional or accidental ingestion of ethylene glycol, and thus, are not suitable for dose-response assessment. Although the 4-day gavage study reported by Hong et al. (1988) identified bone marrow effects at doses of 50–250 mg/kg/day, the biological significance of these effects is considered uncertain in light of the lack of supporting evidence for effects on bone marrow, spleen, or hematology in longer-duration studies of mice and rats exposed to much higher doses (DePass et al. 1986a; Gaunt et al. 1974; Melnick 1984; NTP 1993; Robinson et al. 1990; Schladt et al. 1998). The remaining animal studies collectively identify the developing fetus as the most sensitive target of acute oral exposure to ethylene glycol.

In acute-duration oral developmental toxicity studies in rodents, fetal effects have consistently been observed at doses that are not maternally toxic. Furthermore, the developmental effects observed after ethylene glycol exposure appear to be generally consistent across studies and across rodent species, with the primary end point consisting of skeletal malformations. The incidence of malformations was increased in CD-1 mice at doses of ≥500 mg/kg/day when administered by gavage during gestation

(Gd 6–15) (Neeper-Bradley et al. 1995; Tyl 1989). Embyrotoxicity was also manifested as a reduction in fetal body weight in CD-1 mice given doses of ≥750 mg/kg/day on Gd 6–15 (Neeper-Bradley 1990; Price et al. 1985; Tyl 1989). In rats, doses of ≥1,000 mg/kg/day by gavage on Gd 6–15 resulted in an increased incidence of skeletal malformations in offspring (Neeper-Bradley 1990; Neeper-Bradley et al. 1995; Price et al. 1985). Decreases in pup body weight and increases in both the number of litters with malformations and the number of malformed fetuses per litter were observed in rats treated during Gd 6–15 with doses of ≥2,500 mg/kg/day (Neeper-Bradley 1990; Neeper-Bradley et al. 1995; Price et al. 1985). In mice given doses of 3,000 mg/kg/day during Gd 6–15, neural tube and craniofacial defects were increased and the number of live fetuses per litter was decreased (Price et al. 1985). In contrast to the results in rodents, no developmental effects were observed in rabbits exposed to maternally lethal doses of 2,000 mg/kg/day during gestation (Tyl et al. 1993).

Among the available studies, the study by Neeper-Bradley et al. (1995; Tyl 1989) in mice identified the lowest LOAEL; thus, these data were selected for use in deriving the acute oral MRL for ethylene glycol. This study was well-conducted, using adequate numbers of animals (30/dose), including four dose levels in addition to controls, and evaluating relevant end points (skeletal and other malformations as well as reproductive and litter parameters).

In the mouse study by Neeper-Bradley et al. (1995; Tyl 1989), groups of 30 timed-pregnant CD-1 mice were given doses of 50, 150, 500, or 1,500 mg/kg ethylene glycol daily by gavage on Gd 6–15; vehicle controls were given water on the same schedule. Maternal animals were observed daily for clinical signs and weighed periodically; water intake was measured throughout gestation. At sacrifice on Gd 18, body weight, gravid uterine weight, liver weight, and kidney weight were measured in dams. Kidneys from control and high-dose dams were examined microscopically. Corpora lutea and uterine contents were evaluated, and live fetuses were weighed and sexed. External, visceral, and skeletal malformations and variations in the fetuses were evaluated.

No effects on maternal body weight, water consumption, or liver or kidney weight were observed. There were no significant effects on the number of corpora lutea/dam, number of total, nonviable, or viable implants/litter, or sex ratio. Average fetal body weight per litter was reduced (13% below controls) at 1,500 mg/kg/day. The incidence of individual external or visceral malformations was not significantly increased in any treatment group relative to the vehicle control; however, exencephaly (a malformation observed by Price et al. [1985] at higher doses) was observed in two fetuses in the 500 mg/kg/day group and in three fetuses of the 1,500 mg/kg/day dose group. There was a significant increase in the incidence

of two skeletal malformations (fused ribs or thoracic arches) in the 1,500 mg/kg/day group (15/21 litters with fused ribs vs. 1/19 controls; 8/21 litters with fused thoracic arches vs. 0/19 controls). Further, the incidence of total malformations per litter (external, visceral, and skeletal) was significantly increased both at 500 and 1,500 mg/kg/day (3/19, 7/20, 5/24, 12/24, and 17/21 from control to high dose). The incidences of 23 skeletal variations were increased in the 1,500 mg/kg/day group. One of these variations (bilateral extra rib 14) was also significantly increased at ≥500 mg/kg/day (4/19, 4/20, 6/24, 17/24, and 21/21 in control through high dose groups, respectively). This study identified a developmental NOAEL of 150 mg/kg/day and LOAEL of 500 mg/kg/day for increased incidence of total malformations and bilateral extra rib 14. The high dose (1,500 mg/kg/day) was a NOAEL for maternal effects.

To derive a point of departure for MRL derivation, benchmark dose (BMD) modeling was conducted using the mouse data on the incidence of litters with malformations (of any kind) and on the incidence of one skeletal variation (bilateral extra rib 14). These two end points were observed at lower doses than other observed effects (skeletal malformations, pup body weight reductions). All dichotomous variable models in the EPA Benchmark Dose Software (Version 1.4.1) were fit to the malformation and skeletal variation data. Although one of the end points modeled (total malformations) represents a more serious effect, the group sizes in this study (19–24 litters/dose examined) did not support a benchmark response (BMR) lower than 10%; thus, an extra risk incidence of 10% above controls was selected as the BMR. The multistage and quantal linear models converged on the same model providing the best fit to the data on total malformations; these models both predicted a BMD_{10} of 113.84 mg/kg/day and a $BMDL_{10}$ of 75.59 mg/kg/day. For the data on bilateral extra rib 14, the probit model provided the best fit, and predicted a BMD_{10} of 99.35 mg/kg/day and a $BMDL_{10}$ of 75.56 mg/kg/day. Modeling of both the malformation and skeletal variation end points resulted in the same $BMDL_{10}$, indicating that an acute oral MRL based on this point of departure should provide protection against both effects. The $BMDL_{10}$ of 76 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to derive an MRL of 0.8 mg/kg/day for acute-duration oral exposure to ethylene glycol.

Although some mechanistic information suggests that humans may be less sensitive than rodents to the developmental effects of ethylene glycol, the available data are not adequate to support a lower interspecies uncertainty factor; thus, a full 10-fold uncertainty factor was used for interspecies extrapolation. While *in vitro* data suggest that humans metabolize glycolic acid (the proximate developmental toxicant) more efficiently than rats (Booth et al. 2004; Corley et al. 2005a), NTP-CERHR (2004) observed that the data supporting the glycolic acid metabolic rate in humans are limited. In

addition, NTP-CERHR (2004) reviewed preliminary data indicating that the inverted yolk sac placenta, a stage in placental development that does not exist in humans, tends to concentrate weak acids such as glycolic acid in the embryonic fluids. These data suggest enhanced sensitivity to ethylene glycol developmental effects in rodents compared with humans; however, NTP-CERHR (2004) characterized the available data as inconclusive. A 10-fold uncertainty factor for human variability was also used. Ethylene glycol metabolism is known to involve alcohol dehydrogenase and aldehyde dehydrogenase, and may also involve cytochrome p450 isozymes (NTP-CERHR 2004). Polymorphisms in the genes encoding these enzymes may lead to wide variability in the production and elimination of glycolic acid and other metabolites in humans exposed to ethylene glycol, but data quantifying the range of variability are not currently available (NTP-CERHR 2004). In addition, fetal and/or placental differences in expression of these enzymes over the course of gestation will affect local concentrations of glycolic acid and other metabolites to which the developing conceptus is exposed, yet little is known about these differences (NTP-CERHR 2004).

Corley et al. (2005a) published a PBPK model for rats, but no model has yet been developed for mice, the species used in the study selected for MRL derivation. As a result, available data do not support the use of PBPK modeling to derive an acute oral MRL for ethylene glycol based on developmental toxicity in mice.

A key uncertainty in the acute-duration oral MRL stems from the use of gavage administration in the critical study. Bolus doses from gavage administration lead to higher peak concentrations of glycolic acid in the blood than occur with equivalent doses at slower dose-rates associated with environmentally-relevant exposures (Carney et al. 2001; NTP-CERHR 2004). Because the key study used gavage administration, the dose at which effects were observed may be lower than would be observed with non-bolus dosing. In support of this, Maronpot et al. (1983) observed neither fetal nor maternal toxicity at dietary doses up to 1,000 mg/kg in F344 rats, while Neeper-Bradley et al. (1995) reported skeletal malformations and effects on fetal body weight in CD rats given 1,000 mg/kg via gavage. While strain differences in susceptibility to ethylene glycol cannot be ruled out as the source of the differing results, the data supporting glycolic acid as the proximate toxicant, and the evidence for much lower serum levels of glycolic acid with continuous dosing than with bolus dosing, suggest that the lack of developmental toxicity observed by Maronpot et al. (1983) likely resulted from the difference in dose-rate.

- An MRL of 0.8 mg/kg/day has been adopted for intermediate-duration oral exposure (15–364 days) to ethylene glycol.

Information on the toxicity of intermediate-duration oral exposure to ethylene glycol essentially consists of several well-designed studies in rats (Cruzan et al. 2004; Gaunt et al. 1974; Melnick 1984; Robinson et al. 1990) and mice (Melnick 1984; NTP 1993). Based on generally comprehensive evaluations that included body and organ weights, food and water consumption, hematology, blood chemistry, urinalysis, and histopathology in adequate numbers of animals, these studies consistently showed that the kidney is the predominant and most sensitive target of ethylene glycol toxicity. As summarized below, renal toxicity varied with sex, species, and strain, with males more sensitive than females, rats more sensitive than mice, and Wistar rats more sensitive than other strains of rats.

Renal effects in Sprague-Dawley rats that were exposed to ethylene glycol in drinking water for 90 days included renal tubular oxalate crystal deposition, dilation, and degeneration in males at ≥947 mg/kg/day and females at 3,087 mg/kg/day (Robinson et al. 1990). Key findings in F344 rats exposed for 13 weeks via diet consisted of renal tubular dilation, necrosis, fibrosis, and oxalate crystal deposition in males at ≥2,500 mg/kg/day, mortality in males at 5,000 mg/kg/day, and mild renal lesions (e.g., inflammation and vacuolation) with no crystal deposition or mortality in females at 10,000 mg/kg/day (Melnick 1984). Results of 16-week dietary studies showed that male Wistar rats are approximately twice as sensitive as male F344 rats to ethylene glycol nephrotoxicity (Cruzan et al. 2004), and that kidney lesions in male Wistar rats occurred at average doses as low as 180 mg/kg/day (Gaunt et al. 1974). In a 13-week dietary study in B6C3F1 mice, effects were observed in the kidneys (minimal to mild tubule dilation, cytoplasmic vacuolation, and regenerative hyperplasia, without tubular oxalate crystal deposition) and liver (centrilobular hyaline degeneration) of males at ≥6,450 mg/kg/day, with no effects in females at doses ≤16,000 mg/kg/day (Melnick 1984; NTP 1993).

The 16-week study by Cruzan et al. (2004) compared the renal toxicity of ethylene glycol in male Wistar and F344 rats. The comparison was conducted to confirm an apparent greater sensitivity of the Wistar strain indicated by seemingly inconsistent renal effect levels in key intermediate- and chronic-duration studies (i.e., a LOAEL of 180 mg/kg/day in Wistar rats exposed for 16 weeks [Gaunt et al. 1974] that was lower than a NOAEL of 200 mg/kg/day in F344 rats exposed for 2 years [DePass et al. 1986a]). In the Cruzan et al. (2004) study, groups of 10 male Wistar rats and 10 male F-344 rats were administered ethylene glycol in the diet in constant dose levels of 0, 50, 150, 500, or 1,000 mg/kg/day for 16 weeks. Clinical signs, body weight, and food intake were evaluated throughout the study. Water consumption was measured and urine was collected for urinalysis during the 24 hours prior to sacrifice; parameters included specific gravity, pH, color, appearance, protein, glucose, bilirubin, urobilinogen, ketones, occult blood, leukocytes, nitrites, volume, and microscopy of sediment. Following sacrifice, complete

necropsies were performed, and kidneys were evaluated for organ weight and histological changes and presence of alpha 2-μ-globulin. Effects observed in Wistar rats included reduced body weight gain at 500 and 1,000 mg/kg/day (final weight 9 and 23% lower than controls), and reduced food intake and 2/10 deaths at 1,000 mg/kg/day; these end points were not affected in F344 rats. Significantly increased water intake (~200% higher than controls) and urine volume, decreased urine specific gravity, and increased occurrence of white blood cells in urine occurred in Wistar rats at ≥500 mg/kg/day and F344 rats at 1,000 mg/kg/day. Calcium oxalate crystals in the urine were increased in both strains of rats at ≥150 mg/kg/day; incidences at 0, 50, 150, 500, and 1,000 mg/kg/day were 0/10, 1/10, 5/10, 10/10, and 4/8 in Wistar rats, and 1/10, 0/10, 3/10, 10/10, and 7/10 in F344 rats. Absolute and relative kidney weights were significantly increased at ≥500 mg/kg/day in Wistar rats and 1,000 mg/kg/day in F344 rats. No treatment-related increases in alpha 2-μ-globulin were observed in either strain of rats. The histological examinations showed crystal deposition in the kidneys and associated nephropathy in both strains of rats at ≥500 mg/kg/day, with greater severity in the Wistar rats. Respective incidences of nephropathy with crystal deposition at 0, 50, 150, 500, and 1,000 mg/kg/day were 0/10, 0/10, 0/10, 10/10, and 10/10 in Wistar rats, and 0/10, 0/10, 0/10, 1/10, and 10/10 in F344 rats. Because crystal-induced nephropathy occurred in only 1/10 F344 rats at 500 mg/kg/day (compared to 10/10 Wistar rats at this dose), and six additional F344 rats at 500 mg/kg/day had crystals in the kidney tubules without nephropathy, this dose is a less serious LOAEL in the F344 rats. The severity of the crystal nephropathy in the Wistar rats at 500 mg/kg/day was approximately equivalent to that in the F344 rats at 1,000 mg/kg/day. Due to the higher incidence and greater severity of the crystal nephropathy, as well as the accompanying impairment of kidney function (i.e., compromised kidney water regulation as indicated by increased urine volume and decreased urine specific gravity leading to increased water consumption), 500 mg/kg/day is a serious LOAEL in the Wistar rats. The only effect observed at doses lower than 500 mg/kg/day was calcium oxalate crystals in the urine of both strains at 150 mg/kg; this is a NOAEL because excretion of crystals in the urine reflects a detoxification process and is not considered adverse in the absence of crystal deposition in the renal tubule epithelium and associated histopathology.

The 16-week study by Gaunt et al. (1974) exposed male and female weanling Wistar rats to diets containing 0, 0.05, 0.1, 0.25, or 1.0% ethylene glycol for 2 weeks (5/sex/dose), 6 weeks (5/sex/dose), or 16 weeks (15/sex/dose). Reported calculated average daily chemical intakes were 35, 71, 180, and 715 mg/kg/day in males, and 0, 38, 85, 185, and 1,128 mg/kg/day in females. Intakes were averaged among rats house five per cage and decreased throughout the study because the amount of ethylene glycol in the diet was not adjusted for changing food consumption and body weight. Survival, clinical signs, food and water intake, and body weight were evaluated throughout the exposure period. Hematology

(hemoglobin, hematocrit, packed cell volume, total erythrocytes, reticulocytes, total and differential leukocytes), serum chemistry (urea, glucose, protein, albumin, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, and lactic dehydrogenase), organ weights (including kidneys, liver, spleen, brain, heart, stomach, small intestines, caecum, adrenals, pituitary, thyroid, and gonads), and histology (organs that were weighed and 19 additional tissues) were evaluated at the 2-, 6-, and 16-week sacrifices. Urinalysis (glucose, ketones, bile salts, blood, protein, and presence of oxalic acid crystals, cells and other microscopic constituents) and renal function (urine concentration and dilution tests measuring volume and specific gravity, and cell excretion) were evaluated at weeks 2 and 16. Urine was additionally analyzed for oxalic acid at weeks 2, 6, 12, 14, and 16. There were no clear exposure-related effects on survival, clinical signs, body weight, hematology, or serum chemistry. Pneumonial changes occurred in the lungs of most males and females and salivary adenitis occurred in 90% of the males and 45% of the females, but these effects were not considered exposure-related. Urinary excretion of oxalic acid was significantly increased in males at 715 mg/kg/day at weeks 2–16 and in females at 1,128 mg/kg/day at weeks 6–16, with the magnitude of the effect markedly greater in males (100–500% of control levels) than females (40–100% of control values). Increased absolute kidney weight, oxalic acid crystals in urine, and excretion of a larger volume of urine with a lower specific gravity after a prolonged period (16 hours) without water were observed in the 715 mg/kg/day males at week 16. Exposure-related histopathologic changes occurred only in the kidneys. Incidences of kidney lesions were statistically significantly increased in males at ≥180 mg/kg/day. Specific renal histopathologic findings in the males at 16 weeks included individual nephrons with degenerative changes (incidences of 0/15, 1/15, 1/15, 2/15, and 5/15 [$p<0.05$] in the control to high-dose groups), individual nephrons with degenerative changes and occasional oxalate crystals (0/15, 0/15, 0/15, 1/15, and 4/15 [$p<0.05$]), and generalized tubular damage and heavy oxalate crystals (0/15, 0/15, 0/15, 0/15, and 4/15 [$p<0.05$]). At 0, 35, 71, 180, and 715 mg/kg/day, the total incidence of male rats with oxalate crystals was 0/15, 0/15, 0/15, 1/15, and 10/15 ($p<0.001$), and the total incidence of male rats with renal tubular damage was 0/15, 1/15, 1/15, 4/15 ($p<0.05$), and 15/15 ($p<0.001$). Females had an increased incidence of renal tubular damage at 1,128 mg/kg/day, but the increase was not statistically significant. The histological evaluations of the kidneys in the five rats/sex/dose exposed for 2 or 6 weeks showed no statistically significant increases in incidences of specific changes, although the total incidence of animals with tubular damage was significantly increased in the 715 mg/kg/day males at 6 weeks. Based on the 16-week kidney histopathology data in male Wistar rats, this study identified a NOAEL of 71 mg/kg/day and LOAEL of 180 mg/kg/day for intermediate-duration exposure.

The 16-week studies of Cruzan et al. (2004) and Gaunt et al. (1974) provide dose-response data for the critical effect in the most sensitive species, strain, and sex (i.e., kidney lesions in male Wistar rats). The respective NOAEL and LOAEL values were 150 and 500 mg/kg/day in the Cruzan et al. (2004) study and 71 and 180 mg/kg/day in the Gaunt et al. (1974) study. Although Gaunt et al. (1974) identified a lower apparent LOAEL, this study is not suitable for MRL consideration because the animal care was questionable and the daily dose was not constant. Nearly all of the rats, possibly from the beginning of the study, showed evidence of respiratory infection (pneumonia) and infectious salivary adenitis, either of which could have confounded the results. Also, the rats were fed a constant dietary percentage of ethylene glycol, such that daily consumption varied throughout the study. For example, in the 180 mg/kg/day LOAEL group, the rats were exposed to approximately 300 mg/kg/day for the first 2-weeks, which is a level above the threshold for renal toxicity in male Wistar rats in a 12-month study (Wilson et al. 2005) (see chronic-duration MRL discussion). Further, the rats were housed in groups of five, such that consumption of individual rats among the groups likely varied. Hence, the dose levels in the Gaunt et al. (1974) study are not reliably consistent, unlike the study by Cruzan et al. (2004), which was conducted in the same rat strain and sex for the same duration. An additional reason to use the Cruzan et al. (2004) study for MRL derivation is that the 12-month study (Wilson et al. 2005) showed the same NOAEL of 150 mg/kg/day and a LOAEL of 300 mg/kg/day, thus appearing to substantiate the results of Cruzan et al. (2004) but not Gaunt et al. (1974).

The Cruzan et al. (2004) 16-week study is better basis for intermediate-duration MRL derivation because it identified the lowest reliable LOAEL and has no confounding factors. The incidences of the critical effect, crystal nephropathy, were 0/10, 0/10, 0/10, 10/10, and 10/10 at 0, 50, 150, 500, and 1,000 mg/kg/day, respectively. This data set is not appropriate for BMD analysis because the incidences increased from 0% in the rats exposed to ≤150 mg/kg/day to 100% in the rats exposed to ≥500 mg/kg/day; the lack of a low-response data point(s) limits the accuracy of dose-response modeling. Basing the MRL on the NOAEL of 150 mg/kg/day and using an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) yields an MRL of 1.5 mg/kg/day, but this value is higher than the acute-duration oral MRL of 0.8 mg/kg/day. It is against ATSDR policy to derive an intermediate-duration MRL that is higher than the acute-duration MRL. The acute MRL is based on a NOAEL of 150 mg/kg/day for developmental toxicity in mice (Neeper-Bradley et al. 1995; Tyl 1989), which is the same as the intermediate- and chronic-duration NOAELs for kidney effects in male Wistar rats (Cruzan et al. 2004; Wilson et al. 2005). Because available evidence indicates that the acute-duration oral MRL for ethylene should be protective for kidney effects following longer-term exposure, the acute-duration value of 0.8 mg/kg/day is adopted for intermediate-duration exposure.

An MRL has not been derived for chronic-duration oral exposure (365 days or more) to ethylene glycol. The chronic oral toxicity of ethylene glycol has been evaluated in three studies in rats (Blood 1965; DePass et al. 1986a; Wilson et al. 2005) and two studies in mice (DePass et al. 1986a; NTP 1993) using dietary exposure. As summarized below, the main target organs were the kidneys in rats and liver in mice, and rats were more sensitive than mice.

Male Wistar rats (20/dose) were exposed to ethylene glycol in dietary doses of 0, 50, 150, 300 or 400 mg/kg/day for 12 months (Wilson et al. 2005). Ten rats/group (main group) were used to assess toxicity; end points included clinical observations, body weight, feed and water consumption, urinalysis, organ weights, gross necropsy, and kidney and bladder histopathology. Urinalysis was performed the week prior to study termination and included color, appearance, specific gravity, volume, pH, bilirubin, glucose, proteins, ketones, blood, urobilinogen, and microscopic evaluation for crystal types. Five rats/group were used to determine renal clearance of ^{14}C-oxalate and ^{3}H-inulin, and five rats/group were used to evaluate concentrations of ethylene glycol and its glycolate and oxalate metabolites in blood, urine, and kidneys. No adverse effects occurred at 50 or 150 mg/kg/day, but toxicity was pronounced at 300 and 400 mg/kg/day. Effects at 400 mg/kg/day included mortality (4/10 died or were moribund on days 43-193) and weight loss, which led to early termination of the remaining animals on day 203. Mortality was also increased at 300 mg/kg/day (4/10 died or were moribund on days 111–221). Other effects at ≥300 mg/kg/day included increased water consumption with corresponding increased urine volume and decreased urine specific gravity, increased absolute and relative kidney weights, and gross and histopathological changes in the kidneys and bladder. Gross pathology included calculi, dilatation, and hemorrhage in the bladder at ≥300 mg/kg/day and calculi and dilatation in the renal pelvis and ureter at 400 mg/kg/day. Renal histopathology occurred in the majority of animals at 300 mg/kg/day and in all animals at 400 mg/kg/day; lesions included crystalluria-related nephropathy, tubule dilatation, birefringent crystals (particularly in the pelvic fornix), pelvic dilatation, and transitional cell hyperplasia. Incidences of crystal nephropathy, the most prevalent lesion, were 0/14, 0/15, 0/15, 12/13, and 10/10 at 0, 50, 150, 300, and 400 mg/kg/day, respectively. Histopathological changes in the bladder occurred in the majority of animals at ≥300 mg/kg/day; the basic change was transitional cell hyperplasia, progressing to acute inflammation and hemorrhage in severe cases. The early deaths were considered related to the inflammation and hemorrhage of the bladder wall. Decreased urinary pH and increased urinary oxalate crystals occurred at all treatment levels (≥50 mg/kg/day); these effects were not considered adverse, but rather normal metabolic/physiological consequences of ethylene glycol exposure. There were no treatment-related effects on renal clearance of oxalate or inulin. Urinary levels of oxalate were

unchanged at all doses, urinary ethylene glycol followed a linear dose-response relationship, and urinary glycolic acid was linear between 50 and 150 mg/kg with a disproportionate nonlinear increase at 300 mg/kg/day. Kidney concentrations of glycolate and oxalate were unchanged at 50 and 150 mg/kg/day, but clear nonlinear increases in both of these metabolites occurred at ≥300 mg/kg/day, indicating that the accumulation of calcium oxalate in the kidneys correlated with the appearance of renal toxicity. A NOAEL of 150 mg/kg/day and a LOAEL of 300 mg/kg/day were identified in male Wistar rats based on histopathology in the kidneys (crystal nephropathy) and bladder (inflammation and hemorrhage).

Sprague-Dawley rats (16/sex/dose) were fed ethylene glycol in estimated dietary doses of 0, 75, 150, 375, 750, or 3,000 mg/kg/day for up to 2 years (Blood 1965). Evaluations included food and water consumption, body and organ weights, hematology, urinary protein, and limited histopathology. Decreased body weight gain, increased water consumption, proteinuria, and mortality occurred in males at ≥750 mg/kg/day and females at 3,000 mg/kg/day. Incidences of calcification (oxalate crystal deposition) in the kidneys were increased in both sexes at ≥750 mg/kg/day, and oxalate-containing calculi were increased in males at ≥750 mg/kg/day and females at 3,000 mg/kg/day. Oxalate crystal deposition also occurred in males at 375 mg/kg/day (4/10 compared to 0/7 controls), although the increase was not statistically significantly. The report implied, but did not adequately document, that many of the animals with crystal deposition in the renal tubules also had degenerative changes in the tubular epithelium. Due to the insufficiently reported histopathology findings and lack of a clear (statistically significant) increase in oxalate crystal deposition at 375 mg/kg/day due to small number of animals, this study provides limited evidence that 375 mg/kg/day was a LOAEL for kidney toxicity in male Sprague-Dawley rats; the NOAEL was 150 mg/kg/day.

F344 rats (130/sex/dose) were fed ethylene glycol in approximate dietary doses of 0, 40, 200, or 1,000 mg/kg/day for up to 2 years (DePass et al. 1986a). End points included food and water consumption, body and organ weights, hematology, clinical chemistry, extensive urinalysis, and comprehensive histopathology. No treatment-related or statistically significant changes occurred in the male rats at 40 or 200 mg/kg/day. Toxicity was pronounced in males at 1,000 mg/kg/day as shown by increased mortality from month 9 (100% day 475), and various other effects that included increased water consumption and urine volume, increased blood urea nitrogen (BUN) and serum creatinine, decreased urine specific gravity and pH, increased urinary calcium oxalate crystals, and increased kidney weight and lesions. All 1,000 mg/kg/day males sacrificed at 12 months had calcium oxalate crystalluria and multiple severe renal lesions that included tubular dilation, proteinosis, hyperplasia, glomerular shrinkage, and/or

chronic interstitial nephritis. Most of the 1,000 mg/kg/day males that died during the study or were sacrificed when moribund had oxalate nephrosis, which was the primary cause of death, as well as hydronephrosis. In the male rats at 0, 40, 200, and 1,000 mg/kg/day, the total incidences of oxalate nephrosis were 0/256, 0/129, 0/129, and 95/116, respectively. Non-neoplasic lesions in several non-renal tissues were also significantly increased in the 1,000 mg/kg/day males; these included cellular hyperplasia in the parathyroids and mineralization in the heart (vessels and muscle), lungs (interstitial), stomach, and vascular system. A NOAEL of 200 mg/kg/day and a serious LOAEL of 1,000 mg/kg/day were identified in male F344 rats based on kidney toxicity (oxalate nephrosis)-induced mortality.

In the female F344 rats, effects occurred in kidneys and lymph nodes at 1,000 mg/kg/day and liver at ≥200 mg/kg/day (DePass et al. 1986a). Renal effects in females were limited to increased kidney weight and calcium oxalate crystals and uric acid crystals in the urine at 1,000 mg/kg/day; no kidney histopathology or mortality occurred as in males (DePass et al. 1986a). Hemosiderosis in the mesenteric lymph nodes was increased at 1,000 mg/kg/day. Hepatic effects included increases in mononuclear cell infiltrates at 1,000 mg/kg/day and fatty metamorphosis (slight) at ≥200 mg/kg/day. Total incidences of liver fatty metamorphosis in the 0, 40, 200, and 1,000 mg/kg/day females were 34/256, 16/129, 27/125, and 35/128, respectively; the increases at 200 and 1,000 mg/kg/day were statistically significant. The liver fatty metamorphosis is not considered to be adverse because the effect was slight and there was no other evidence of hepatotoxicity; at no time (6, 12, 18, or 24 months) was there an increase in liver function parameters (serum chemistry) or in liver weight, even in animals dosed at 1,000 mg/kg/day. Additionally, this was the only long-term study (intermediate- or chronic-duration) to find liver lesions in rats, and the mode of action supports the kidney as the critical target. Based on the kidney effects, a NOAEL of 200 mg/kg/day and a LOAEL of 1,000 mg/kg/day were identified in female F344 rats.

CD-1 mice (80/sex/dose) were fed ethylene glycol in approximate dietary doses of 0, 40, 200, or 1,000 mg/kg/day for up to 2 years (DePass et al. 1986a). Evaluations were limited to clinical signs, body weight, food consumption, and comprehensive histopathology. No clear treatment-related effects were observed in either sex, indicating that this study identified a NOAEL of 1,000 mg/kg/day and no LOAEL in CD-1 mice. In the other mouse study, B6C3F1 mice (60/sex/dose) were exposed to ethylene glycol in the diet for up to 2 years (NTP 1993). Estimated average doses were 0, 1,500, 3,000, and 6,000 mg/kg/day in males and 0, 3,000, 6,000, and 12,000 mg/kg/day in females. Evaluations included hematology, clinical chemistry, organ weights (limited), and comprehensive histopathology. Effects were essentially limited to increased incidences of hepatocellular hyaline degeneration in males at ≥3,000 mg/kg/day and females at 12,000 mg/kg/day, and medial hyperplasia of the pulmonary arterioles

in females at ≥3,000 mg/kg/day; the biological significance of the pulmonary lesion was unclear (NTP 1993). Small numbers of oxalate-like crystals and/or calculi were noted in the renal tubules, urethrae, and urinary bladder in a few males at 6,000 mg/kg/day. A NOAEL of 1500 mg/kg/day and LOAEL of 3,000 mg/kg/day for liver histopathology were identified in male B6C3F1 mice.

Key findings in the chronic toxicity studies were kidney lesions (oxalate nephrosis) and mortality at ≥300 mg/kg/day in male Wistar rats (Wilson et al. 2005), kidney lesions (oxalate crystal deposition and implied degenerative changes) at ≥375 mg/kg/day and mortality at 750 mg/kg/day in male Sprague-Dawley rats (Blood 1965), kidney lesions (oxalate nephrosis) and mortality at 1,000 mg/kg/day in male F344 rats (DePass et al. 1986a), no kidney or liver histopathology in male or female CD-1 mice at 1,000 mg/kg/day (DePass et al. 1986a), and liver lesions (hepatocellular hyaline degeneration) in male B6C3F1 mice at ≥3,000 mg/kg/day (NTP 1993). The kidney lesions and mortality in male rats occurred at doses that were NOAELs in mice, indicating that rats were more sensitive than mice and the most appropriate species for MRL consideration.

Chronic effect levels for kidney lesions and mortality in male rats included a NOAEL of 150 mg/kg/day and a serious LOAEL of 300 mg/kg/day in Wistar males (Wilson et al. 2005), a NOAEL of 200 mg/kg/day and a serious LOAEL of 1,000 mg/kg/day in F344 males (DePass et al. 1986a), and a NOAEL of 150 mg/kg/day and a serious LOAEL of 750 mg/kg/day in Sprague-Dawley males (Blood 1965). An apparent increase in kidney lesions without mortality occurred in Sprague-Dawley males at 375 mg/kg/day (Blood 1965), suggesting that this dose was a less serious LOAEL for renal effects in this strain of rats. The 150 mg/kg/day NOAEL for renal effects in Wistar males (Wilson et al. 2005) and Sprague-Dawley males (Blood 1965) is consistent with the 200 mg/kg/day NOAEL for renal effects in F344 males (DePass et al. 1986a).

The study in male Wistar rats (Wilson et al. 2005) is the most appropriate basis for chronic MRL derivation because it identified the lowest LOAEL (300 mg/kg/day) and is the only study providing information on effects of chronic exposure in Wistar rats, a strain shown to be approximately twice as sensitive as F344 rats to kidney toxicity in a 16-week study (Cruzan et al. 2004) (see intermediate-duration MRL discussion). The incidences of the critical chronic effect, oxalate nephropathy, were 0/14, 0/15, 0/15, 12/13, and 10/10 at 0, 50, 150, 300, and 400 mg/kg/day, respectively (Wilson et al. 2005). This data set is not appropriate for BMD analysis because the incidences increased from 0% in the rats exposed to ≤150 mg/kg/day to 92% at 300 mg/kg/day and 100% at 400 mg/kg/day; the lack of a low-response data point(s) limits the accuracy of dose-response modeling. Basing the MRL on the NOAEL of

150 mg/kg/day and using an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) yields an MRL of 1.5 mg/kg/day, but this value is higher than the acute-duration oral MRL of 0.8 mg/kg/day. It is against ATSDR policy to derive a chronic-duration MRL that is higher than the acute-duration MRL. The acute MRL is based on a NOAEL of 150 mg/kg/day for developmental toxicity in mice (Neeper-Bradley et al. 1995; Tyl 1989), which is the same as the intermediate- and chronic-duration NOAELs for kidney effects in male Wistar rats (Cruzan et al. 2004; Wilson et al. 2005). The available evidence therefore indicates that the acute-duration oral MRL should be protective for chronic kidney effects.

2. RELEVANCE TO PUBLIC HEALTH

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3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of ethylene glycol. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

The general population may be exposed to ethylene glycol. Ethylene glycol is widely sold in grocery stores and in automobile supply, discount, drug, and other stores throughout the United States for general use as an antifreeze/coolant in automobile radiators. Additionally, it is used in the manufacturing or blending of polyester products; aircraft and runway de-icing fluids; heat transfer fluids used in heating, ventilation, and air conditioning systems; humectants; polyester and alkyd resins; plasticizers; electrolytic capacitors; low freeze dynamite; and brake and shock solutions.

Dermal exposure, through activities such as changing antifreeze, is the most likely route of exposure to ethylene glycol, but dermal exposure is not likely to lead to toxic effects. Only oral exposure, through accidental or intentional ingestion, is likely to lead to such effects, and then only if a sufficient amount is swallowed at one time. A review of the literature for ethylene glycol indicated that the stages of oral ethylene glycol poisoning in humans are well understood and documented. There is adequate knowledge of ethylene glycol metabolism to permit successful treatment of ethylene glycol intoxication, and substantial information concerning pathology and pathophysiology of the organ systems involved is available. Although the majority of the studies in humans represent descriptions of case studies of accidental or intentional poisoning, or exposure in industrial settings, they have been collected for a period of >60 years. Animal studies corroborate human findings and were used to provide quantitative data to support observations made in humans.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive,

developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into “less serious” or “serious” effects. “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

Information regarding health effects of ethylene glycol following inhalation exposure is limited. Health effects in humans were found in only a few studies (Bond et al. 1985; Troisi 1950; Wills et al. 1974). Animal studies were described by Tyl (1985, 1988a).

3.2.1.1 Death

No studies were located regarding death in humans after inhalation exposure to ethylene glycol.

Mortality occurred in 1/15 rats, 3/15 guinea pigs, 1/3 rabbits, 0/3 dogs, and 0/3 monkeys that were continuously whole-body exposed to 12 mg/m^{3}of ethylene glycol aerosol for 90 days, although none of the affected animals showed "any specific signs of toxicity" (Coon et al. 1970). This concentration is not a reliable LOAEL for mortality because intake of ethylene glycol from ingestion of aerosol deposited on the fur likely significantly contributed to total dose (Tyl et al. 1995a, 1995b). Exposure to 10 or 57 mg/m^3 ethylene glycol aerosol for 8 hours/day, 5 days/week for 6 weeks caused no mortality in rats (15/concentration), guinea pigs (15/concentration), rabbits (3/concentration), dogs (2/concentration), or monkeys (2/concentration) (Coon et al. 1970).

3.2.1.2 Systemic Effects

No studies were located regarding gastrointestinal, musculoskeletal, endocrine, dermal, ocular, body weight, or metabolic effects in humans or respiratory, gastrointestinal, musculoskeletal, dermal, or body weight effects in animals after inhalation exposure to ethylene glycol. The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category for ethylene glycol after inhalation exposure are reported in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Tolerable nose and throat irritation were occasional complaints in 19 volunteers (incidence and frequency not reported) who were exposed to ethylene glycol aerosol for 20–22 hours/day for 30 days in a controlled study (Wills et al. 1974). The average mean weekly exposure concentration was 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30. Sessions in which the concentration was increased for short periods during the last 10 days of the study showed that upper respiratory tract irritation became common at approximately 140 mg/m^3, and caused exposure to be tolerated for only 15 minutes at 188 mg/m^3, 2 minutes at 244 mg/m^3, and one or two breaths at 308 mg/m^3 due to symptoms that included a burning sensation in the trachea and a burning cough. The NOAEL of 23 mg/m^3 for

Table 3-1 Levels of Significant Exposure to Ethylene Glycol - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Systemic								
1	Human	15 min	Resp		140 M (respiratory tract irritation)	188 M (intolerable respiratory tract irritation)	Wills et al. 1974	
2	Human	14 d 20-22 hr/d	Resp	23 M[b]			Wills et al. 1974	
			Hemato	23 M				
			Hepatic	23 M				
			Renal	23 M				
Neurological								
3	Human	14 d 20-22 hr/d		23 M			Wills et al. 1974	
INTERMEDIATE EXPOSURE								
Systemic								
4	Human	30 d 20-22 hr/d	Resp	30 M			Wills et al. 1974	
			Hemato	30 M				
			Hepatic	30 M				
			Renal	30 M				
Neurological								
5	Human	30 d 20-22 hr/d		30 M			Wills et al. 1974	

a The number corresponds to entries in Figure 3-1.

b Used to derive an acute-duration inhalation minimal risk level (MRL) of 2 mg/m3; the NOAEL of 23 mg/m3 was divided by an uncertainty factor of 10 for human variability.

d = day(s); Gastro = gastrointestinal; Gd = gestational day; Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory

Figure 3-1 Levels of Significant Exposure to Ethylene Glycol - Inhalation

Acute (≤14 days)

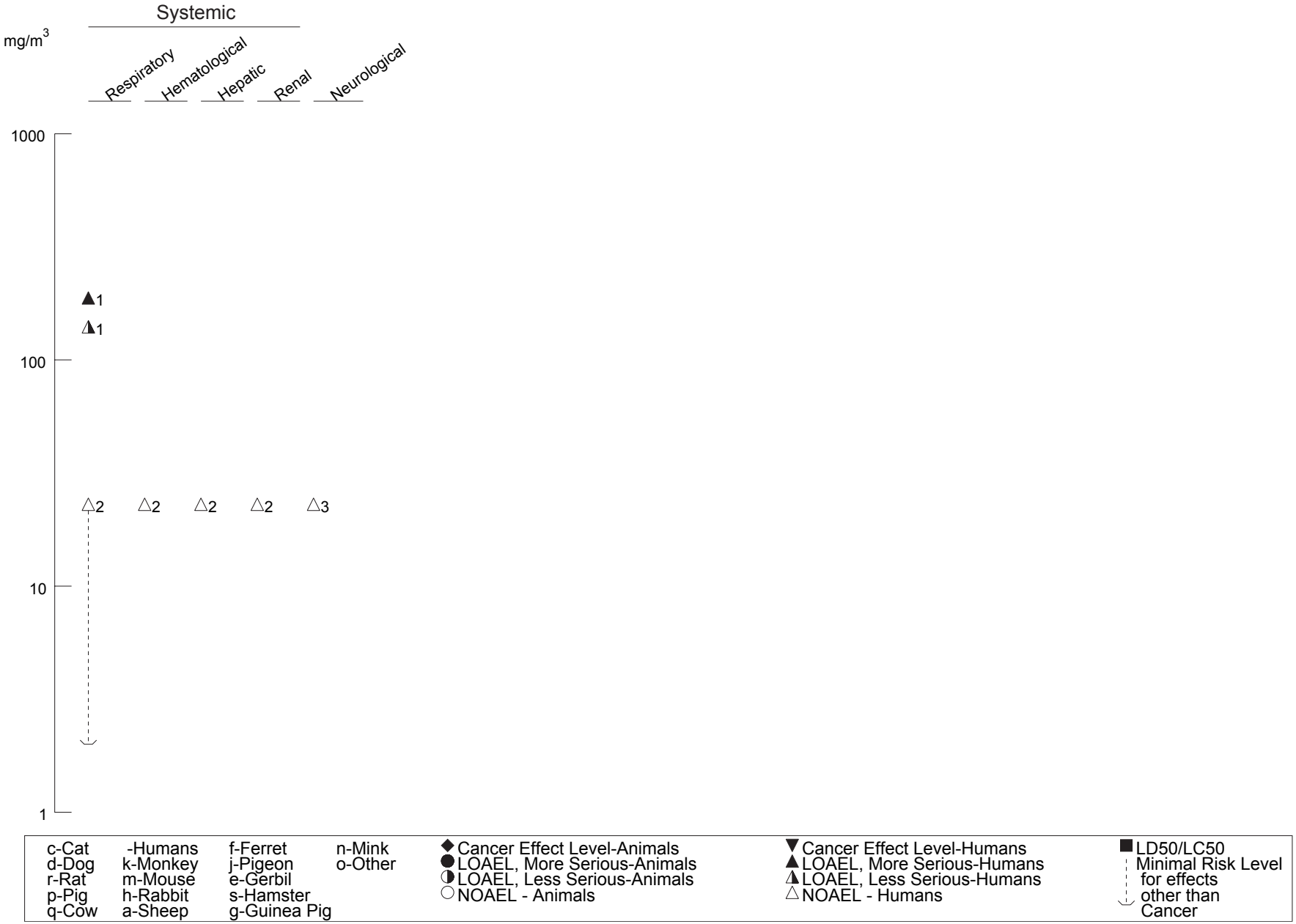
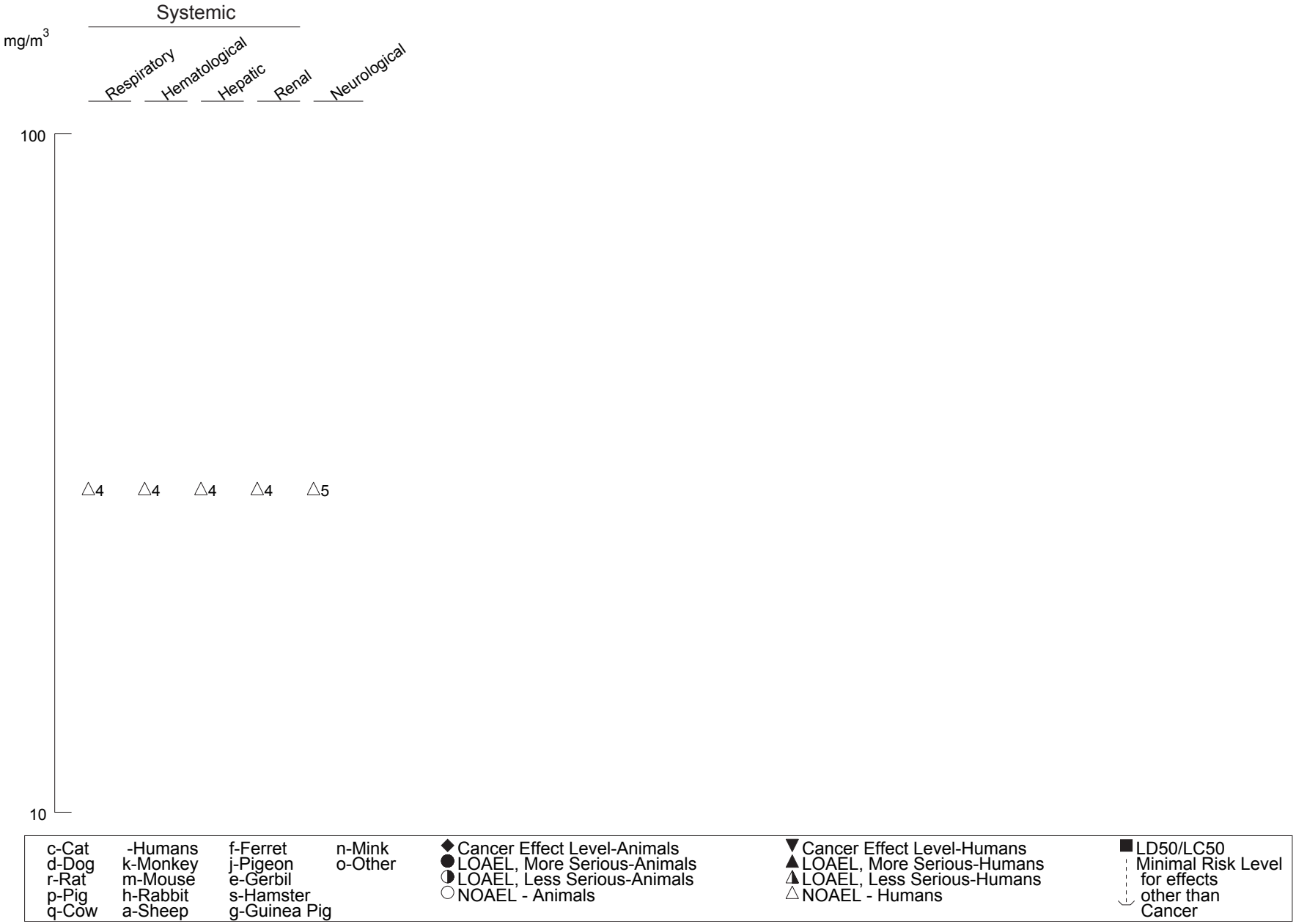


Figure 3-1 Levels of Significant Exposure to Ethylene Glycol - Inhalation *(Continued)*

Intermediate (15-364 days)



days 1–14 was used to derive an acute-duration inhalation MRL of 2 mg/m^3 as indicated in the footnote to Table 3-1 and discussed in Chapter 2 and Appendix A.

Cardiovascular Effects. Electrocardiographs conducted after 14 and 30 days of exposure were normal in a controlled study of 19 volunteers who were exposed to ethylene glycol aerosol for 20–22 hours/day for 30 days (Wills et al. 1974). The average mean weekly exposure concentration was 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30.

No gross or histological effects in the heart occurred in rats (15/concentration), guinea pigs (15/concentration), rabbits (3/concentration), dogs (2/concentration), or monkeys (2–3/concentration) that were whole-body exposed to apparently aerosolized ethylene glycol in concentrations of 10 or 57 mg/m^3 for 8 hours/day, 5 days/week for 6 weeks, or 12 mg/m^3 continuously for 90 days (Coon et al. 1970). The histology examinations in the rats and guinea pigs were limited to eight animals each. This study identified NOAELs of 57 mg/m^3 (intermittent exposure) and 12 mg/m^3 (continuous exposure) for cardiovascular effects, but the relevance of these NOAELs is unclear because intake of ethylene glycol from ingestion of aerosol deposited on the fur likely significantly contributed to total dose (Tyl et al. 1995a, 1995b).

Hematological Effects. No significant hematologic alterations were found in a controlled study of 19 volunteers who were exposed to ethylene glycol aerosol for 20–22 hours/day for 30 days (Wills et al. 1974). The average mean weekly exposure concentration was 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30. Evaluations were performed approximately every 2–3 days and included hematocrit, hemoglobin, total and differential leucocyte counts, and prothrombin time.

No treatment-related hematological effects were found in rats (15/concentration), guinea pigs (15/concentration), rabbits (3/concentration), dogs (2/concentration), or monkeys (2–3/concentration) that were whole-body exposed to apparently aerosolized ethylene glycol in concentrations of 10 or 57 mg/m^3 for 8 hours/day, 5 days/week for 6 weeks, or 12 mg/m^3 continuously for 90 days (Coon et al. 1970). Evaluations included hemoglobin concentration, packed erythrocyte volume, total leukocyte counts, and prothrombin time. This study identified NOAELs of 57 mg/m^3 (intermittent exposure) and 12 mg/m^3 (continuous exposure) for hematological effects, but the relevance of these NOAELs is unclear because intake of ethylene glycol from ingestion of aerosol deposited on the fur likely significantly contributed to total dose (Tyl et al. 1995a, 1995b).

Hepatic Effects. No significant alterations in serum hepatic end points were found in a controlled study of 19 volunteers who were exposed to ethylene glycol aerosol for 20–22 hours/day for 30 days (Wills et al. 1974). The average mean weekly exposure concentration was 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30. Clinical chemistry was evaluated approximately every 2–3 days and included serum glutamate-oxaloacetate transferase (SGOT), alkaline phosphatase, cholesterol, and bilirubin.

No treatment-related hepatic effects were found in rats (15/concentration), guinea pigs (15/concentration), rabbits (3/concentration), dogs (2/concentration), or monkeys (2–3/concentration) that were whole-body exposed to apparently aerosolized ethylene glycol in concentrations of 10 or 57 mg/m^3 for 8 hours/day, 5 days/week for 6 weeks, or 12 mg/m^3 continuously for 90 days (Coon et al. 1970). Evaluations included serum enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase, and lactate dehydrogenase), gross pathology, and histopathology; the histology examinations in the rats and guinea pigs were limited to eight animals each. This study identified NOAELs of 57 mg/m^3 (intermittent exposure) and 12 mg/m^3 (continuous exposure) for hepatic effects, but the relevance of these NOAELs is unclear because intake of ethylene glycol from ingestion of aerosol deposited on the fur likely significantly contributed to total dose (Tyl et al. 1995a, 1995b).

Renal Effects. No significant alterations in renal end points were found in a controlled study of 19 volunteers who were exposed to ethylene glycol aerosol for 20–22 hours/day for 30 days (Wills et al. 1974). The average mean weekly exposure concentration was 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30. End points included daily examination of urine for occurrence of oxalate crystals and erthyrocytes, twice weekly evaluations of urine volume, specific gravity, color, clarity, pH, amino acid nitrogen, and creatinine, and evaluation of blood urea nitrogen every 2–3 days. There also was no indication of renal impairment in 33 aviation workers who were intermittently exposed to ethylene glycol during airplane de-icing operations over a 2-month winter period (Gérin et al. 1997). Personal exposures to ethylene glycol vapor and aerosol were <2.5–22 and <17–190 mg/m^3, respectively. Pre- and post-shift measurements of urinary albumin, β-*N*-acetyl-glucosaminidase, β-2-microglobulin, and retinol-binding protein were used to assess kidney function.

No gross or histological effects occurred in the kidneys of rats (15/concentration), guinea pigs (15/concentration), rabbits (3/concentration), dogs (2/concentration), or monkeys (2–3/concentration) that were whole-body exposed to apparently aerosolized ethylene glycol in concentrations of 10 or 57 mg/m^3 for 8 hours/day, 5 days/week for 6 weeks, or 12 mg/m^3 continuously for 90 days (Coon et al. 1970). The histology examinations in the rats and guinea pigs were limited to eight animals each. This study

identified NOAELs of 57 mg/m^3 (intermittent exposure) and 12 mg/m^3 (continuous exposure) for kidney effects, but known sensitive renal end points for ethylene glycol (e.g., organ weight and urinalysis parameters) were not evaluated. The relevance of these NOAELs is additionally unclear because intake of ethylene glycol from ingestion of aerosol deposited on the fur likely significantly contributed to total dose (Tyl et al. 1995a, 1995b).

Endocrine Effects. No gross or histological effects occurred in the adrenals in dogs (2/concentration) or monkeys (2–3/concentration), or thyroid in dogs (2/concentration), following whole-body exposure to apparently aerosolized ethylene glycol in concentrations of 10 or 57 mg/m^3 for 8 hours/day, 5 days/week for 6 weeks, or 12 mg/m^3 continuously for 90 days (Coon et al. 1970). Additional endocrine end points were not evaluated. The relevance of these NOAELs is unclear because intake of ethylene glycol from ingestion of aerosol deposited on the fur likely significantly contributed to total dose (Tyl et al. 1995a, 1995b).

Ocular Effects. Rats (15/concentration), guinea pigs (15/concentration), rabbits (3/concentration), dogs (2/concentration), and monkeys (2–3/concentration) were exposed to apparently aerosolized ethylene glycol in concentrations of 10 or 57 mg/m^3 for 8 hours/day, 5 days/week for 6 weeks, or 12 mg/m^3 continuously for 90 days (Coon et al. 1970). No signs of ocular or nasal irritation were observed in any of the animals that were intermittently exposed to ≤57 mg/m^3 in the 6-week study. In the 90-day study, continuous exposure to 12 mg/m^3 caused moderate to severe eye irritation (erythema, edema, and discharge) in all (3/3) rabbits and corneal opacity with possible blindness in 2/15 rats; both species were affected within 8 days of the initial exposure.

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located specifically regarding adverse immunological effects in humans or animals after inhalation exposure to ethylene glycol. There were no significant alterations in total or differential white blood cell counts in a controlled study of 19 volunteers who were exposed to ethylene glycol aerosol for 20–22 hours/day for 30 days (Wills et al. 1974). The average mean weekly exposure concentration was 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30 (Wills et al. 1974).

3.2.1.4 Neurological Effects

Slight headache and backache were occasional complaints in 19 volunteers (incidence and frequency not reported) who were exposed to ethylene glycol aerosol for 20–22 hours/day for 30 days in a controlled

study (Wills et al. 1974). The average mean weekly exposure concentration was 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30. No effects were seen in electroencephalographs or a battery of psychological tests conducted after 14 and 30 days of exposure; the tests evaluated simple reaction time, reaction time with discrimination, visual-motor coordination, depth perception, and mental ability (encoding and subtraction accuracy).

No gross or histological effects in the brain or spinal cord occurred in dogs (2/concentration) that were whole-body exposed to apparently aerosolized ethylene glycol in concentrations of 10 or 57 mg/m^3 for 8 hours/day, 5 days/week for 6 weeks, or 12 mg/m^3 continuously for 90 days (Coon et al. 1970). This study identified NOAELs of 57 mg/m^3 (intermittent exposure) and 12 mg/m^3 (continuous exposure) for neurological effects based on small numbers of animals. The relevance of these NOAELs is unclear because neurobehavioral function was not evaluated and intake of ethylene glycol from ingestion of aerosol deposited on the fur likely significantly contributed to total dose (Tyl et al. 1995a, 1995b).

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category for ethylene glycol after inhalation exposure are reported in Table 3-1, and plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to ethylene glycol.

Acute-duration developmental toxicity studies of inhaled ethylene glycol in mice and rats are available, but all of the studies are confounded by concurrent ingestion exposure to ethylene glycol deposited on the fur. Whole-body exposure of pregnant CD-1 mice to 150–2,500 mg/m^3 aerosolized ethylene glycol for 6 hours/day on gestation days (Gd) 6–15 caused a decrease in the number of live fetuses per litter at ≥1,000 mg/m^3, but no effect on reproductive parameters was observed in CD rats dosed under the same regimen (Tyl 1985, 1988a; Tyl et al. 1995a). Both the mouse and rat studies were confounded by ingestion of ethylene glycol deposited on the fur of exposed animals and consumed during grooming; the authors estimated that ingestion comprised the majority of exposure. In a companion study, nose-only exposure of CD-1 mice to 500–2,500 mg/m^3 aerosolized ethylene glycol using the same study design resulted in no effects on pre- or postimplantation loss (Tyl 1988a; Tyl et al. 1995a). Although this study was aimed at reducing confounding from concurrent ingestion exposure, the authors noted that the

animals in the nose-only experiment were also exposed by ingestion of ethylene glycol deposited on the face during nose-only exposure.

As a result of confounding from exposure via ingestion, NTP-CERHR (2004) characterized the developmental toxicity studies as inadequate for the purpose of identifying effect levels for inhalation exposure; thus, there are no reliable NOAEL or LOAEL values.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans after inhalation exposure to ethylene glycol.

Acute-duration developmental toxicity studies of inhaled ethylene glycol in mice and rats are available, but all of the studies are confounded by concurrent ingestion exposure to ethylene glycol deposited on the fur. Groups of 25 pregnant CD-1 mice and CD rats were exposed (whole-body) to target concentrations of 0, 150, 1,000, or 2,500 mg/m^3 aerosolized ethylene glycol (mass median aerodynamic diameter [MMAD] of 2.3 μm) for 6 hours/day on Gd 6–15 (Tyl 1985, 1988a; Tyl et al. 1995a). Fetal evaluations included litter size, fetal weight, and external, visceral, and skeletal malformations. Maternal toxicity (e.g., increased liver weight in rats and reduced body weight gain in mice) was evident at 2,500 and 1,000 mg/m^3 in rats and mice, respectively (Tyl et al. 1995b). In mice, significant decreases in the number of live fetuses per litter and in the weight of live fetuses, as well as increases in the number of late resorptions per litter and the incidence of external, visceral, and skeletal malformations were observed at target concentrations of ≥1,000 mg/m^3. In rats, reduced ossification at some sites in the axial skeleton was observed with exposure to 1,000 and 2,500 mg/m^3 (Tyl 1985; Tyl et al. 1995a); however, in an Expert Panel Review, NTP-CERHR (2004) concluded that the relationship of this effect to treatment was uncertain due to the lack of a dose-response relationship. This study was confounded by significant ingestion of ethylene glycol deposited on the fur and consumed during grooming; the authors estimated that the ingestion dose comprised the majority of exposure.

In a follow-up study aimed at reducing the confounding from ingestion exposure, pregnant CD-1 mice were exposed nose-only to 0, 500, 1,000, or 2,500 mg/m^3 aerosolized ethylene glycol (MMAD 2.6 μm) (Tyl 1988a; Tyl et al. 1995b). Fetal weight and incidence of external, visceral, and skeletal malformations were evaluated in the offspring. In maternal animals, there were no effects other than changes in kidney weights. Absolute kidney weight was significantly increased at 1,000 and

2,500 mg/mg^3, and relative kidney weight was increased at 2,500 mg/m^3; however, the increases were small (6.6–9.5% higher than controls) and microscopic examination of kidneys showed no histopathological changes. At 2,500 mg/m^3, live fetal body weight was significantly reduced, and there was a significant increase in the one type of skeletal malformation (fused ribs). Increases in some skeletal variations were observed at 2,500 mg/m^3, and one type (extra ossification sites in the sagittal suture) was significantly increased at all concentrations. Although this study used restraint to minimize oral exposure, the authors noted that oral exposure was possible via grooming of the face after exposure. In addition, NTP-CERHR (2004) noted that stress from restraint during nose-only exposure (struggling was observed) may have contributed to the developmental effects observed with ethylene glycol, which were similar in nature to effects observed in a study of restrained nose-only exposure to water vapor (Tyl et al. 1994).

All of the available studies of developmental effects after inhalation exposure to ethylene glycol are confounded by concurrent ingestion exposure and the single nose-only exposure study is also confounded by stress related to restraint during exposure. In its review of these studies, NTP-CERHR (2004) concluded that the data are insufficient to identify effect levels from inhaled ethylene glycol in animals; thus, there are no reliable NOAEL or LOAEL values.

3.2.1.7 Cancer

An epidemiologic study on renal cancer mortality examined the work and health histories of 1,666 chemical plant employees and found no elevation in the odds ratio for workers exposed to ethylene glycol (Bond et al. 1985), although the sample size was quite small. Exposure was presumed to be by inhalation.

No studies were located regarding cancer effects in animals after inhalation exposure to ethylene glycol.

3.2.2 Oral Exposure

Ethylene glycol is a colorless, water-soluble liquid with a sweet taste and little or no odor, most commonly used as an antifreeze fluid. The ready availability of antifreeze mixtures makes ethylene glycol intoxication a significant medical and veterinary problem. Antifreeze mixtures contain up to 95% ethylene glycol (Mallya et al. 1986; Siew et al. 1975a). The exposure route most commonly associated with adverse effects is oral ingestion.

3.2.2.1 Death

The American Association of Poison Control Centers (AAPCC) reported 25 fatalities for 2005 due to ethylene glycol ingestion (Lai et al. 2006). Other fatal ethylene glycol poisonings included case reports of deaths resulting from accidental or intentional ingestion of ethylene glycol or antifreeze containing 99% ethylene glycol (Froberg et al. 2006; Godolphin et al. 1980; Gordon and Hunter 1982; Hantson et al. 2002; Hewlett et al. 1986; Jacobsen et al. 1984; Leth and Gregersen 2005; Siew et al. 1975a; Zeiss et al. 1989). A 22-year-old male who ingested 300 mL of antifreeze (approximately 4,071 mg/kg ethylene glycol) lapsed into a coma 24 hours after hospital admission and died 24 hours later (Siew et al. 1975a). A dose of 7,850 mg/kg can be estimated in the case of a 73-year-old male who consumed 500 mL of 95% ethylene glycol and died of myocardial failure after 68 hours (Gordon and Hunter 1982). A 25-year-old male who ingested ethylene glycol-based antifreeze in an apparent amount of 225 cc (approximately 3,600 mg/kg) died within 24 hours of hospital admission (Froberg et al. 2006). In five other fatal cases of accidental or intentional poisoning, the amount of ingested ethylene glycol ranged from 150 to 1,500 mL (2,379–23,786 mg/kg) (Karlson-Stiber and Persson 1992; Walton 1978). Thus, oral dose of ethylene glycol required to cause death in humans is not well defined in the literature. The minimum lethal dose for adults is thought to be 1.4 mL/kg of 95% ethylene glycol, or about 1,330 mg ethylene glycol/kg body weight (Parry and Wallach 1974; Robinson and McCoy 1989; Siew et al. 1975a).

A single dose oral LD_{50} of 4,000 mg/kg was determined in female F344 rats (Clark et al. 1979). Male Wistar rats administered 12,900 mg/kg ethylene glycol in a single oral dose had 55% mortality within 48 hours (Richardson 1973). Pregnant CD-1 mice given 11,090 mg/kg/day ethylene glycol orally on Gd 7–14 showed 10% mortality (Schuler et al. 1984) and pregnant rabbits exhibited 42% mortality after receiving 2,000 mg/kg/day ethylene glycol orally on Gd 6–19 (Tyl et al. 1993). Cats administered a single 4,440–8,880 mg/kg dose by gavage had 100% mortality within 20–36 hours (Penumarthy and Oehme 1975). A single gavage dose of 4,180–12,540 mg/kg/day caused 17–100% mortality in dogs within 72 hours (Kersting and Nielsen 1965). Dogs administered a single oral dose of 4,880 mg/kg in food had 100% mortality within 6 days (Beckett and Shields 1971).

Intermediate-duration dietary exposure to 1,000 mg/kg/day for 16 weeks caused 20% mortality in male Wistar rats, with no deaths occurring in similarly treated male F344 rats; females were not tested (Cruzan et al. 2004). Male F344/N rats fed 5,000 mg/kg/day ethylene glycol had 40% mortality after 13 weeks, whereas similarly treated females did not die (Melnick 1984). A chronic dietary study of ethylene glycol in Sprague-Dawley rats found 100% mortality after 12–24 months in males at 750 mg/kg/day and females

at 3,000 mg/kg/day (Blood 1965). Male F344 rats given 1,000 mg/kg/day ethylene glycol in the feed all died within 16 months (DePass et al. 1986a; Woodside 1982). In a 12-month dietary study in male Wistar rats, exposure to 300 mg/kg/day caused 40% mortality (died or were moribund) on days 111–221 (Wilson et al. 2005).

All reliable LOAEL and LD_{50} values for death in each species and duration category for ethylene glycol after oral exposure are reported in Table 3-2, and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located regarding hematological, musculoskeletal, endocrine, hepatic, dermal, ocular, or body weight effects in humans after oral exposure to ethylene glycol.

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category for ethylene glycol after oral exposure are reported in Table 3-2 and Figure 3-2.

Respiratory Effects. Respiratory system involvement occurs 12–24 hours after ingestion of sufficient amounts of ethylene glycol and is considered to be part of a second stage in ethylene glycol poisoning (Davis et al. 1997; Hess et al. 2004; Vale 1979). The symptoms include hyperventilation (Godolphin et al. 1980; Gordon and Hunter 1982), shallow rapid breathing (Woolf et al. 1992; Zeiss et al. 1989), and generalized pulmonary edema with calcium oxalate crystals occasionally present in the lung parenchyma (Friedman et al. 1962; Johnson et al. 1999; Leth and Gregersen 2005; Pellegrino et al. 2006; Vale 1979). Respiratory system involvement appears to be dose-dependent and occurs concomitantly with cardiovascular changes. Pulmonary infiltrates and other changes compatible with adult respiratory distress syndrome (ARDS) may characterize the second stage of ethylene glycol poisoning (Bey et al. 2002; Piagnerelli et al. 1999; Taylor et al. 1997). Pulmonary edema can be secondary to cardiac failure, ARDS, or aspiration of gastric contents (Walder and Tyler 1994). Symptoms related to acidosis such as hyperpnea and tachypnea are frequently observed; however, major respiratory morbidities such as pulmonary edema and bronchopneumonia are relatively rare and usually only observed with extreme poisoning (e.g., in only 5 of 36 severely poisoned cases) (Friedman et al. 1962; Johnson et al. 1999; Karlson-Stiber and Persson 1992; Leth and Gregersen 2005; Parry and Wallach 1974; Piagnerelli et al. 1999; Verrilli et al. 1987). In one case, respiratory failure occurred in a woman who had consumed 9,771 mg/kg ethylene glycol (as antifreeze) (Blakeley et al. 1993).

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Human	once				7070 M (death 68 hours after ingestion of ethylene glycol)	Gordon and Hunter 1982	
2	Human	once				4071 M (death 48 hours after ingestion)	Siew et al. 1975a	
3	Human	once				2379 (death in 6/11)	Walton 1978	
4	Rat (Fischer 344)	once (G)				4000 F (24-hour LD50)	Clark et al. 1979	
5	Mouse (Swiss CD-1)	8 d Gd 7-14 1 x/d (G)				11090 F (5/50 died)	Schuler et al. 1984	
6	Rabbit (New Zealand)	14 d Gd 6-19 1 x/d (GW)				2000 F (8/19 died)	Tyl et al. 1993	
Systemic								
7	Human	once	Metab			4332 M (severe metabolic acidosis)	Cheng et al. 1987	

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
8	Human	once	Resp			7070 M (hyperventilation)	Gordon and Hunter 1982	
			Cardio			7070 M (myocardial failure)		
			Renal			7070 M (renal failure)		
			Metab			7070 M (metabolic acidosis)		
9	Human	once	Renal			11238 F (calcium oxalate crystalluria)	Heckerling 1987	
			Metab			11238 F (metabolic acidosis)		
10	Human	once	Renal			2714 M (renal failure)	Mallya et al. 1986	
11	Human	once	Cardio			3171 M (tachycardia, ventricular gallop)	Parry and Wallach 1974	
			Renal			3171 M (calcium oxalate crystalluria, renal failure)		
			Metab			3171 M (metabolic acidosis)		
12	Human	once	Renal			7600 M (ethylene glycol in urine)	Peterson et al. 1981	
			Metab			7600 M (metabolic acidosis)		

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
13	Human	once	Cardio			4071 M (ventricular tachycardia, cardiac arrest)	Siew et al. 1975a	
			Renal			4071 M (oxalate nephrosis)		
			Metab			4071 M (metabolic acidosis)		
14	Human	once (W)	Gastro			12840 M (upper gastrointestinal bleeding)	Spillane et al. 1991	
			Renal			12840 M (renal failure)		
			Metab			12840 M (metabolic acidosis)		
15	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GW)	Bd Wt		2500 F (treatment period weight gain decreased 27%; gestational weight gain decreased 13%)		Marr et al. 1992	

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
16	Rat (CD)	10 d Gd 6-15 1 x/d (GW)	Hepatic	2500 F			Neeper-Bradley 1990, Neeper-Bradley et al. 1995	Hepatic NOAEL for organ weight. Liver and kidney histopathology not evaluated.
			Renal	1000 F	2500 F (increased absolute and relative kidney weight)			
			Bd Wt	1000 F	2500 F (26% decreased body weight)			
17	Rat (CD)	10 d Gd 6-15 1 x/d (GW)	Hepatic	5000 F			Price et al. 1985	Absolute but not relative liver weight 11% decreased at 5000 mg/kg/day. No change in absolute kidney weight. Histopathology not evaluated.
			Renal	1250 F	2500 F (increased relative kidney weight)			
			Bd Wt		1250 F (17% decreased body weight)			

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
18	Rat (Sprague-Dawley)	10 d (W)	Resp	7327 F			Robinson et al. 1990	Musc/skel NOAEL is for muscle histopathology. Endocrine NOAEL is for histopathology of adrenals, pancreas and pituitary.
			Gastro	7327 F				
			Hemato	2953 F	7327 F (Decreased hemoglobin, hematocrit, erythrocytes and total leukocytes)			
			Musc/skel	7327 F				
			Hepatic	7327 F				
			Renal	1343 M		2615 M (tubular oxalate crystals, dilation, degeneration and necrosis)		
			Endocr	7327 F				
			Dermal	7327 F				
			Bd Wt	2615 M		5279 M (13% body weight loss)		

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
19	Mouse (B6C3F1)	4 d (GW)	Resp	250			Hong et al. 1988	Biological significance of bone marrow effects is uncertain. NOAELs are for organ weight and histopath; endocrine NOAEL for adrenals.
			Cardio	250				
			Gastro	250				
			Hemato	50 F	100 F (bone marrow hypocellularity)			
20	Mouse (CD-1)	10 d Gd 6-15 1 x/d (GW)	Hepatic	3000 F			Price et al. 1985	Hepatic NOAEL for liver weight. Histopathology not evaluated.
			Bd Wt	750 F	1500 F (31% reduced weight gain)			
21	Mouse (CD-1)	10 d Gd 6-15 1 x/d (GW)	Hepatic	1500 F			Tyl 1989; Neeper-Bradley et al. 1995	Hepatic NOAEL for liver weight; liver histopathology not evaluated. Renal NOAEL for kidney weight and histopathology.
			Renal	1500 F				
			Bd Wt	1500 F				

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
22	Dog	once (F)	Renal			10743	(renal failure, oxalate nephrosis)	Grauer et al. 1987	
23	Rabbit (New Zealand)	14 d Gd 6-19 1 x/d (GW)	Hepatic	2000 F				Tyl et al. 1993	Hepatic NOAEL for liver weight; liver histopathology not evaluated.
			Renal			2000 F	(intraluminal oxalate crystals, epithelial necrosis, and tubular dilatation and degeneration of the cortical tubules)		
			Bd Wt	2000 F					
24	Cat	once (G)	Renal			4440	(oxalate nephrosis)	Penumarthy and Oehme 1975	
Immuno/ Lymphoret									
25	Rat (Sprague-Dawley)	10 d (W)		2615 M	5279 M	(decreased spleen and thymus weights)		Robinson et al. 1990	No histopathology in spleen, thymus or lymph nodes.
26	Mouse (B6C3F1)	4 d (GW)		250				Hong et al. 1988	NOAEL is for histopathology of spleen and thymus.

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
27	Mouse CBA	once (G)			12000 M (increased mortality from E. coli infection, decreased number of spleen CFUs, and inhibited antibody formation)		Zabrodskii and Germanchuk 2000	
28	Mouse CBA	once			12000 M (reduced natural killer cell activity)		Zabrodskii et al. 2003	
Neurological								
29	Human	once				9771 F (unresponsive, incontinent, no corneal, gag, or deep-tendon reflexes)	Blakeley et al. 1993	
30	Human	once				4332 M (tremors, agitation)	Cheng et al. 1987	
31	Human	once				11238 F (unresponsive to deep pain, delayed pupillary light reflex, no deep tendon or corneal reflex)	Heckerling 1987	

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
32	Human	once				2714 M (bilateral facial paralysis, hearing loss, absent gag reflex, unilateral facial numbness)	Mallya et al. 1986	
33	Human	once				3171 M (ataxia, somnolence, slurred speech, stupor, seizures, bilateral 6th nerve paralysis, lethargy)	Parry and Wallach 1974	
34	Human	once				4071 (stupor, loss of consciousness, coma)	Siew et al. 1975a	
35	Human	once				12840 M (unresponsive, depressed mental status, dysfunction of cranial nerves 9 and 10)	Spillane et al. 1991	
36	Rat (Sprague-Dawley)	10 d (W)		7327 F			Robinson et al. 1990	NOAEL is for histopathology of brain and sciatic nerve.
37	Dog	once (F)				10743 (depression, ataxia)	Grauer et al. 1987	

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
38	Cat	once (G)				4440 (convulsions and coma)	Penumarthy and Oehme 1975	
Reproductive								
39	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GW)		2500 F			Marr et al. 1992	
40	Rat (CD)	10 d Gd 6-15 1 x/d (GW)		2500 F			Neeper-Bradley 1990, Neeper-Bradley et al. 1995	
41	Rat (Sprague-Dawley)	10 d (W)		5279 M 7327 F			Robinson et al. 1990	NOAELs are for histopathology of testis, prostate, epididymis, seminal vesicles, ovary, uterus, and preputial and clitoral glands.
42	Mouse (B6C3F1)	4 d (GW)		250			Hong et al. 1988	NOAEL is for histopathology of testis and uterus.
43	Mouse (CD-1)	10 d Gd 6-15 1 x/d (GW)		1500			Tyl 1989; Neeper-Bradley et al. 1995	

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Developmental								
44	Rat (Fischer 344)	10 d Gd 6-15 (F)		1000 F			Maronpot et al. 1983	
45	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GW)				2500 F (increased skeletal malformations, decreased extent of ossification)	Marr et al. 1992	
46	Rat (CD)	10 d Gd 6-15 1 x/d (GW)		500 F		1000 F (increased skeletal malformations)	Neeper-Bradley 1990, Neeper-Bradley et al. 1995	
47	Rat (CD)	10 d Gd 6-15 1 x/d (GW)		1250 F		2500 F (increased skeletal malformations, decreased live fetuses/litter)	Price et al. 1985	
48	Mouse (Swiss Crl:CD-1)	7 d Gd 8-14 1 x/d (GW)		750 F	2500 (decreased pup body weight on ppd 1 and 4)		Harris et al. 1992	
49	Mouse (CD-1)	10 d Gd 6-15 1 x/d (GW)				750 F (increased skeletal malformations)	Price et al. 1985	

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
50	Mouse (Swiss CD-1)	8 d Gd 7-14 1 x/d (G)				11090 F (decreased numbers of viable litters and live pups per litter)	Schuler et al. 1984	
51	Mouse (CD-1)	10 d Gd 6-15 1 x/d (GW)		150[b] F		500 (increased total malformations)	Tyl 1989; Neeper-Bradley et al. 1995	
52	Rabbit (New Zealand)	14 d Gd 6-19 1 x/d (GW)		2000 F			Tyl et al. 1993	
INTERMEDIATE EXPOSURE								
Death								
53	Rat (Wistar)	16 wk (F)				1000 M (2/10 deaths)	Cruzan et al. 2004	
54	Rat (Fischer 344/N)	13 wk (F)				5000 M (4/10 deaths)	Melnick 1984; NTP 1993	
55	Mouse (CD-1)	2 gen (W)				2826 M (18% mortality in F1 males)	NTP 1986; Morrissey et al. 1989; Bolon et al. 1997	

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
56	Rat (Wistar)	16 wk (F)	Renal	150[c] M		500 M (oxalate crystal nephropathy with impaired kidney function)	Cruzan et al. 2004	
			Bd Wt	500 M	1000 M (23% reduced body weight gain)			
57	Rat (Fischer- 344)	16 wk (F)	Renal	150 M	500 M (oxalate crystals in tubles with normal kidney function)		Cruzan et al. 2004	
			Bd Wt	1000 M				
58	Rat (Fischer 344)	3 gen (F)	Renal	1000			DePass et al. 1986b	
			Bd Wt	1000				

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
59	Rat (Wistar)	16 wk (F)	Resp	1128 F			Gaunt et al. 1974	NOAELs mainly for histopathology; tissues included adrenals, pituitary, pancreas, lungs, heart, aorta, skeletal muscle, and GI tract.
			Cardio	1128 F				
			Gastro	1128 F				
			Hemato	1128 F				
			Musc/skel	1128 F				
			Hepatic	1128 F				
			Renal	71 M	180 M (renal tubular damage)			
				1128 F				
			Endocr	1128 F				
			Ocular	1128 F				
			Bd Wt	1128 F				

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
60	Rat (Fischer 344/N)	13 wk (F)	Cardio	10000 F			Melnick 1984; NTP 1993	Musc/skel NOAEL is for histopath of bone and marrow. Endocrine NOAEL is for histopath of adrenals, pancreas, pituitary, thyroid and parathyroids.
			Gastro	10000 F				
			Musc/skel	10000 F				
			Hepatic	10000 F				
			Renal	1250 M 2500 F	5000 F (increased relative kidney weight)	2500 M (renal tubular oxalate crystals, dilation, necrosis, and fibrosis)		
			Endocr	10000 F				
			Bd Wt	1250 M	2500 M (13% reduced body weight gain)			
61	Rat (CD)	15 d Gd 6-20 1 x/d (GW)	Renal	250		1250 (renal tubular dilatation and degeneration)	NTP 1988	
			Bd Wt	1250	2250 (21% decreased weight gain)			

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
62	Rat (Sprague-Dawley)	90 d (W)	Resp	5744 F			Robinson et al. 1990	Musc/skel NOAEL is for muscle histopathology. Endocrine NOAEL is for histopathology of adrenals, pancreas and pituitary.
			Gastro	5744 F				
			Hemato		597 F (decreased leukocyte level)			
			Musc/skel	5744 F				
			Hepatic	5744 F				
			Renal	407 M 1145 F		947 M (increased kidney weight and tubular oxalate crystals, dilation and degeneration) 3087 F (tubular lesions lower in frequency and severity than in males)		
			Endocr	5744 F				
			Dermal	5744 F				
			Bd Wt	947 M	3134 M (17% reduced body weight gain)			

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
63	Rat (Wistar)	33 d 1 x/d (GW)	Cardio	2000			Schladt et al. 1998	Endocrine NOAEL is for adrenal histopathology.
			Hepatic	2000				
			Renal		2000 (oxalate crystals in renal tubules and pelvis, tubulopathy, epithelial hyperplasia in renal pelvis)			
			Endocr	2000				
			Bd Wt	2000				
64	Mouse (Swiss Crl:CD-1)	17 d 1 x/d (GW)	Hepatic	2500 M			Harris et al. 1992	Hepatic and renal NOAELs for organ weight and histopathology.
			Renal	2500 M				
			Bd Wt	2500 M				

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
65	Mouse (B6C3F1)	13 wk (F)	Resp	16000 F			Melnick 1984; NTP 1993	Musc/skel NOAEL is for histopath of bone and marrow. Endocrine NOAEL is for histopath of adrenals, pancreas, pituitary, thyroid and parathyroids.
			Cardio	16000 F				
			Gastro	16000 F				
			Musc/skel	16000 F				
			Hepatic	3230 M	6450 M (hyaline degeneration of centrilobular hepatocytes)			
			Renal	3230 M 16000 F	6450 M (mild nephrosis and regenerative hyperplasia)			
			Endocr	16000 F				
			Bd Wt	16000 F				
66	Mouse (CD-1)	2 gen (W)	Hepatic	2826			NTP 1986; Morrissey et al. 1989; Bolon et al. 1997	Liver and kidney histopathology was evaluated in F0 and F1 males and females.
			Renal	1798 M		2826 M (tubular oxalate crystals, dilation and degeneration in F0 males)		

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Immuno/ Lymphoret								
67	Rat (Wistar)	16 wk (F)		1128 F			Gaunt et al. 1974	NOAEL for histopathology of lymph nodes, spleen and thymus.
68	Rat (Fischer 344/N)	13 wk (F)		10000 F			Melnick 1984; NTP 1993	NOAEL is for histopathology of lymph nodes.
69	Rat (Sprague-Dawley)	90 d (W)		5744 F			Robinson et al. 1990	No effects on spleen or thymus weights or histopathology of spleen, thymus or lymph nodes.
70	Rat (Wistar)	33 d 1 x/d (GW)		2000			Schladt et al. 1998	NOAEL is for spleen histopathology.
71	Mouse (B6C3F1)	13 wk (F)		16000 F			Melnick 1984; NTP 1993	NOAEL is for histopathology of lymph nodes.
Neurological								
72	Rat (Wistar)	16 wk (F)		1128 F			Gaunt et al. 1974	NOAEL for clinical signs of neurotoxicity and histopathology of brain, spinal cord and sciatic nerve.

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
73	Rat (Fischer 344\N)	13 wk (F)		2500 M		5000 M (calcium oxalate deposits in brain blood vessel walls)	Melnick 1984; NTP 1993	NOAEL is for brain histopathology.
74	Rat (Sprague-Dawley)	90 d (W)		5744 M			Robinson et al. 1990	NOAEL is for histopathology of brain and sciatic nerve.
75	Rat (Wistar)	33 d 1 x/d (GW)		2000 F			Schladt et al. 1998	NOAEL is for brain histopathology.
76	Mouse (B6C3F1)	13 wk (F)		16000 F			Melnick 1984; NTP 1993	NOAEL is for brain histopathology.
Reproductive								
77	Rat (Fischer 344)	3 gen (F)		1000			DePass et al. 1986b	
78	Rat (Wistar)	16 wk (F)		715 M 1128 F			Gaunt et al. 1974	NOAEL for histopathology of testes, seminal vesicles, prostate and uterus.
79	Rat (Fischer 344/N)	13 wk (F)		5000 M 10000 F			Melnick 1984; NTP 1993	NOAELs are for histopathology of testes, prostate, ovaries and uterus.

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
80	Rat (CD)	15 d Gd 6-20 1 x/d (GW)		1250 F		2250	decreased postnatal viability)	NTP 1988	
81	Rat (Sprague-Dawley)	90 d (W)		3134 M 5744 F				Robinson et al. 1990	NOAELs are for histopathology of testis, prostate, epididymis, seminal vesicles, ovary, uterus, and preputial and clitoral glands.
82	Rat (Wistar)	33 d 1 x/d (GW)		2000				Schladt et al. 1998	NOAEL is for histopathology of testes and ovaries. histopathology.
83	Mouse (Swiss Crl:CD-1)	17 d 1 x/d (GW)		2500 M				Harris et al. 1992	NOAEL for testicular and epididymal weight and histopathology, sperm count and motility, and reproductive function.
84	Mouse (Swiss Crl:CD-1)	20 d 1 x/d (GW)		750		2500	(decreased live fetuses, increased dead implants, 2/6 litters totally resorbed)	Harris et al. 1992	

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
85	Mouse (Swiss CD-1)	15-18 wk (W)		840			1640	(slightly decreased numbers of litters/mating pair and live pups/litter)	Lamb et al. 1985	
86	Mouse (B6C3F1)	13 wk (F)		12900 M 16000 F					Melnick 1984; NTP 1993	NOAELs are for histopathology of testes, prostate, ovaries and uterus.
87	Mouse (CD-1)	2 gen (W)		897 M 1798 F	1798 M	(reduced seminal vesicle and epididymis weights and sperm motility in F1 males)	2826	(testicular degeneration in F0 and F1 males; reduced live F0 female and total pups per litter)	NTP 1986; Morrissey et al. 1989; Bolon et al. 1997	
Developmental										
88	Rat (CD)	15 d Gd 6-20 1 x/d (GW)		1250 F			2250	(decreased postnatal viability, increased malformations in axial skeleton)	NTP 1988	
89	Mouse (Swiss CD-1)	15-18 wk (W)		840 F	1640 F	(reduced live litter size, skeletal defects)			Lamb et al. 1985	

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
90	Mouse (CD-1)	2 gen (W)			897 F (reduced pup weight in F0 females)		NTP 1986; Morrissey et al. 1989; Bolon et al. 1997	
CHRONIC EXPOSURE								
Death								
91	Rat (Sprague-Dawley)	2 yr (F)				750 M (100% mortality within 100 days)	Blood 1965	
						3000 F (100% mortality during second year)		
92	Rat (Fischer 344)	16 mo (F)				1000 M (100% dead or moribund by day 475)	DePass et al. 1986a; Woodside 1982	
93	Rat (Wistar)	12 mo (F)				300 M (4/10 died or were moribund on days 111-221)	Wilson et al. 2005	

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
94	Rat (Sprague-Dawley)	2 yr (F)	Gastro	3000 F			Blood 1965	Endocrine NOAEL for adrenal histopathology.
			Hepatic	3000 F				
			Renal	150 M 375 F	375 M (renal tubular oxalate crystal deposition and degenerative changes)			
					750 F (renal tubular oxalate crystal deposition and degenerative changes)			
			Endocr	3000 F				
			Bd Wt	375 M	750 M (30% decreased body weight gain within 100 days)			

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
95	Rat (Fischer 344)	24 mo (F)	Resp	200 M	1000 M (mineralization in lungs)		DePass et al. 1986a; Woodside 1982	Musc/skel NOAEL is for histopathology of skeletal muscle and bone. Endocr NOAEL is for histopath of adrenals, pituitary, thyroid and parathyroids.
			Cardio	200 M	1000 M (mineralization in heart vessels and muscle)			
			Gastro	200 M	1000 M (mineralization in stomach)			
			Hemato	200 M	1000 M (mineralization in vascular system, decreased erythrocytes, hematocrit and hemoglobin)			
			Musc/skel	1000				
			Hepatic	40 F	200 F (slight fatty metamorphosis)			
			Renal	200	1000 F (increased kidney weight and crystalluria without lesions)	1000 M (oxalate nephrosis)		
			Endocr	1000				
			Dermal	1000				
			Ocular	1000				
			Bd Wt	200 M	1000 M (15% decreased body weight gain)			

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
96	Rat (Wistar)	12 mo (F)	Renal	150 M		300 M (oxalate nephrosis and bladder inflammation and hemorrhage)	Wilson et al. 2005	
			Bd Wt	300 M	400 M (31% reduced body weight gain on day 197)			

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
97	Mouse (CD-1)	24 mo (F)	Resp	1000			DePass et al. 1986a; Woodside 1982	Musc/skel NOAEL is for histopathology of skeletal muscle and bone. Endocr NOAEL is for histopath of adrenals, pituitary, thyroid and parathyroids.
			Cardio	1000				
			Gastro	1000				
			Musc/skel	1000				
			Hepatic	1000				
			Renal	1000				
			Endocr	1000				
			Dermal	1000				
			Ocular	1000				
			Bd Wt	1000				

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
98	Mouse (B6C3F1)	24 mo (F)	Resp		3000 F (medial hyperplasia of pulmonary arterioles)		NTP 1993	Musc/skel NOAEL is for skeletal bone and marrow. Endocr NOAEL is for histopathology of adrenals, pancreas, thyroid, parathyroid and pituitary.
			Cardio	12000 F				
			Gastro	12000 F				
			Hemato	12000 F				
			Musc/skel	12000 F				
			Hepatic	1500 M	3000 M (hepatocellular hyaline degeneration)			
			Renal	3000 M	6000 M (oxalate-like crystals and calculi in tubules)			
				12000 F				
			Endocr	12000 F				
			Dermal	12000 F				
			Bd Wt	12000 F				
Immuno/ Lymphoret								
99	Rat (Sprague-Dawley)	2 yr (F)		3000 F			Blood 1965	NOAEL is for histopathology of spleen.

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
100	Rat (Fischer 344)	24 mo (F)		200 F	1000 F (hemosiderosis in mesenteric lymph nodes)		DePass et al. 1986a; Woodside 1982	NOAEL is for histopathology of spleen and lymph nodes.
101	Mouse (CD-1)	24 mo (F)		1000			DePass et al. 1986a; Woodside 1982	NOAEL is for histopathology of spleen and lymph nodes.
102	Mouse (B6C3F1)	24 mo (F)		12000 F			NTP 1993	NOAEL is for histopathology of spleen, thymus and lymph nodes.
Neurological								
103	Rat (Sprague-Dawley)	2 yr (F)		3000 F			Blood 1965	NOAEL is for histopathology of brain.
104	Rat (Fischer 344)	24 mo (F)		1000			DePass et al. 1986a; Woodside 1982	NOAEL is for histopathology of brain and spinal cord.
105	Mouse (CD-1)	24 mo (F)		200			DePass et al. 1986a; Woodside 1982	NOAEL is for histopathology of brain and spinal cord.

Table 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
106	Mouse (B6C3F1)	24 mo (F)		12000 F			NTP 1993	NOAEL is for histopathology of brain.
Reproductive								
107	Rat (Fischer 344)	24 mo (F)		1000			DePass et al. 1986a; Woodside 1982	NOAEL is for histopathology of testis, epididymis, prostate, uterus, ovaries and oviduct.
108	Mouse (CD-1)	24 mo (F)		1000			DePass et al. 1986a; Woodside 1982	NOAEL is for histopathology of testis, epididymis, prostate, uterus, ovaries and oviduct.
109	Mouse (B6C3F1)	24 mo (F)		6000 M 12000 F			NTP 1993	NOAEL is for histopathology of testis, seminal vesicles, epididymis, prostate, ovary and uterus.

a The number corresponds to entries in Figure 3-2.

b An acute-duration oral minimal risk level (MRL) of 0.8 mg/kg/day was derived from a BMDL10 of 76 mg/kg/day based on benchmark dose analysis of the incidences of litters with total malformations and incidences of bilateral extra rib 14; the BMDL10 was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability.

c An intermediate-duration oral MRL of 1.5 mg/kg/day was calculated by dividing the NOAEL of 150 mg/kg/day for kidney lesions by an an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). This value is higher than the acute-duration oral MRL of 0.8 mg/kg/day. It is against ATSDR policy to derive an intermediate-duration MRL that is higher than the acute-duration MRL. Because available evidence indicates that the acute-duration MRL should be protective for kidney effects following longer-term exposure, the acute-duration value of 0.8 mg/kg/day was adopted for intermediate-duration exposure.

Bd Wt = body weight; Cardio = cardiovascular; CFU = colony forming unit; d = day(s); (F)= feed; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; GI = gastrointestinal; (GW) = gavage in water; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); Metab = metabolic; NOAEL = no-observed-adverse-effect level; ppd = post-parturition day; Resp = respiratory; x = time(s); (W) = drinking water; wk = week(s); yr = year(s)

Figure 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral

Acute (≤14 days)

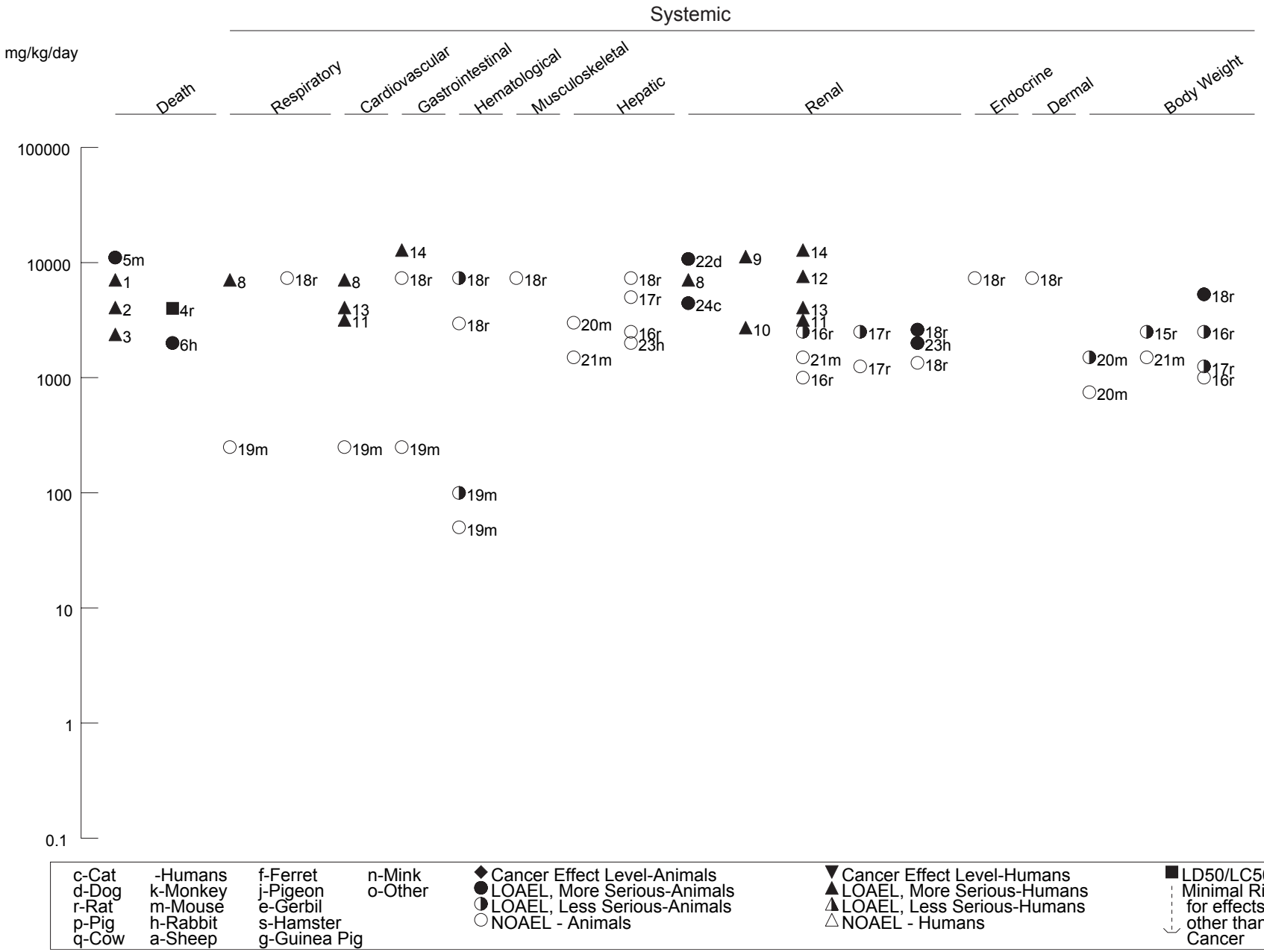


Figure 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral *(Continued)*

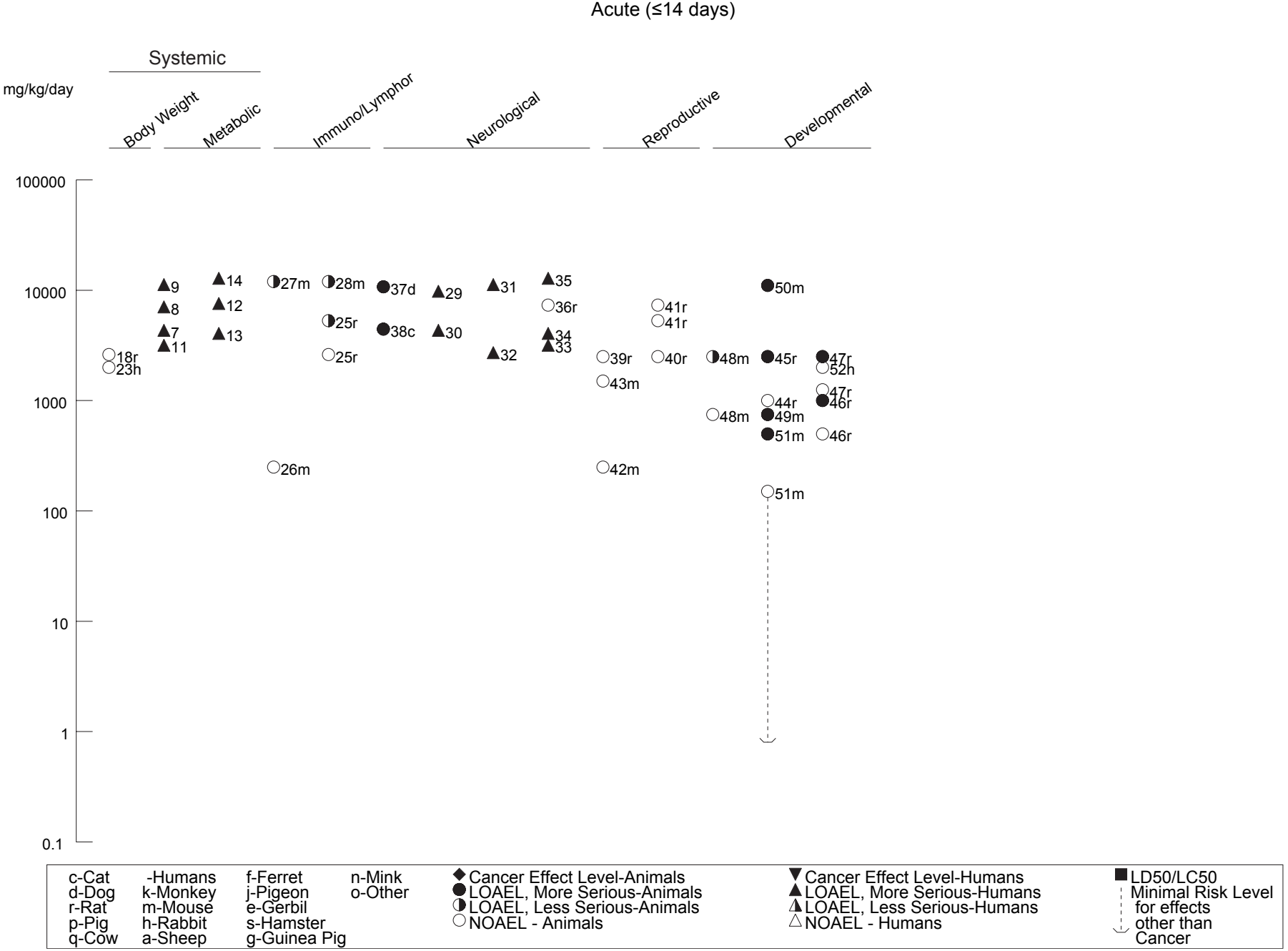


Figure 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral *(Continued)*

Intermediate (15-364 days)

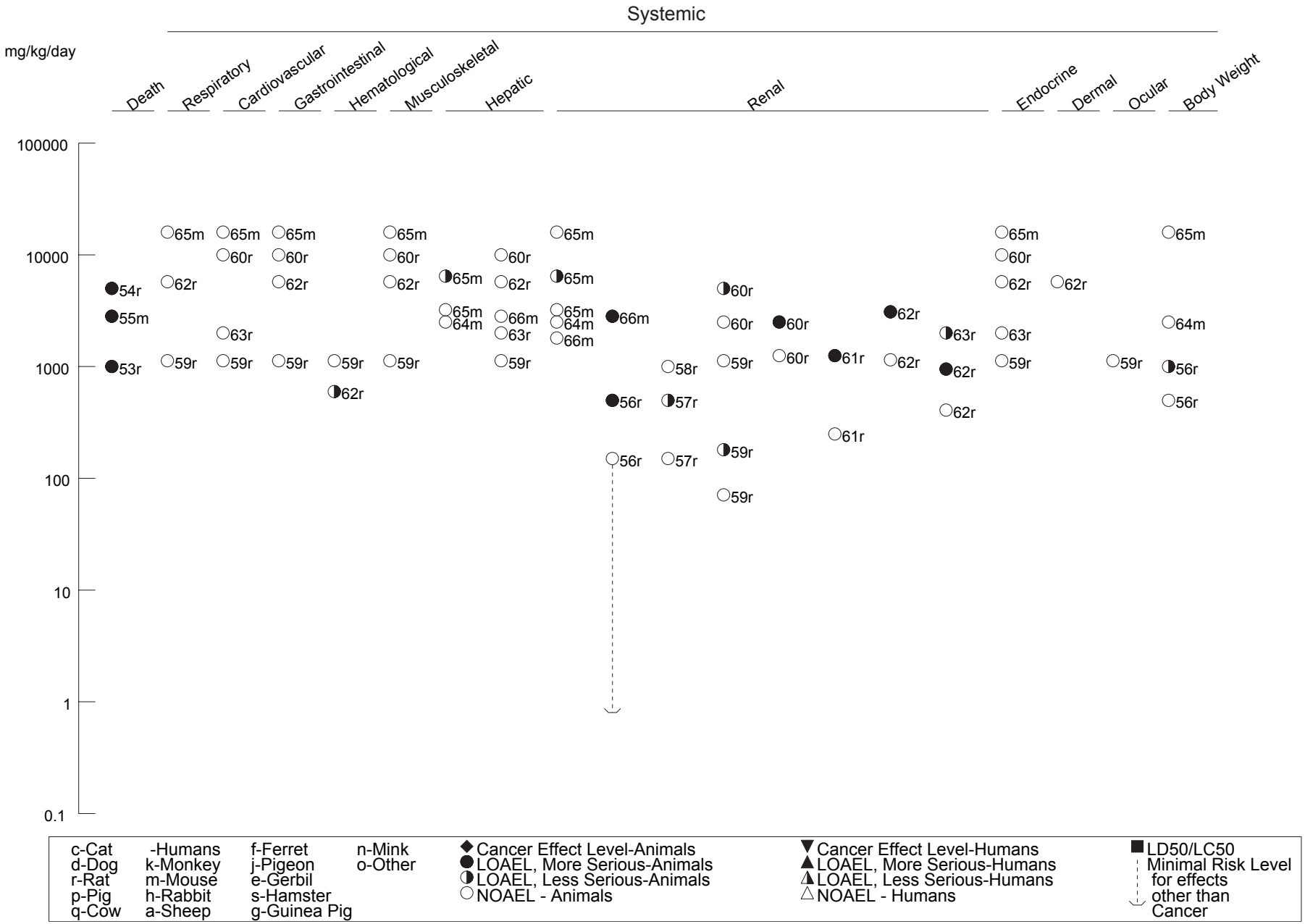


Figure 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral *(Continued)*

Intermediate (15-364 days)

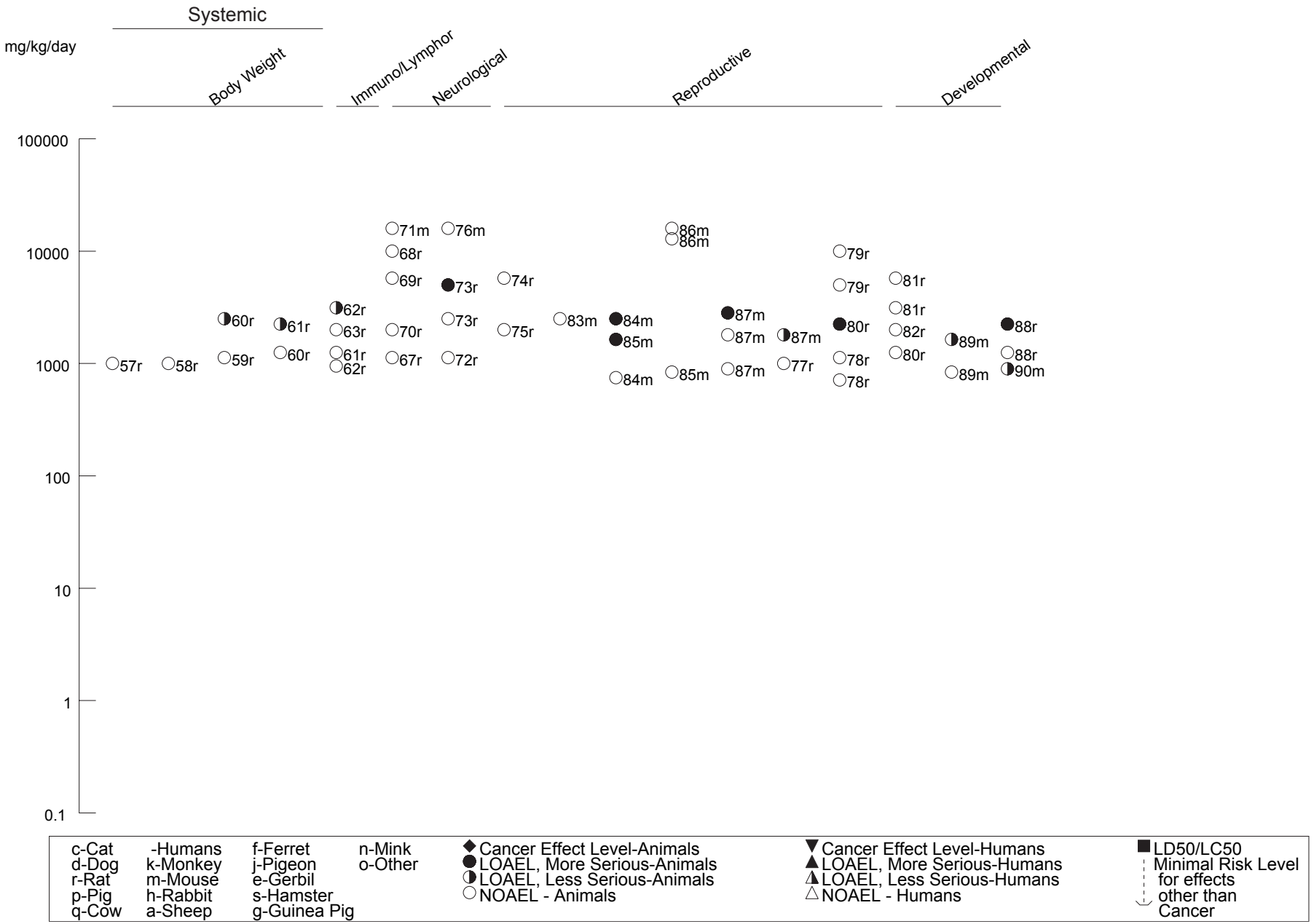


Figure 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral *(Continued)*

Chronic (≥365 days)

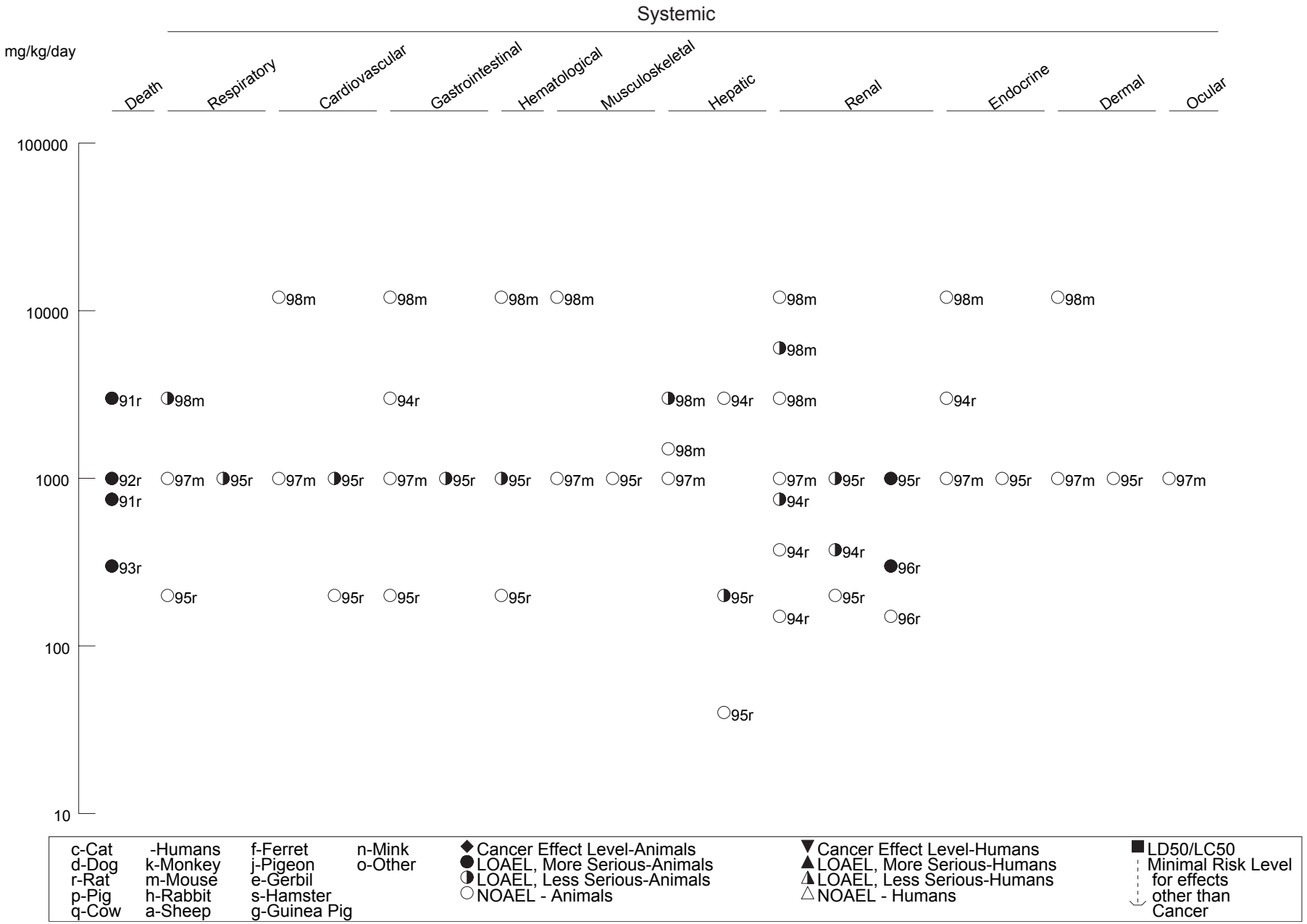
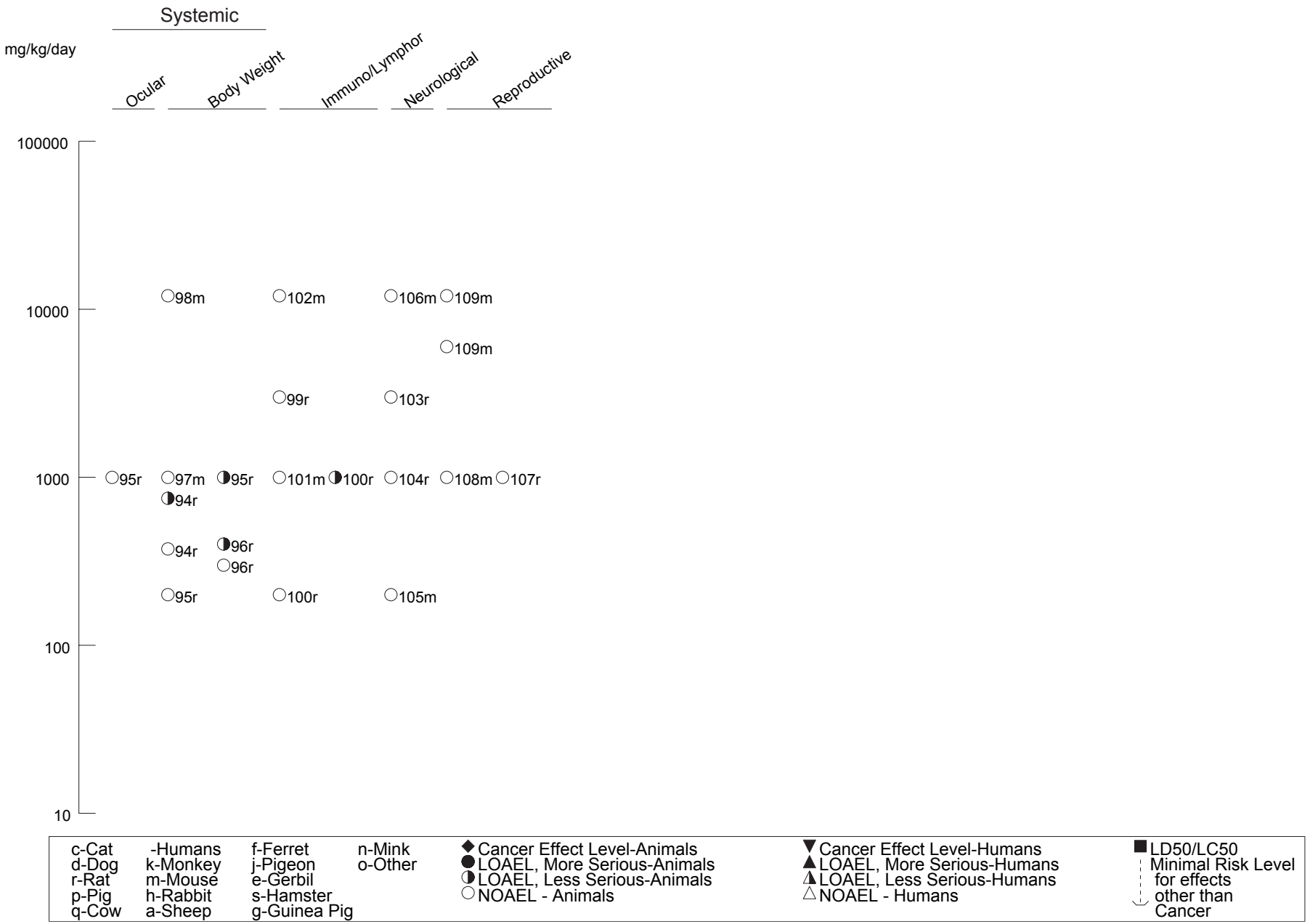


Figure 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral *(Continued)*

Chronic (≥365 days)



Pulmonary hyperemia and edema were frequent findings in dogs that ingested unknown lethal amounts of ethylene glycol in cases of antifreeze poisoning (Kersting and Nielsen 1965). A generalized soft tissue mineralization that included the lungs (interstitial) occurred in male F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside 1982). Histological examinations of the lungs showed no effects in Sprague-Dawley rats exposed to ≤7,327 mg/kg/day in drinking water for 10 days or ≤5,744 mg/kg/day in drinking water for 90 days (Robinson et al. 1990), Wistar rats exposed to ≤2,000 mg/kg/day by gavage for 4 weeks (Schladt et al. 1998), Wistar rats exposed to ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), F344 rats exposed to ≤10,000 mg/kg/day in the diet for 13 weeks (Melnick 1984), Sprague-Dawley rats exposed to ≤3,000 mg/kg/day in the diet for 2 years (Blood 1965), B6C3F1 mice exposed to ≤250 mg/kg/day by gavage for 4 days (Hong et al. 1988), B6C3F1 mice exposed to ≤16,000 mg/kg/day in the diet for 13 weeks or ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside 1982). The 10- and 90-day drinking water studies in rats also found no histopathological changes in the nasal cavity or turbinates (Robinson et al. 1990).

Cardiovascular Effects. Cardiovascular system involvement in humans occurs at the same time as respiratory system involvement, during the second phase of oral ethylene glycol poisoning, which is 12–24 hours after acute exposure (Vale 1979). The symptoms of cardiac involvement include tachycardia, ventricular gallop (Morgan et al. 2000; Parry and Wallach 1974; Siew et al. 1975a), and cardiac enlargement (Friedman et al. 1962; Vale 1979; Verrilli et al. 1987). Repeated cardiac arrhythmias were observed prior to cardiac arrest and death in a 22-year-old man who ingested 4,071 mg/kg of ethylene glycol (Siew et al. 1975a). Ingestion of ethylene glycol may also cause hypertension or hypotension, which may progress to cardiogenic shock (Chung and Tuso 1989; Jobard et al. 1996; Morgan et al. 2000; Rasic et al. 1999; Walder and Tyler 1994). Episodes of hypotension were observed prior to renal failure and death in a 73-year-old man who ingested 7,850 mg/kg ethylene glycol, contained in antifreeze (Gordon and Hunter 1982). Myocarditis has been observed at autopsy in cases of people who died following acute ingestion of ethylene glycol (Friedman et al. 1962). As in the case of respiratory effects, cardiovascular involvement occurs with ingestion of relatively high doses of ethylene glycol. Nevertheless, circulatory disturbances are a rare occurrence, having been reported in only 8 of 36 severely poisoned cases (Karlson-Stiber and Persson 1992). Therefore, it appears that acute exposure to high levels of ethylene glycol can cause serious cardiovascular effects in humans. The effects of a long-term, low-dose exposure are unknown.

Edema of the heart was occasionally observed in dogs that ingested unknown lethal amounts of ethylene glycol in cases of antifreeze poisoning (Kersting and Nielsen 1965). A generalized soft tissue mineralization that included the heart (vessels and muscle) occurred in male F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside 1982). Histological examinations of the heart showed no effects in Wistar rats exposed to ≤2,000 mg/kg/day by gavage for 4 weeks (Schladt et al. 1998), F344 rats exposed to ≤10,000 mg/kg/day in the diet for 13 weeks (Melnick 1984), Wistar rats exposed to ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), B6C3F1 mice exposed to ≤250 mg/kg/day by gavage for 4 days (Hong et al. 1988), B6C3F1 mice exposed to ≤16,000 mg/kg/day in the diet for 13 weeks or ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside 1982).

Gastrointestinal Effects. Nausea, vomiting with or without blood, pyrosis, and abdominal cramping and pain are common early effects of acute ethylene glycol ingestion (Davis et al. 1997; Johnson et al. 1999; Moossavi et al. 2003; Singh et al. 2001; Verrilli et al. 1987). Hemorrhagic areas in the gastric mucosa were observed at autopsy in a case of fatal oral poisoning with ethylene glycol (Hantson et al. 2002). Ischemic hemorrhagic necrosis of the colon was possibly ethylene glycol-related in a case of acute oral poisoning due to the absence of any other apparent causes (Singh et al. 2001). Acute effects of ethylene glycol ingestion in another patient included intermittent diarrhea and abdominal pain, which were attributed to mild colonic ischemia; severe abdominal pain secondary to colonic stricture and perforation developed 3 months after ingestion, and histology of the resected colon showed birefringent crystals highly suggestive of oxalate deposition (Gardner et al. 2003, 2004).

A 33-year-old man who drank a quart of ethylene glycol (12,840 mg/kg) developed upper gastrointestinal tract bleeding secondary to multiple gastric lesions (Spillane et al. 1991). It is not clear whether or not the gastric lesions were a pre-existing condition in this patient.

A generalized soft tissue mineralization that included the stomach, but not other parts of the gastrointestinal tract, occurred in male F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside 1982). Histological examinations of the gastrointestinal tract showed no effects in Sprague-Dawley rats exposed to ≤7,327 mg/kg/day in drinking water for 10 days or ≤5,744 mg/kg/day in drinking water for 90 days (Robinson et al. 1990), F344 rats exposed to ≤10,000 mg/kg/day in the diet for 13 weeks (Melnick 1984), Wistar rats exposed to ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), Sprague-Dawley rats exposed to ≤3,000 mg/kg/day in the diet

for 2 years (Blood 1965), B6C3F1 mice exposed to ≤250 mg/kg/day by gavage for 4 days (Hong et al. 1988), B6C3F1 mice exposed to ≤16,000 mg/kg/day in the diet for 13 weeks or ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside 1982).

Hematological Effects. Initial laboratory findings in cases of acute ethylene glycol poisoning may include moderate leukocytosis with a predominance of polymorphonuclear neutrophils and a normal hematocrit (Davis et al. 1997; Parry and Wallach 1974; Reddy et al. 2007; Verrilli et al. 1987).

No effects were observed on hematology parameters, but dose-related effects on bone marrow and erythropoietic parameters were observed when gavage doses up to 250 mg/kg/day ethylene glycol were given for 4 consecutive days to B6C3F1 mice (Hong et al. 1988). Granulocyte-macrophage progenitor formation was suppressed in males exposed to 50 mg/kg/day and in both sexes at higher doses. Ethylene glycol treatment resulted in bone marrow hypocellularity in both sexes up to 14 days after dosing at 100 mg/kg/day. Iron uptake in the bone marrow was suppressed in males exposed to 250 mg/kg/day; erythroid precursor colony-forming units were not significantly affected in mice at any dose. The biological significance of the bone marrow effects is uncertain in the absence of supporting data from other studies, as summarized below.

No histological changes in the bone marrow were observed in mice or rats exposed to higher doses of ethylene glycol for longer durations; these included F344 rats exposed to ≤10,000 mg/kg/day in the diet for 13 weeks (Melnick 1984), Sprague-Dawley rats exposed to ≤7,327 mg/kg/day in drinking water for 10 or 90 days (Robinson et al. 1990), and B6C3F1 mice exposed to ≤16,000 mg/kg/day in the diet for 13 weeks or ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993). Results of routine hematology evaluations in these studies were unremarkable except for some alterations in 10- and 90-day studies in rats. In the 10-day study, statistically significant decreases in hemoglobin, hematocrit, erythrocytes, and total leukocytes (7.3, 8.9, 8.5, and 34.8% less than controls, respectively) occurred in female rats at 7,327 mg/kg/day (Robinson et al. 1990). In the 90-day study, total leukocyte counts were significantly reduced in female rats at 597, 3,087 and 5,744 mg/kg/day (32, 30, and 50% less than controls, respectively) (Robinson et al. 1990). Results of differential counts were not reported and no clear hematological changes occurred in male rats in either study. Hematology evaluations were also negative in studies that did not examine bone marrow histology; these included studies of Wistar rats exposed to ≤2,000 mg/kg/day by gavage for 33 days (Schladt et al. 1998), Wistar rats exposed to ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), and CD-1 mice exposed to

≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a). Hematological changes (decreased erythrocyte count and hematocrit and hemoglobin concentration, and increased neutrophil count) were observed in male F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year, although this dose was a serious LOAEL for renal toxicity and mortality (DePass et al. 1986a).

Musculoskeletal Effects. Reported musculoskeletal effects in cases of acute ethylene glycol poisoning have included diffuse muscle tenderness and myalgias associated with elevated serum creatinine phosphokinase levels, and myoclonic jerks and tetanic contractions associated with hypocalcemia (Davis et al. 1997; Friedman et al. 1962; Parry and Wallach 1974; Verrilli et al. 1987). In some of these cases, autopsies showed interstitial and parenchymatous myositis in skeletal muscle (Friedman et al. 1962; Verrilli et al. 1987).

Histological examinations of skeletal muscle and/or bone in acute-, intermediate- and chronic-duration studies of ethylene glycol showed no effects in rats or mice. These studies included Sprague-Dawley rats exposed to ≤7,327 mg/kg/day in drinking water for 10 days or ≤5,744 mg/kg/day in drinking water for 90 days (Robinson et al. 1990), Wistar rats exposed to ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), F344 rats exposed to ≤10,000 mg/kg/day in the diet for 13 weeks (Melnick 1984) or 1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside 1982), B6C3F1 mice exposed to ≤16,000 mg/kg/day in the diet for 13 weeks or ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside 1982).

Hepatic Effects. Central hydropic or fatty degeneration, parenchymal necrosis, and calcium oxalate crystals in the liver have been observed at autopsy in cases of people who died following acute ingestion of ethylene glycol (Friedman et al. 1962; Leth and Gregersen 2005; Verrilli et al. 1987).

Acute-duration studies of ethylene glycol showed no effects on liver weight or liver histology in Sprague-Dawley rats exposed to ≤7,327 mg/kg/day in drinking water for 10 days (Robinson et al. 1990) or B6C3F1 mice exposed to ≤250 mg/kg/day by gavage for 4 days (Hong et al. 1988). Developmental toxicity studies found no effect on maternal liver weight (histology not examined) in CD rats exposed to ≤5,000 mg/kg/day by gavage on Gd 6–15 (Neeper-Bradley 1990; Neeper-Bradley et al. 1995; Price et al. 1985), CD-1 mice exposed to ≤3,000 mg/kg/day by gavage on Gd 6–15 (Neeper-Bradley et al. 1995; Price et al. 1985; Tyl 1989), or New Zealand rabbits exposed to ≤2,000 mg/kg/day by gavage on Gd 6–19 (Tyl et al. 1993).

Histopathologic changes in the liver were reported in one intermediate-duration study in mice (Melnick 1984), one chronic study in mice (NTP 1993), and one chronic study in rats (DePass et al. 1986a; Woodside 1982).

A centrilobular degenerative change occurred in the liver of male B6C3F1 mice exposed to ethylene glycol in estimated dietary doses of 6,450 or 12,900 mg/kg/day for 13 weeks (Melnick 1984; NTP 1993). This effect was characterized by the accumulation of a non-birefringent eosinophilic hyaline material in the cytoplasm of hepatocytes adjacent to or close to the central veins, and was not observed in females similarly exposed to ≤16,000 mg/kg/day (Melnick 1984; NTP 1993). No liver lesions or changes in liver weight were observed in CD-1 mice exposed to ≤2,500 mg/kg/day by gavage for 17 days (Harris et al. 1992) or ≤2,826 mg/kg/day in the diet for one or two generations (Bolon et al. 1997; Morrissey et al. 1989; NTP 1986), Wistar rats exposed to ≤2,000 mg/kg/day by gavage for 33 days (Schladt et al. 1998) or ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), F344 rats exposed to ≤10,000 mg/kg/day in the diet for 13 weeks (Melnick 1984), or Sprague-Dawley rats exposed to ≤5,744 mg/kg/day in drinking water for 90 days (Robinson et al. 1990). There were no effects on serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (AP), lactate dehydrogenase (LDH), cholesterol, and/or bilirubin in the 33- and 90-day studies in rats (Robinson et al. 1990; Schladt et al. 1998); clinical chemistry was not evaluated in the other intermediate-duration studies.

A 2-year study of ethylene glycol in B6C3F1 mice found significantly increased incidences of centrilobular hepatocyte hyaline degeneration in males at estimated dietary doses of 3,000 and 6,000 mg/kg/day (45 and 67% compared to 0% in controls) and females at 12,000 mg/kg/day (52% compared to 0% in controls) (NTP 1993). The lesions appeared similar to the hyaline degeneration in the 13-week study by the same investigators (Melnick 1984; NTP 1993) and consisted of cytoplasmic accumulations of non-birefringent, eosinophilic, granular to globular material resembling erythrocytes in size, shape, and tinctorial properties. Severity did not increase with dose. In another chronic study, CD-1 mice and F344 rats of both sexes were exposed to doses as high as 1,000 mg/kg/day in the diet for up to 2 years (DePass et al. 1986a; Woodside 1982). There were no effects on liver weight or histopathology in mice of either sex or male rats, or on serum parameters of liver function in male or female rats (not evaluated in mice). The female F344 rats had significantly increased incidences of slight liver fatty metamorphosis at ≥200 mg/kg/day and liver mononuclear cell infiltrates at 1,000 mg/kg/day; the incidences of slight fatty metamorphosis were 13% (34/256), 12% (16/129), 22% (27/125), and 27% (35/128) at 0, 40, 200, and 1,000 mg/kg/day, respectively. The biological significance of these minor

hepatic lesions is questionable because of the lack of effects on liver weight and liver function measures, even at the highest dose. A 2-year dietary study in Sprague-Dawley rats found no effects on liver weight or histopathology in males at ≤375 mg/kg/day (higher doses caused early mortality) or females at ≤3,000 mg/kg/day (Blood 1965).

Renal Effects. Adverse renal effects after ethylene glycol ingestion in humans can be observed during the third stage of ethylene glycol toxicity 24–72 hours after acute exposure (Davis et al. 1997; Hess et al. 2004). The hallmark of renal toxicity is the presence of birefringent calcium oxalate monohydrate crystals deposited in renal tubules and their presence in urine after ingestion of relatively high amounts of ethylene glycol (CDC 1987; Baum et al. 2000; Blakeley et al. 1993; Boyer et al. 2001; Chung and Tuso 1989; Davis et al. 1997; Factor and Lava 1987; Froberg et al. 2006; Godolphin et al. 1980; Hantson et al. 2002; Heckerling 1987; Huhn and Rosenberg 1995; Leth and Gregersen 2005; Olivero 1993; Parry and Wallach 1974; Rasic et al. 1999; Rothman et al. 1986; Siew et al. 1975a; Takayesu et al. 2006; Underwood and Bennett 1973). In addition to birefringent oxalate crystals in the tubular lumens, other signs of nephrotoxicity can include tubular cell degeneration and necrosis and tubular interstitial inflammation (Davis et al. 1997; Factor and Lava 1987; Froberg et al. 2006; Hantson et al. 2002; Rasic et al. 1999; Tobe et al. 2002). In a case study of a 38-year-old female who consumed 240 mL of antifreeze (3,454 mg ethylene glycol/kg/day), crystalluria was not present upon hospital admission (about 12 hours after ingestion). Within 5 hours, excretion of calcium oxalate dihydrate crystals was evident, although monohydrate crystals became the primary form in the urine thereafter (2–3 hours) (Jacobsen et al. 1988). In the course of ethylene glycol intoxication, serum creatinine (Factor and Lava 1987; Spillane et al. 1991) and serum blood urea nitrogen (BUN) (Chung and Tuso 1989; Factor and Lava 1987) levels may be increased. If untreated, the degree of renal damage caused by high doses of ethylene glycol progresses and leads to hematuria (Baum et al. 2000; CDC 1987; Davis et al. 1997; Rothman et al. 1986; Underwood and Bennett 1973), proteinuria (Davis et al. 1997; Rothman et al. 1986), decreased renal function, oliguria, anuria (Davis et al. 1997; Mallya et al. 1986; Parry and Wallach 1974; Spillane et al. 1991; Woolf et al. 1992; Zeiss et al. 1989), and ultimately renal failure (Chung and Tuso 1989; Gordon and Hunter 1982; Jacobsen et al. 1984; Johnson et al. 1999; Mallya et al. 1986; Takayesu et al. 2006). These changes in the kidney are linked to acute tubular necrosis (Factor and Lava 1987), but normal or near normal renal function can return with adequate supportive therapy (see Section 3.11, Methods for Reducing Toxic Effects).

In acute-duration studies in rats, kidney effects occurred at doses as low as 1,250 mg/kg/day by gavage and 1,400 mg/kg/day in drinking water. Renal tubular dilation and regeneration were increased in female

Sprague-Dawley rats that were exposed to 1,250 or 2,250 mg/kg/day ethylene glycol by gavage on Gd 6–20 and examined on postnatal day (Pnd) 1 (NTP 1988). Increased relative and absolute kidney weights, but no renal histopathology, occurred in female CD rats exposed to 2,500 mg/kg/day by gavage on Gd 6–15 and examined on Gd 21 (Neeper-Bradley 1990; Neeper-Bradley et al. 1995). In a 10-day drinking water systemic toxicity study, the incidence and severity of renal lesions were significantly increased in male Sprague-Dawley rats exposed to 2,615 and 5,270 mg/kg/day, but not at doses ≤1,343 mg/kg/day; lesions included tubular dilation, degeneration, necrosis, and intratubular calcium oxalate crystals (Robinson et al. 1990). Exposure to 1,400 mg/kg/day in the drinking water for 15–29 days caused renal tubular oxalate deposits, but apparently no nephrosis, in male Sprague-Dawley rats (Khan et al. 1993). Mice that were administered doses ≤1,000 mg/kg by gavage for 4 days had no histopathological changes in the kidneys (Hong et al. 1988). Renal toxicity occurred in female New Zealand white rabbits that were exposed to 2,000 mg/kg/day by gavage on Gd 6–19 and examined on Gd 30; lesions that included tubule dilatation and regeneration, epithelial necrosis, and intraluminal oxalate crystal deposition were increased at this dose level, but not at doses ≤1,000 mg/kg/day (Tyl et al. 1993).

Limited data are available on acute renal effects in other species. A single oral dose of 4,440 mg/kg in cats (Penumarthy and Oehme 1975) or 4,880 or 10,743 mg/kg in dogs (Beckett and Shields 1971; Grauer et al. 1987) caused kidney damage leading to oliguria and renal failure. Dogs administered a single dose of 10,600 mg/kg ethylene glycol as antifreeze or as reagent-grade ethylene glycol in feed exhibited polyuria, azotemia, and renal failure (Dial et al. 1994). Serum BUN and creatinine were not increased in two dogs given a single gavage dose of approximately 1,000 mg/kg/day, suggesting that renal function was not altered (Hewlett et al. 1989). Histopathological changes in the kidneys of dogs given a single 3,300 mg/kg gavage dose of ethylene glycol first appeared at 12 hours post-dosing; effects were most common in the proximal convoluted tubules and included interstitial edema, tubular dilation, and cellular degeneration and necrosis (Smith et al. 1990). Crystal formation was observed mainly within tubular lumina (most frequently in the proximal convoluted tubules), but generally not before 24 hours post-dosing. In male macaque monkeys exposed to ethylene glycol in drinking water, five of seven animals receiving doses ranging from 1,665 to 146,520 mg/kg/day for 6–13 days had calcium oxalate crystals and evidence of necrosis in the kidneys (Roberts and Seibold 1969).

The renal effects of intermediate-duration oral exposure to ethylene glycol are well characterized in a number of studies in rats and mice (Cruzan et al. 2004; Gaunt et al. 1974; Melnick 1984; NTP 1993; Robinson et al. 1990). As summarized below, the results of these studies indicate that renal toxicity

varies with sex, species, and strain, with males more sensitive than females, rats more sensitive than mice, and Wistar rats more sensitive than other strains of rats.

In a 90-day drinking water study with Sprague-Dawley rats (Robinson et al. 1990), incidences of renal lesions were significantly increased in males at ≥947 mg/kg/day and females at ≥3,087 mg/kg/day. Males showed a greater number and severity of lesions than females; lesions included tubular dilation and degeneration, acute and subacute inflammation, calcium oxalate crystals in tubules and pelvis epithelium, dilation of urinary pelvis, and hyperplasia and degeneration of pelvis epithelium. The male rats also had increases in relative kidney weight and serum creatinine at ≥947 mg/kg/day and BUN at 3,134 mg/kg/day. A 13-week dietary study in F344 rats (Melnick 1984) found renal effects that included increased relative kidney weight at ≥2,500 mg/kg/day in males and ≥5,000 mg/kg/day in females, increased BUN and serum creatinine in males at ≥2,500 mg/kg/day, and histopathology in males ≥2,500 mg/kg/day and females at 10,000 mg/kg/day. The lesions were more severe in the males (e.g., dilation, necrosis, fibrosis, and crystal deposition in renal tubules) than in the females (e.g., inflammation and vacuolation without crystal deposition). The NOAELs for renal toxicity in this study were 1,250 mg/kg/day in males and 2,500 mg/kg/day in females.

In a 16-week dietary study in Wistar rats (Gaunt et al. 1974), renal findings in males included no effects at 71 mg/kg/day, increased incidences of kidney lesions at ≥180 mg/kg/day, and oxalic acid crystals in urine, increased absolute kidney weight, increased urine volume, and decreased urine specific gravity at 715 mg/kg/day. The lesions ranged from degenerative changes in individual nephrons with occasional oxalate crystals to generalized tubular damage with heavy crystal deposition. At the 0, 35, 71, 180, and 715 mg/kg/day dose levels for the male rats in this study, the overall incidence of renal tubular damage was 0/15, 1/15, 1/15, 4/15, and 15/15, respectively. The only effect observed in females was a non-statistically significant increase in kidney lesions at 1,128 mg/kg/day, the highest tested dose. Limitations of this study include questionable animal care and dose levels that were not constant. Most of the rats showed evidence of respiratory infection (pneumonia) and infectious salivary adenitis, either of which could have confounded the results. The dose levels decreased throughout the exposure period because the amount of ethylene glycol in the diet was not adjusted for changing food consumption and body weight. For example, at the apparent LOAEL of 180 mg/kg/day, the rats were exposed to approximately 300 mg/kg/day for the first 2 weeks of the study; this level is above the threshold for renal toxicity in male Wistar rats shown in a 12-month study (Wilson et al. 2005). Further, the rats were housed in groups of five, such that consumption of individual rats among the groups likely varied.

In another 16-week dietary study (Cruzan et al. 2004), male Wistar and male F344 rats were exposed to dose levels of 0, 50, 150, 500, or 1,000 mg/kg/day. Effects included calcium oxalate crystals in the urine of both strains of rats at ≥150 mg/kg/day and increased absolute and relative kidney weights, increased water intake, increased urine volume, and decreased urine specific gravity at ≥500 mg/kg/day in Wistar rats and 1,000 mg/kg/day in F344 rats. No treatment-related increases in alpha 2-μ-globulin were observed in the kidneys of either strain of rats. No histological effects occurred in the kidneys of either strain of rats at 50 or 150 mg/kg/day. At higher doses, histopathological findings included calcium oxalate crystal deposition in the renal tubules with associated nephropathy in all Wistar rats (10/dose) at ≥500 mg/kg/day. Histological findings in the F344 rats included crystals in the tubules without nephropathy in 6/10 animals at 500 mg/kg/day, and crystal nephropathy in 1/10 animals at 500 mg/kg/day and 10/10 animals at 1,000 mg/kg/day. The severity of the crystal nephropathy in the Wistar rats at 500 mg/kg/day was approximately equivalent to that in the F344 rats at 1,000 mg/kg/day. Although the male Wistar rats were more sensitive than the male F344 rats, the LOAEL for kidney toxicity was 500 mg/kg/day in both strains. The NOAEL in both strains of rats is 150 mg/kg/day because the only effect at this dose, crystalluria, reflects a detoxification process and is not adverse in the absence of crystal deposition in the renal tubule epithelium and associated histopathology.

Information on the intermediate-duration renal toxicity of ethylene glycol is also available in mice. In a 13-week dietary study in B6C3F1 mice, effects were observed in the kidneys of males at ≥6,450 mg/kg/day (minimal to mild tubule dilation, cytoplasmic vacuolation, and regenerative hyperplasia, without tubular oxalate crystal deposition), with no effects on kidney histology or urinalysis in females at doses ≤16,000 mg/kg/day (Melnick 1984; NTP 1993). No histopathological changes were observed in the kidneys of male CD-1 mice that were administered doses as high as 2,500 mg/kg/day by gavage for 17 days (Harris et al. 1992). Kidney weight and histology were evaluated in F_0 and F_1 parental male and female CD-1 mice that were exposed to 2,826 mg/kg/day in the drinking water in a two-generation reproduction study (Bolon et al. 1997; Morrissey et al. 1989; NTP 1986). The exposure period of both generations included 14 weeks of cohabitation through gestation and lactation. Kidney lesions occurred in 60% of the F_0 male mice; the lesions included tubular degeneration, dilation, and regeneration, as well as a low incidence of oxalate crystal deposition (3/20 treated vs. 0/21 controls). There was no effect on kidney weight in the F_0 males or on kidney weight or histology in the F_0 females or F_1 males or females.

A 1-year study in rats (Wilson et al. 2005) and 2-year studies in rats (Blood 1965; DePass et al. 1986a) and mice (DePass et al. 1986a; NTP 1993) provide information on chronic renal toxicity of ethylene

glycol. Males were more sensitive than females, rats were more sensitive than mice, and Wistar rats appear to be the most sensitive strain to ethylene glycol nephrotoxicity.

Male Wistar rats were exposed to ethylene glycol in dietary doses of 0, 50, 150, 300, or 400 mg/kg/day for 12 months (Wilson et al. 2005). Decreased urinary pH and increased urinary oxalate crystals occurred at all dose levels; these effects were not considered adverse, but rather normal metabolic/physiological consequences of ethylene glycol exposure. Effects at ≥300 mg/kg/day included increased water consumption with corresponding increased urine volume and decreased urine specific gravity, increased absolute and relative kidney weights, and gross and histopathological changes in the kidneys and bladder. Gross pathology included calculi, dilatation, and hemorrhage in the bladder at ≥300 mg/kg/day and calculi and dilatation in the renal pelvis and ureter at 400 mg/kg/day. Renal histopathology occurred in the majority of animals at 300 mg/kg/day and in all animals at 400 mg/kg/day; lesions included crystalluria-related nephropathy, tubule dilatation, birefringent crystals (particularly in the pelvic fornix), pelvic dilatation, and transitional cell hyperplasia. Incidences of crystal nephropathy, the most prevalent lesion, were 0/14, 0/15, 0/15, 12/13, and 10/10 at 0, 50, 150, 300, and 400 mg/kg/day, respectively. Histopathological changes in the bladder occurred in the majority of animals at ≥300 mg/kg/day; the basic change was transitional cell hyperplasia, progressing to acute inflammation and hemorrhage in severe cases. Inflammation and hemorrhage of the bladder wall apparently contributed to mortality at ≥300 mg/kg/day. There were no treatment-related effects on renal clearance of oxalate or inulin. Kidney concentrations of glycolate and oxalate were unchanged at 50 and 150 mg/kg/day, but clear nonlinear increases in both of these metabolites occurred at ≥300 mg/kg/day, indicating that the accumulation of calcium oxalate in the kidneys correlated with the appearance of renal toxicity. A NOAEL of 150 mg/kg/day and a LOAEL of 300 mg/kg/day were identified in male Wistar rats based on histopathology in the kidneys (crystal nephropathy) and bladder (inflammation and hemorrhage).

In Sprague-Dawley rats that were fed ethylene glycol for 2 years, effects included increased water consumption, proteinuria, and mortality in males at ≥750 mg/kg/day and females at 3,000 mg/kg/day. Incidences of calcification (oxalate crystal deposition) in the kidneys were increased in both sexes at ≥750 mg/kg/day, and oxalate-containing calculi were increased in males at ≥750 mg/kg/day and females at 3,000 mg/kg/day. The incidences of oxalate crystal deposition in the males were 0/7, 0/12, 0/10, 4/10, 7/7, and 15/15 at 0, 75, 150, 375, 750 and 3,000 mg/kg/day; the increase at 375 mg/kg/day was not statistically significant. The report implied, but did not adequately document, that many of the animals with crystal deposition in the renal tubules also had degenerative changes (mainly cytoplasmic vacuolation) in the tubular epithelium. Due to the insufficiently reported histopathology findings and lack

of a clear (statistically significant) increase in oxalate crystal deposition at 375 mg/kg/day due to small numbers of animals, this study provides limited evidence that 375 mg/kg/day was a chronic LOAEL for kidney toxicity in male Sprague-Dawley rats.

F344 rats (130/sex/dose) were fed ethylene glycol in the dietary concentrations that yielded reported approximate doses of 0, 40, 200, or 1,000 mg/kg/day for up to 2 years (DePass et al. 1986a). No treatment-related or statistically significant changes occurred in the male rats at 40 or 200 mg/kg/day. A number of renal effects were observed in the 1,000 mg/kg/day males after 12 months (subsequent sacrifices at this dose level were precluded by early mortality), including increased water consumption and urine volume, decreased urine specific gravity and pH, increased urinary calcium oxalate crystals, increased BUN and serum creatinine. Increases in absolute and relative kidney weights and incidences of kidney lesions were increased at 1,000 mg/kg/day at 6 and 12 months. At 6 months, incidences of the following renal lesions were significantly increased in the 1,000 mg/kg/day males: calcium oxalate crystalluria, tubular hyperplasia, tubular dilation, and peritubular nephritis. All of the 1,000 mg/kg/day males that were sacrificed at 12 months had calcium oxalate crystalluria as well as multiple severe renal lesions that included tubular dilation, proteinosis and hyperplasia, glomerular shrinkage, and/or chronic interstitial nephritis. Most of the 1,000 mg/kg/day males that died during the study or were sacrificed when moribund had oxalate nephrosis, which was the primary cause of death, and hydronephrosis. The female rats were less sensitive to kidney toxicity than the males as shown by renal effects that were limited to increases in kidney weight and calcium oxalate crystals and uric acid crystals in the urine at 1,000 mg/kg/day; no histopathological changes occurred in the kidneys. A NOAEL of 200 mg/kg/day and serious LOAEL of 1,000 mg/kg/day were identified in male F344 rats based on kidney toxicity (oxalate nephrosis)-induced mortality.

CD-1 mice (80/sex/dose) were also fed ethylene glycol in the dietary concentrations that yielded reported approximate doses of 0, 40, 200, or 1,000 mg/kg/day for up to 2 years (DePass et al. 1986a). No treatment-related kidney histopathology occurred in either sex; water consumption, clinical chemistry, urinalysis, and organ weight were not evaluated as in the companion study in rats. B6C3F1 mice (60/sex/dose) were exposed to ethylene glycol in the diet for up to 2 years at estimated doses as high as 6,000 mg/kg/day in males and 12,000 mg/kg/day in females (NTP 1993). Histopathological evaluations of the kidneys showed effects that were limited to small numbers of oxalate-like crystals and/or calculi were noted in the renal tubules, urethrae, and urinary bladder in a few males at 6,000 mg/kg/day.

Endocrine Effects. Histological examinations of endocrine organs in acute-, intermediate- and chronic-duration studies of ethylene glycol showed no effects in rats or mice. As indicated in Table 3-2, the evaluations included the adrenals, pancreas, pituitary, thyroid, and/or parathyroids in Sprague-Dawley rats exposed to ≤7,327 mg/kg/day in drinking water for 10 days or ≤5,744 mg/kg/day in drinking water for 90 days (Robinson et al. 1990), Wistar rats exposed to ≤2,000 mg/kg/day by gavage for 4 weeks (Schladt et al. 1998), F344 rats exposed to ≤10,000 mg/kg/day in the diet for 13 weeks (Melnick 1984), Wistar rats exposed to ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), Sprague-Dawley rats exposed to ≤3,000 mg/kg/day in the diet for 2 years (Blood 1965), F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside 1982), B6C3F1 mice exposed to ≤250 mg/kg/day by gavage for 4 days (Hong et al. 1988), B6C3F1 mice exposed to ≤16,000 mg/kg/day in the diet for 13 weeks or ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside 1982). None of these studies included assessments of endocrine function.

Dermal Effects. Histological examinations of the skin showed no effects in Sprague-Dawley rats exposed to ≤7,327 mg/kg/day in drinking water for 10 days or ≤5,744 mg/kg/day in drinking water for 90 days (Robinson et al. 1990), F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside 1982), B6C3F1 mice exposed to ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside 1982).

Ocular Effects. Histological examinations of the eyes showed no effects in Wistar rats exposed to ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), or in F344 rats or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 1–2 years (DePass et al. 1986a; Woodside 1982).

Body Weight Effects. In an acute-duration study, male Sprague-Dawley rats exposed to 5,279 mg/kg/day ethylene glycol in the diet for 10 days experienced 13% body weight loss; no effect occurred in females at doses as high as 7,327 mg/kg/day (Robinson et al. 1990). Administration of ethylene glycol by gavage during gestation (Gd 6–15 or 6–20) caused 17–31% decreases in maternal body weight gain in CD and Sprague-Dawley rats exposed to 1,250–2,500 mg/kg/day and B6C3F1 mice exposed to 1,500 mg/kg/day (Marr et al. 1992; Neeper-Bradley 1990, Neeper-Bradley et al. 1995; NTP 1988; Price et al. 1985). Body weight gain corrected for gravid uterine weight was generally similar to controls, indicating that intrauterine loss was a significant contributor to the reduced maternal weight gain

during pregnancy. New Zealand white rabbits showed no changes in maternal body weight after gavage exposure to 2,000 mg/kg/day ethylene glycol on Gd 6–19 (Tyl et al. 1993).

In intermediate-duration studies, body weight gain was 9–30% lower than controls in Wistar rats exposed to 500 mg/kg/day in the diet for 16 weeks, Sprague-Dawley rats exposed to 750 mg/kg/day in the diet or 3,134 mg/kg/day in drinking water for 90–100 days, and F344 rats exposed to 2,500 mg/kg/day in the diet for 13 weeks (Blood 1965; Cruzan et al. 2004; Melnick 1984; NTP 1993; Robinson et al. 1990). No adverse effects on body weight occurred in CD-1 mice exposed to 2,500 mg/kg/day by gavage for 17 days (Harris et al. 1992) or B6C3F1 mice exposed 16,000 mg/kg/day in the diet for 13 weeks (Melnick 1984; NTP 1993).

Chronic (2-year) dietary studies of ethylene glycol found decreased body weight gain (15% less than controls) in male F344 rats at 1,000 mg/kg/day, but not in male F344 or Sprague-Dawley rats at 200–375 mg/kg/day (Blood 1965; DePass et al. 1986b); decreased body weight gain in female Sprague-Dawley rats at 3,000 mg/kg/day, but not in female Sprague-Dawley or F344 rats at 750–1,000 mg/kg/day (Blood 1965; DePass et al. 1986b); and no effects on body weight in CD-1 or B6C3F1 mice at 1,000–12,000 mg/kg/day (DePass et al. 1986a; Melnick 1984; NTP 1993; Woodside 1982). In a 12-month dietary study in male Wistar rats, body weight gain was reduced 8.4% on day 365 at 300 mg/kg/day and 31.3% on day 197 at 400 mg/kg/day (Wilson et al. 2005).

Metabolic Effects. One of the major adverse effects following acute oral exposure of humans to ethylene glycol involves metabolic changes. These changes occur as early as 12 hours after ethylene glycol exposure. Ethylene glycol intoxication is accompanied by metabolic acidosis which is manifested by decreased pH and bicarbonate content of serum and other bodily fluids caused by accumulation of excess glycolic acid (CDC 1987; Berger and Ayyar 1981; Blakeley et al. 1993; Cheng et al. 1987; Chung and Tuso 1989; Gordon and Hunter 1982; Heckerling 1987; Jacobsen et al. 1988; Parry and Wallach 1974; Pellegrino et al. 2006; Siew et al. 1975a; Spillane et al. 1991; Takayesu et al. 2006; Woolf et al. 1992; Zeiss et al. 1989). There is an inverse relationship between the decreasing plasma pH and increasing plasma glycolic acid concentrations (Clay and Murphy 1977). The normal level of bicarbonate of 24 mmol/L can be depleted in cases of severe ethylene glycol intoxication to reach concentrations as low as 2 mmol/L (Jacobsen et al. 1984). This decrease in base concentration indicates that a similar quantity of acid has to be present to achieve such a depletion. Glycolic acid is the only acidic metabolite present in such quantities. Humans highly intoxicated with ethylene glycol had glycolate concentrations of 17–29 and <1 mmol of glyoxalate and oxalate, respectively (Jacobsen et al. 1984). Similar

observations were made in animals. Metabolic acidosis due to glycolate accumulation was observed after acute oral exposure of dogs to 1,000–1,360 mg/kg of ethylene glycol (Hewlett et al. 1989) and of rats to 1,000 mg/kg (Marshall 1982). These results indicate that glycolic acid is the major toxic metabolite causing metabolic acidosis, and that its high serum levels are likely responsible for systemic toxicity observed after ethylene glycol exposure.

Other characteristic metabolic effects of ethylene glycol poisoning are increased serum anion gap, increased osmolal gap, and hypocalcemia. Serum anion gap is calculated from concentrations of sodium, chloride, and bicarbonate, is normally 12–16 mM, and is typically elevated after ethylene glycol ingestion due to increases in unmeasured metabolite anions (mainly glycolate) (Chung and Tuso 1989; Curtin et al. 1992; Davis et al. 1997; Factor and Lava 1987; Heckerling 1987; Hess et al. 2004; Jacobsen et al. 1984; Pellegrino et al. 2006; Spillane et al. 1991; Takayesu et al. 2006; Taylor et al. 1997; Walder and Tyler 1994; Zeiss et al. 1989). Osmolal gap represents the difference between the measured and calculated osmolalities and is also typically elevated during ethylene glycol intoxication (Baum et al. 2000; Boyer et al. 2001; Curtin et al. 1992; Davis et al. 1997; Taylor et al. 1997; Walder and Tyler 1994). The normal value for osmolal gap in humans is 10–15 mOsm/kg water (Egbert and Abraham 1999; Leth and Gregersen 2005; Scalley et al. 2002). Each 16 mM (100 mg/dL) increment in ethylene glycol concentration contributes to about 16 mOsm/kg water (Hess et al. 2004). Amounts of ethylene glycol causing these in humans effects have ranged from 1,628 to 12,840 mg/kg/day (Chung and Tuso 1989; Heckerling 1987; Spillane et al. 1991). Dogs receiving a single dose of 10,600 mg/kg ethylene glycol as antifreeze or as reagent grade ethylene glycol in feed exhibited metabolic acidosis and hyperosmolality (Dial et al. 1994). Although a high anion gap metabolic acidosis and an increased osmolal gap are common metabolic changes associated with ethylene glycol intoxication, clinically significant ingestions are possible without substantially elevating either of these parameters (Davis et al. 1997; Huhn and Rosenberg 1995; Moossavi et al. 2003; Pellegrino et al. 2006; Taylor et al. 1997). One case report presented a patient who developed recurrent severe anion gap metabolic acidosis with no osmolar gap consequent to episodic ethylene glycol ingestion (Moossavi et al. 2003). Hypocalcemia is occasionally reported and occurs when oxalate chelates with calcium ions forming insoluble calcium oxalate monohydrate crystals (Davis et al. 1997; Takayesu et al. 2006). This affects the overall ion concentration and can lead to an imbalance of divalent ion concentrations (Zeiss et al. 1989).

3.2.2.3 Immunological and Lymphoreticular Effects

No specific information was located regarding immunological and lymphoreticular effects in humans orally exposed to ethylene glycol. Moderate leukocytosis has been observed in some cases of acute oral poisoning (Davis et al. 1997; Parry and Wallach 1974; Reddy et al. 2007; Verrilli et al. 1987).

Immune responses were investigated in male CBA mice that were treated with a single 12,000 mg/kg dose of ethylene glycol by gavage (Zabrodskii and Germanchuk 2000; Zabrodskii et al. 2003). Exposure-related effects were observed on all tested end points; results included increased mortality from *Escherichia coli*-induced infection (peritonitis), decreased number of spleen colony-forming units, decreased numbers of antibody-producing cells in spleen to sheep erythrocytes (T cell-dependent antigen) and Vi-agglutinin (T cell-independent antigen), decreased activity of natural killer cells, decreased antibody-dependent cytotoxicity of splenocytes to sheep erythrocytes, and decreased delayed-type hypersensitivity to sheep erythrocytes.

No information is available on immune function in animals following intermediate- or chronic-duration exposure to ethylene glycol.

Histological examinations of immune and lymphoreticular system tissues in acute-, intermediate-, and chronic-duration studies of ethylene glycol showed no effects in rats or mice. As indicated in Table 3-2, the evaluations included spleen, lymph nodes, and/or thymus in Wistar rats exposed to ≤2,000 mg/kg/day by gavage for 4 weeks (Schladt et al. 1998), Sprague-Dawley rats exposed to ≤7,327 mg/kg/day in drinking water for 10 days or ≤5,744 mg/kg/day in drinking water for 90 days (Robinson et al. 1990), Wistar rats exposed to ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), F344 rats exposed to ≤10,000 mg/kg/day in the diet for 13 weeks (Melnick 1984), F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside 1982), Sprague-Dawley rats exposed to ≤3,000 mg/kg/day in the diet for 2 years (Blood 1965), B6C3F1 mice exposed to ≤250 mg/kg/day by gavage for 4 days (Hong et al. 1988), B6C3F1 mice exposed to ≤16,000 mg/kg/day in the diet for 13 weeks or ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside 1982).

Leukocyte counts were generally unaffected in the acute-, intermediate- and chronic-duration studies of ethylene glycol cited above. Exceptions included statistically significant decreased total leukocyte counts in female Sprague-Dawley rats exposed to 7,327 mg/kg/day for 10 days (34.8% less than controls) or

597–5,744 mg/kg/day for 90 days (30–50% less than controls) (Robinson et al. 1990), and significantly increased neutrophil count (38% higher than controls) in male F344 rats exposed to 1,000 mg/kg/day for 1 year (DePass et al. 1986a).

The highest NOAEL values and all reliable LOAEL values for immunological and lymphoreticular effects in rats after intermediate-duration oral exposure to ethylene glycol are reported in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

Adverse neurological reactions are among the first symptoms to appear in humans after ethylene glycol ingestion. These early neurotoxic effects are also the only symptoms attributed to unmetabolized ethylene glycol. Together with metabolic changes, they occur during the period of 30 minutes to 12 hours after exposure and are considered to be part of the first stage in ethylene glycol intoxication (Davis et al. 1997; Hess et al. 2004; Robinson and McCoy 1989; Vale 1979). In cases of acute intoxication, in which a large amount of ethylene glycol is ingested over a very short time period, there is a progression of neurological manifestations which, if not treated, may lead to generalized seizures and coma (Chung and Tuso 1989; Froberg et al. 2006; Hantson et al. 2002; Jobard et al. 1996; Leth and Gregersen 2005; Olivero 1993; Siew et al. 1975a; Takayesu et al. 2006; Zeiss et al. 1989). Ataxia, slurred speech, confusion, and somnolence are common during the initial phase of ethylene glycol intoxication (Boyer et al. 2001; Buell et al. 1998; CDC 1987; Parry and Wallach 1974; Reddy et al. 2007; Takayesu et al. 2006; Tobe et al. 2002; Zeiss et al. 1989), as are irritation, restlessness, and disorientation (Cheng et al. 1987; Factor and Lava 1987; Gordon and Hunter 1982; Rothman et al. 1986; Woolf et al. 1992), and semiconsciousness and unresponsiveness (Blakeley et al. 1993; Chung and Tuso 1989; Heckerling 1987; Spillane et al. 1991; Underwood and Bennett 1973). In an unusual case of ethylene glycol poisoning, initial neurological symptoms of confusion, slurred speech, and somnolence were followed by the development of deafness, dysphagia, and dysarthria after 7 days and full paralysis after 12 days (Tobe et al. 2002). The patient was completely unresponsive to any stimulus, all brainstem reflexes were absent, clinical neurophysiological examination showed a severe axonal polyneuropathy, and sural nerve biopsy findings showed severe axonal degeneration and oxalate deposits.

Cerebral edema and crystalline deposits of calcium oxalate in the walls of small blood vessels in the brain were found at autopsy in people who died after acute ethylene glycol ingestion (Friedman et al. 1962; Froberg et al. 2006; Hantson et al. 2002; Leth and Gregersen 2005; Zeiss et al. 1989). In one case of fatal

ethylene glycol poisoning, the development of rapid cerebral edema was documented by computed tomography (CT) scan and was accompanied by definitive evidence of calcium oxalate crystals within walls of central nervous system blood vessels, with associated inflammation and edema (Froberg et al. 2006). In a case of nonfatal poisoning, CT showed a marked edema and leukoencephalopathy of both cerebral hemispheres on the tenth day after ingestion that conformed to a toxic or inflammatory encephalopathy (Chung and Tuso 1989). The cerebral edema decreased drastically during the next 20 days, although some residual temporal lobe dysfunctions and auditory verbal agnosis developed. Unusual brain findings in other cases of ethylene glycol poisoning included bilateral pallidal hemorrhage (Capparros-Lefebvre et al. 2005) and acute hemorrhagic necrosis of the basal ganglia that resulted in acute Parkinson's syndrome (Reddy et al. 2007).

Effects on cranial nerves appear late (generally 5–20 days post-ingestion), are relatively rare, and according to some investigators constitute a fourth, late cerebral phase in ethylene glycol intoxication (Chung and Tuso 1989; Gardner et al. 2004; Lewis et al. 1997). Clinical manifestations of the cranial neuropathy commonly involve lower motor neurons of the facial and bulbar nerves and are reversible over many months. In one case, facial paralysis and bilateral optic nerve dysfunction were noted in a patient 13 days after ethylene glycol ingestion (Factor and Lava 1987). Delay in treatment may have contributed to the development of these symptoms; the patient was not treated until 3 days after ingesting ethylene glycol. Severe cranial nerve dysfunction including nerves VII, IX, and X was noted in a man 5 days after he ingested 12,840 mg/kg of ethylene glycol (Spillane et al. 1991). In another case of ethylene glycol poisoning, bilateral facial paralysis and peripheral neurosensory hearing loss were observed in a patient 18 days after ingestion of 2,714 mg/kg of ethylene glycol; this effect was only partially reversible (Mallya et al. 1986). Bilateral paralysis of cranial nerve VII, as well as bilateral dysfunction of cranial nerves II, V, VIII, IX, X, and XII, developed in a woman 10–11 days after dyspnea, nausea, confusion, metabolic acidosis, and other initial effects of acute toxic ingestion; the cranial neuropathy resolved over a period of 11 months (Lewis et al. 1997). In another case, a CT scan 3 days after ingestion of approximately 24,000 mg/kg of ethylene glycol showed low density areas in the basal ganglia, thalami, midbrain, and upper pons (Morgan et al. 2000). Clinical findings reflected dysfunction in all the areas of hypodensity on the CT scan and included gaze-directed nystagmus with bilateral sixth cranial nerve palsies that developed 7 days following ingestion. Although a magnetic resonance imaging (MRI) of the brain 24 days after ingestion revealed bilateral putamen necrosis, the patient's neurologic sequelae resolved over the following 4 months.

Information on the neurotoxicity of ethylene glycol in orally-exposed animals is essentially limited to results of clinical observations and histopathology evaluations, as summarized below; tests of neurobehavioral function have not been conducted.

Ataxia, convulsions, and/or central nervous system depression occurred in dogs given a single nonlethal dose of 4,880–10,743 mg/kg ethylene glycol in food (Beckett and Shields 1971; Dial et al. 1994; Grauer et al. 1987). Clinical signs of neurotoxicity were observed prior to death in cats given a single 4,440 mg/kg dose by gavage; effects included abnormal gait, loss of reflexes, central nervous system depression (symptoms not specified), and convulsions (Penumarthy and Oehme 1975). In F344 rats, a single gavage dose of 4,000 mg/kg ethylene glycol caused ataxia and coma prior to death (Clark et al. 1979). There were no clinical signs of neurotoxicity or histopathological changes in brain or sciatic nerve tissue in Sprague-Dawley rats exposed to 7,327 mg/kg/day in drinking water for 10 days (Robinson et al. 1990).

Calcium oxalate crystals were observed in the brain of male F344 rats exposed to 5,000 mg/kg/day ethylene glycol in the diet for 13 weeks (Melnick 1984). The authors reported no significant tissue response to the crystals or clinical signs of neurotoxicity, and the effect did not occur in males at 2,500 mg/kg/day or in females at doses as high as 10,000 mg/kg/day (highest tested dose). As indicated above, a similar effect occurred in humans who died from acute ethylene glycol poisoning (Friedman et al. 1962; Froberg et al. 2006; Hantson et al. 2002; Leth and Gregersen 2005; Zeiss et al. 1989).

There were no clinical signs of neurotoxicity or histopathological changes in nervous system tissue in other intermediate- or chronic-duration studies of ethylene glycol in rats or mice. As indicated in Table 3-2, the histopathological evaluations included brain, spinal cord, and/or sciatic nerve in Wistar rats exposed to ≤2,000 mg/kg/day by gavage for 4 weeks (Schladt et al. 1998), Sprague-Dawley rats exposed ≤5,744 mg/kg/day in drinking water for 90 days (Robinson et al. 1990), Wistar rats exposed to ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside 1982), Sprague-Dawley rats exposed to ≤3,000 mg/kg/day in the diet for 2 years (Blood 1965), B6C3F1 mice exposed to ≤16,000 mg/kg/day in the diet for 13 weeks or ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside 1982).

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category for ethylene glycol after oral exposure are reported in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to ethylene glycol.

Ethylene glycol treatment did not affect gestational length in CD rats given 2,500 mg/kg/day ethylene glycol by gavage administration on Gd 6–15 (Marr et al. 1992). Testis and uterine weights and histopathology were not affected in B6C3F1 mice treated with ethylene glycol for 4 consecutive days at doses up to 250 mg/kg/day and evaluated 1 day later (Hong et al. 1988).

Reproductive function after intermediate-duration oral exposure to ethylene glycol has been tested in three multi-generation studies (one in rats and two in mice) and several shorter studies (15–20 days in rats and mice). In these studies, effects on fertility, fetal viability, and male reproductive organs were observed in mice, while the only effect in rats was an increase in gestational duration.

In a continuous breeding study in which CD-1 mice were exposed to ethylene glycol in drinking water, there were slight, but statistically significant, reductions in the number of litters per fertile pair and in the mean number of live pups per litter at 1,640 mg/kg/day of the F_0 generation (Lamb et al. 1985; Morrissey et al. 1989). In mated F_1 offspring, there were no differences between high-dose and control groups in fertility or live litter size. In a follow-up to this study using the same overall protocol, the number of live female pups and the number of live pups per litter were significantly reduced at 2,826 mg/kg/day in the F_0 generation of mice, but there were no effects on reproductive parameters in the F_1 generation (Morrissey et al. 1989; NTP 1986). Ethylene glycol treatment did not affect mating or fertility rate in either generation, or in F_0 parents used in a crossover mating trial (20/sex high dose mice mated to 20/sex controls) (Morrissey et al. 1989; NTP 1986). Female Swiss CD-1 mice given ethylene glycol at 2,500 mg/kg/day by gavage for 20 days including a 5-day mating period (days 8–12) with concurrently treated males had significantly fewer live and significantly more dead implants as well as complete resorption of two of six litters (Harris et al. 1992). Total number of implantation sites was not affected.

In a three-generation reproductive toxicity and dominant lethality study in F344 rats exposed via the diet, no treatment-related effects on fertility index, gestation index, gestation survival index, or days from first

mating to litter were observed in any generation at doses up to 1,000 mg/kg/day (DePass et al. 1986b). Number of implantation sites was not affected at doses up to 2,250 mg/kg/day in timed pregnant CD rats given gavage doses of ethylene glycol on Gd 6–20 (NTP 1988).

Effects on the male reproductive system, manifested mainly as changes in sperm parameters and testicular lesions, occurred in CD-1 mice exposed to ethylene glycol in drinking water in a continuous breeding study (Morrissey et al. 1989; NTP 1986). Sperm number was decreased in F_1 males at doses as low as 897 mg/kg/day, but the effect did not exhibit a dose-response relationship. Sperm motility, absolute seminal vesicle weight, relative epididymis weight, and absolute and relative testis weights were significantly reduced in F_1 males at ≥1,798 mg/kg/day. Effects at 2,826 mg/kg/day included increased incidence of abnormal sperm and decreased sperm motility in F_0 males, and increased incidence and severity of testicular and epididymal lesions in F_0 males (seminiferous tubule degeneration, loss of spermatozoa, spermatic, spermatogonia and spermocytes, vacuolization of epithelial cells, and interstitial cell hyperplasia) and F_1 males (seminiferous tubule degeneration and intersitital cell hyperplasia). An Expert Panel review of this study (NTP-CERHR 2004) concluded that, while this study provided some evidence for testicular changes and effects on sperm parameters, the high incidence of testicular effects in the control animals limited the ability to draw conclusions about the relationship of this effect to treatment. Ethylene glycol treatment did not affect testis weight, epididymis weight, sperm count, sperm motility, or microscopic findings in testis or epididymis of male Swiss CD-1 mice treated by gavage at doses up to 2,500 mg/kg/day for 17 days (Harris et al. 1992). In a three-generation reproductive toxicity study in F344 rats exposed via the diet, no treatment-related effects on histopathology of male reproductive organs were observed in any generation at doses up to 1,000 mg/kg/day (DePass et al. 1986b).

Limited information is available on reproductive effects of ethylene glycol in female animals. In a continuous breeding study in which CD-1 mice were exposed to ethylene glycol in drinking water, there were no effects on estrous cyclicity, or weights or microscopic findings in the ovary, uterus, or vagina in either generation (Morrissey et al. 1989; NTP 1986). Bolon et al. (1997) sectioned the ovaries of 10 female mice/dose from the NTP continuous breeding study (Morrissey et al. 1989; NTP 1986) and evaluated differential follicular counts (small, growing, and antral), observing no difference in follicular counts attributable to ethylene glycol treatment. In a three-generation reproductive toxicity study in F344 rats exposed via the diet, no treatment-related effects on gestation index or on histopathology of female reproductive organs were observed in any generation at doses up to 1,000 mg/kg/day (DePass et

al. 1986b). Average gestational length was significantly longer in pregnant CD rats given gavage doses of 1,250 and 2,250 mg/kg/day on Gd 6–20 (NTP 1988).

In summary, oral exposure to ethylene glycol can affect fertility and fetal viability at high doses (≥1,640 mg/kg/day in mice and ≥2,500 mg/kg/day in rats), and there is suggestive evidence for an effect on male reproductive function in mice at doses ≥897 mg/kg/day and on gestational duration in rats exposed to ≥1,250 mg/kg/day. The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category for ethylene glycol after oral exposure are reported in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to ethylene glycol.

The developmental toxicity of ethylene glycol has been assessed in several acute-duration studies using mice, rats, and rabbits. Available studies indicate that malformations, especially skeletal malformations occur in both mice and rats exposed during gestation; mice are apparently more sensitive to the developmental effects of ethylene glycol. Other evidence of embyrotoxicity in laboratory animals exposed to ethylene glycol exposure includes reduction in fetal body weight.

Studies in laboratory animals indicate that acute-duration exposure to high doses of ethylene glycol during gestation can affect fetal viability and postimplantation loss. Of 37 pregnancies in CD-1 mice receiving gavage doses of 11,090 mg/kg/day on Gd 7–14, only 15 litters had at least 1 live-born pup, compared with 29/29 control pregnancies (Schuler et al. 1984). In the treated group, there was a significant decrease in the number of live pups per litter and a significant increase in the number of dead pups per litter at birth. Ethylene glycol treatment (up to 2,500 mg/kg/day) of mated female Swiss CD-1 mice during Gd 8–14 did not affect the number of females littering, number of implantation sites, or number of live pups at birth (Harris et al. 1992). The percentage of postimplantation loss per litter was significantly increased in CD rats treated by gavage on Gd 6–15 with 5,000 mg/kg/day and the number of live fetuses per litter was reduced at both 2,500 and 5,000 mg/kg/day (Price et al. 1985). There were no significant effects of treatment on total implantations, preimplantation loss, or litter size when pregnant F344 rats were given ethylene glycol in the diet at target doses of up to 1,000 mg/kg/day on Gd 6–15 (Maronpot et al. 1983). In New Zealand white rabbits given gavage doses of up to 2,000 mg/kg/day

ethylene glycol on Gd 6–19, the numbers of pre- or post-implantation losses were not increased in any treatment group, although 42% of the high-dose dams died prior to sacrifice (Tyl et al. 1993).

The most sensitive indicator of the developmental toxicity of acute oral exposure to ethylene glycol appears to be an increased incidence of malformations, primarily skeletal malformations, in both mice and rats. Available data suggest that malformations appear in mice at lower gavage doses than those that cause malformations in rats. The incidence of skeletal and other malformations was increased at all doses when groups of at least 20 timed-pregnant CD-1 mice were treated by gavage with doses of 0, 750, 1,500, or 3,000 mg/kg/day ethylene glycol on Gd 6–15 (Price et al. 1985). The percentages of malformed fetuses per litter and of litters with one or more malformed fetuses were significantly increased at all doses. The malformations primarily consisted of neural tube, craniofacial, and axial skeletal defects, with skeletal defects comprising the majority. Minimal maternal toxicity (decreased body weight gain and liver weight) was observed at doses of 1,500 mg/kg/day and higher. In a later study aimed at identifying a NOAEL for developmental effects in CD-1 mice, an increased incidence of malformations was observed at doses of ≥500 mg/kg/day by gavage on Gd 6–15 (Neeper-Bradley et al. 1995; Tyl 1989). The incidence of total malformations per litter (external, visceral, and skeletal) was significantly increased at both 500 and 1,500 mg/kg/day. There was a significant increase in the incidence of two skeletal malformations (fused ribs or thoracic arches) in the 1,500 mg/kg/day group, and the incidences of 23 skeletal variations were also increased in this group. One of these variations (bilateral extra rib 14) was also significantly increased at 500 mg/kg/day. The incidence of individual external or visceral malformations was not significantly increased in any treatment group relative to the vehicle control; however, exencephaly (a malformation observed by Price et al. [1985] at higher doses) was observed in two fetuses in the 500 mg/kg/day group and in three fetuses of the 1,500 mg/kg/day dose group (Neeper-Bradley et al. 1995; Tyl 1989). No evidence of maternal toxicity was observed at any dose in this study.

In rats, gestational doses of at least 1,000 mg/kg/day by gavage were required to induce malformations. The number of litters with malformations, number of malformed fetuses per litter, and number of litters with skeletal malformations were increased at doses of ≥2,500 mg/kg/day in CD rats treated by gavage on Gd 6–15 (Price et al. 1985). At 5,000 mg/kg/day, the number of litters with fetuses having external and visceral malformations (primarily neural tube and craniofacial defects) was also increased. The authors reported a significant increase in visceral malformations at 1,250 mg/kg/day, but NTP-CERHR (2004) classified the observed effects (hydroureter, hydronephrosis, and great artery anomalies) as variations rather than malformations, and characterized the 1,250 mg/kg/day dose as a developmental NOAEL. In later studies using lower doses, the incidence of litters with fetuses having two skeletal malformations

(missing thoracic arch and missing ribs) was increased in CD rats exposed by gavage to ≥1,000 mg/kg/day on Gd 6–15 (Neeper-Bradley 1990; Neeper-Bradley et al. 1995). The incidences of total skeletal malformations and skeletal variations (delayed ossification) were also significantly increased at ≥1,000 mg/kg/day. The highest dose (2,500 mg/kg/day) was associated with increased frequencies of visceral and external malformations, including gastroschisis, hydrocephaly, lateral ventricle dilation, umbilical hernia, and atelectasis (Neeper-Bradley 1990; Neeper-Bradley et al. 1995). No evidence of maternal toxicity was observed at doses as high as 1,000 mg/kg/day in this study.

Reduced ossification of the vertebral centra was observed in the 1,000 mg/kg/day dose group when F344 rats were given ethylene glycol in the diet on Gd 6–15 (Maronpot et al. 1983). However, an Expert Panel Review of this study (NTP-CERHR 2004) identified the high dose (1,000 mg/kg/day) as a developmental NOAEL, noting the lack of other findings (change in body weights or consistent alterations in skeletal integrity) to support the authors' suggestion that reduced ossification was indicative of minimal embryotoxicity. No maternal toxicity was observed (Maronpot et al. 1983).

When developmental effects were assessed over the course of postnatal development, there were significant reductions in percentages of total ossification, sternebral ossification, and vertebral centra ossification on Gd 20 and at all postnatal evaluations up to ppd 63 in CD rats given 2,500 mg/kg/day ethylene glycol by gavage administration on Gd 6–15 (Marr et al. 1992). The percent of malformed fetuses per litter was also significantly increased at all scheduled sacrifice times other than ppd 63. The percent of litters with skeletal malformations (primarily skeletal axial defects) was 100% in the treated litters at all time points other than ppd 63 (Marr et al. 1992). Maternal toxicity (reduced weight gain) was also observed at this dose (2,500 mg/kg/day) (Marr et al. 1992).

Fetal body weight and postnatal weight gain are also sensitive indicators of embyrotoxicity after ethylene glycol treatment, albeit at higher doses than skeletal and other malformations in mice. Average fetal body weight per litter was significantly decreased in CD-1 mice treated by gavage with doses of ≥750 mg/kg/day ethylene glycol on Gd 6–15 (Price et al. 1985), although in a later study in the same strain, average fetal body weight per litter was reduced only at gavage doses of 1,500 mg/kg/day on Gd 6–15 (Neeper-Bradley et al. 1995; Tyl 1989). Ethylene glycol treatment of Swiss Crl:CD-1 mice by gavage at 2,500 mg/kg/day on Gd 8–14 resulted in decreased pup body weight on ppd 1 and 4 (Harris et al. 1992). There were significant decreases in pup birth weight as well as pup weight gain and survival over ppd 1–3 in CD-1 mice given 11,090 mg/kg/day ethylene glycol by gavage on Gd 7–14 (Schuler et al. 1984). In CD rats, fetal body weight was significantly decreased at gavage doses of ≥1,000 mg/kg/day

(Neeper-Bradley 1990; Neeper-Bradley et al. 1995) or ≥2,500 mg/kg/day (Price et al. 1985) on Gd 6–15. In another study, when CD rats were given 2,500 mg/kg/day ethylene glycol by gavage on Gd 6–15, mean pup body weight per litter was significantly lower than controls on ppd 1, but was not different from controls at later postnatal evaluations (Marr et al. 1992).

A single study in rabbits suggests that, in this species, developmental toxicity may not occur at acute doses that are not maternally toxic. There was significant mortality (42%) at 2,000 mg/kg/day in New Zealand white rabbits given gavage doses of ethylene glycol on Gd 6–19 (Tyl et al. 1993). One doe aborted and three delivered early at this dose, but there was no evidence of developmental toxicity in live litters at any exposure level.

One gestational exposure study, a 20-day exposure study, and three multi-generation reproductive toxicity studies with some developmental toxicity end points are available to assess developmental effects of intermediate-duration exposure to ethylene glycol. As with acute exposure, intermediate-duration exposure was associated with malformations, decreases in pup body weight, and effects on fetal viability in both rats and mice.

In a continuous breeding study, skeletal evaluation of F_1 offspring of CD-1 mice exposed to 1,640 mg/kg/day exhibiting facial anomalies indicated a pattern of skeletal defects affecting the skull, sternebrae, ribs, and vertebrae in both sexes (Lamb et al. 1985; Morrissey et al. 1989). Bone morphology, but not histology, differed in the affected mice. In a follow-up study using a similar design, similar facial abnormalities were observed in F_1 mice treated with 1,798 or 2,826 mg/kg/day (Morrissey et al. 1989; NTP 1986). There was a significant increase in the incidence of skeletal malformations (rib, sternebral, and vertebral defects) at 2,250 mg/kg/day ethylene glycol when CD rats were given gavage doses on Gd 6–20; the authors noted that 9/443 pups in this group also had hydrocephaly (NTP 1988). In this study, evidence of maternal effects (increased gestational length and renal lesions) occurred at a lower dose (1,250 mg/kg/day) (NTP 1988).

Average pup weight was reduced in the F_0 generation at 1,640 mg/kg/day in a continuous breeding study in CD-1 mice (Lamb et al. 1985; Morrissey et al. 1989), but female pup body weights and pup weight adjusted for litter size were significantly reduced at doses as low as 897 mg/kg/day in both F_0 and F_1 generations in a follow-up study (Morrissey et al. 1989; NTP 1986). In a crossover mating trial using the F_0 parents, pup body weight were reduced when 2,826 mg/kg/day females were mated to control males (Morrissey et al. 1989; NTP 1986). In studies on the postnatal effects of intrauterine exposure,

average pup body weights were not affected on ppd 4, 14, or 21 in F344 rats exposed via the diet to doses up to 1,000 mg/kg/day in a three-generation reproductive toxicity study (DePass et al. 1986b); however, pup body weights were lower than controls at various times between ppd 1 and 22 when CD rats were given gavage doses of 2,250 mg/kg/day ethylene glycol on Gd 6–20 (NTP 1988). Postnatal decreases in kidney weight (1,250 and 2,250 mg/kg/day groups) and brain weight (2,250 mg/kg/day group), without corresponding histopathology changes, have also been observed in the offspring of rats exposed in utero (Gd 6–20) to ethylene glycol (NTP 1988).

Dams exposed to 2,500 mg/kg/day ethylene glycol had significantly fewer live implants and significantly more dead implants as well as complete resorption of two of six litters in a study exposing female Swiss CD-1 mice by gavage at doses up to 2,500 mg/kg/day for 20 days including a period of mating to concurrently treated males (Harris et al. 1992). In a study of postnatal effects of intrauterine exposure, cumulative pup mortality was significantly higher on ppd 1 and 4 in CD rats exposed to gavage doses of 2,250 mg/kg/day ethylene glycol on Gd 6–20 (NTP 1988).

In summary, there is a substantial database demonstrating developmental toxicity at ethylene glycol doses that are not maternally toxic. Mice appear to be more vulnerable to the developmental effects of ethylene glycol, responding at lower doses than rats. Skeletal and other malformations appear to be the most sensitive indicators of toxicity, with effects observed at bolus doses of ≥500 mg/kg/day in mice and ≥1,000 mg/kg/day in rats. Effects on fetal body weight and fetal viability occur at higher doses. The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category for ethylene glycol after oral exposure are reported in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

No studies were located regarding carcinogenicity in humans after oral exposure to ethylene glycol.

Comprehensive histopathological evaluations showed no evidence of carcinogenicity in Sprague-Dawley rats exposed to ≤3,000 mg/kg/day in the diet for 2 years (Blood 1965), F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside 1982), B6C3F1 mice exposed to ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside 1982).

3.2.3 Dermal Exposure

Dermal exposure, through activities such as changing antifreeze, is the most likely route of exposure to ethylene glycol, but dermal exposure is not likely to lead to toxic effects.

3.2.3.1 Death

No studies were located regarding death in humans or animals after dermal exposure to ethylene glycol.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, body weight, or metabolic effects in humans or respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, endocrine, or metabolic effects in animals after dermal exposure to ethylene glycol.

The highest NOAEL values for systemic effects in each species and duration category for ethylene glycol after dermal exposure are reported in Table 3-3.

Hepatic Effects. Maternal liver weight was not affected in female CD-1 mice exposed to 3,549 mg/kg/day ethylene glycol for 6 hours/day on Gd 6–15 by occluded dermal application; liver histopathology was not evaluated (Tyl 1988b; Tyl et al. 1995c).

Renal Effects. Evaluations of maternal kidney weight and kidney histopathology showed no effects female CD-1 mice exposed to 3,549 mg/kg/day ethylene glycol for 6 hours/day on Gd 6–15 by occluded dermal (Tyl 1988b; Tyl et al. 1995c).

Dermal Effects. Minimal skin irritation occurred in New Zealand white rabbits 24–72 hours after application of 0.5 mL (550 mg) ethylene glycol to shaved skin (Clark et al. 1979). Female mice exposed to 3,549 mg/kg/day ethylene glycol for 6 hours/day on Gd 6–15 by occluded dermal application showed no dermal effects (Tyl 1988b; Tyl et al. 1995c).

Ocular Effects. Ocular instillation of 0.1 mL (110 mg) ethylene glycol caused mild eye irritation in rabbits; transient conjunctival redness was the most prominent response (Clark et al. 1979).

Table 3-3 Levels of Significant Exposure to Ethylene Glycol - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL		Reference Chemical Form	Comments
				Less Serious	Serious		
ACUTE EXPOSURE							
Systemic							
Mouse (CD-1)	10 d Gd 6-15 6 hr/d	Hepatic	3549 F mg/kg			Tyl 1988b; Tyl et al. 1995c	Hepatic NOAEL is for liver weight; liver histopathology not evaluated. Renal NOAEL is for kidney weight and histopathology.
		Renal	3549 F mg/kg/day				
		Dermal	3549 F mg/kg				
		Bd Wt	3549 F mg/kg				
Rabbit (New Zealand)	once	Dermal	550 F mg			Clark et al. 1979	
Rabbit (New Zealand)	once	Ocular	110 F mg			Clark et al. 1979	
Reproductive							
Mouse (CD-1)	10 d Gd 6-15 6 hr/d		3549 mg/kg/day			Tyl 1988b; Tyl et al. 1995c	
Developmental							
Mouse (CD-1)	10 d Gd 6-15 6 hr/d		3549 mg/kg/day			Tyl 1988b; Tyl et al. 1995c	

Bd Wt = body weight; d = day(s); F = Female; Gd = gestational day; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory

Body Weight Effects. Maternal CD-1 mice showed no changes in body weight after exposure to 3,549 mg/kg/day ethylene glycol for 6 hours/day on Gd 6–15 by occluded dermal application (Tyl 1988b; Tyl et al. 1995c).

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans or animals after dermal exposure to ethylene glycol.

3.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans or animals after dermal exposure to ethylene glycol.

3.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after dermal exposure to ethylene glycol.

Reproductive function has not been assessed in animals exposed dermally to ethylene glycol. In an acute-duration developmental toxicity study, pregnant CD-1 mice exposed to ethylene glycol at doses up to 3,549 mg/kg on Gd 6–15 by occluded dermal application exhibited no adverse effects on the number of resorptions or number of total, viable, or nonviable implants per litter (Tyl 1988b; Tyl et al. 1995c).

The highest NOAEL value for reproductive effects in mice for the acute-duration category for ethylene glycol after dermal exposure is reported in Table 3-3.

3.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans after dermal exposure to ethylene glycol.

In the single acute-duration dermal study of developmental toxicity, groups of 30 pregnant CD-1 mice were treated with 6-hour daily dermal exposures to ethylene glycol by occluded cutaneous application on Gd 6–15 (Tyl et al. 1995c). The authors estimated the applied doses to be 404, 1,677, or

3,549 mg/kg/day. Neither implantations nor resorptions were affected by treatment. The incidence of malformations (individual or total external, visceral, or skeletal) was not significantly increased in any ethylene glycol dermal treatment group, but was significantly increased in the positive (ethylene glycol by gavage) control group. There were significant increases in the incidence of litters with two skeletal variations (reduced ossification of the skull bone and phalanges) at 3,549 mg/kg/day.

The highest NOAEL value for developmental effects in mice for the acute-duration category for ethylene glycol after dermal exposure is reported in Table 3-3.

3.2.3.7 Cancer

No studies were located regarding cancer effects in humans or animals after dermal exposure to ethylene glycol.

3.3 GENOTOXICITY

Studies in humans have not addressed the genotoxic effects of ethylene glycol. However, available *in vivo* and *in vitro* laboratory studies provide consistently negative genotoxicity results for ethylene glycol.

In F344 rats that received oral doses of 40, 200, and 1,000 mg/kg/day for three generations, there were no dominant lethal mutations (DePass et al. 1986b).

Table 3-4 summarizes information from available *in vitro* studies. Ethylene glycol produced consistently negative results in the Ames assay for reverse mutation in several strains of *Salmonella typhimurium* (Clark et al. 1979; Kubo et al. 2002; McCann et al. 1975; Pfeiffer and Dunkelberg 1980; Zeiger et al. 1987). No growth inhibition due to DNA damage by ethylene glycol was observed in a battery of *E. coli* repair-deficient strains (McCarroll et al. 1981). Negative results were also obtained in two sets of studies when ethylene glycol was tested for gene mutation in the yeast, *Schizosaccharomyces pombe* (Abbondandolo et al. 1980), and for aneuploidy induction in the fungus, *Neurospora crassa* (Griffiths 1979, 1981). Ethylene glycol did not induce gene mutations in L5178Y mouse lymphoma cells (McGregor et al. 1991) deoxyribonucleic acid (DNA) strand breaks in primary rat hepatocytes (Storer et al. 1996).

Two recent genotoxicity assays have been developed and tested against results of standard genotoxicity assays for a variety of chemicals, including ethylene glycol. Ethylene glycol did not induce gene mutation

Table 3-4. Genotoxicity of Ethylene Glycol *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium (Ames test in strains TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	–	–	Clark et al. 1979
S. typhimurium (Ames test in strains TA97, TA98, TA100)	Gene mutation	–	–	Kubo et al. 2002
S. typhimurium (Ames test in strains TA98, TA100, TA1535, TA1537)	Gene mutation	–	No data	McCann et al. 1975
S. typhimurium (Ames test in strains TA98, TA100, TA1535, TA1537)	Gene mutation	No data	–	Pfeiffer and Dunkelberg 1980
S. typhimurium (Ames test in strains TA97, TA98, TA100, TA1535, TA1537)	Gene mutation	–	–	Zeiger et al. 1987
S. typhimurium (Forward mutation in TA100 derived 5-fluorouracil-resistant strain)	Gene mutation	–	–	Miller et al. 2005
Escherichia coli	DNA damage	–	–	McCarroll et al. 1981
Eukaryotic organisms:				
Yeast:				
Schizosaccharomyces pombe	Gene mutation	–	–	Abbondandolo et al. 1980
Fungi:				
Neurospora crassa	Aneuploidy induction	No data	–	Griffiths 1979, 1981
Mammalian cells:				
Mouse (L5178Y cells)	Gene mutation	–	–	McGregor et al. 1991
Rat (hepatocytes)	DNA breaks	No data	–	Storer et al. 1996
Human (GreenScreen HC assay using TK6 cell line)	DNA damage	No data	–	Hastwell et al. 2006

— = negative result

in a low volume, high-throughput forward mutation assay using a TA100-derived 5-fluorouracil-resistant strain of *S. typhimurium* (Miller et al. 2005). Ethylene glycol did not induce DNA damage in a high-throughput (GreenScreen HC) assay that links the regulation of the human GADD45a gene to the production of Green Fluorescent Protein (Hastwell et al. 2006).

3.4 TOXICOKINETICS

Ethylene glycol is quickly and extensively absorbed through the gastrointestinal tract. Limited information suggests that it is also absorbed through the respiratory tract; dermal absorption is apparently slow. Following absorption, ethylene glycol is distributed throughout the body according to total body water. In most mammalian species, including humans, ethylene glycol is initially metabolized by alcohol dehydrogenase to form glycolaldehyde, which is rapidly converted to glycolic acid and glyoxal by aldehyde oxidase and aldehyde dehydrogenase. These metabolites are oxidized to glyoxylate; glyoxylate may be further metabolized to formic acid, oxalic acid, and glycine. Breakdown of both glycine and formic acid can generate CO_2, which is one of the major elimination products of ethylene glycol. In addition to exhaled CO_2, ethylene glycol is eliminated in the urine as both the parent compound and glycolic acid. Elimination of ethylene glycol from the plasma in both humans and laboratory animals is rapid after oral exposure; elimination half-lives are in the range of 1–4 hours in most species tested.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Limited information suggests that ethylene glycol is absorbed across the human respiratory tract. Inhalation of aerosolized ethylene glycol from an automobile heater resulted in a blood ethylene glycol level of 28 mg/dL (Wezorek et al. 1995). When two male volunteers inhaled ^{13}C-labeled ethylene glycol vapor (estimated to result in inhaled doses of 0.96 and 1.51 mg/kg body weight), labeled ethylene glycol and glycolic acid were detected in the plasma and urine, providing evidence of absorption (Carstens et al. 2003). No increase, as compared to controls, in serum or urinary levels of ethylene glycol was recorded in men exposed to 17–49 mg/m^3 ethylene glycol aerosol for 30 days (Wills et al. 1974). However, in a review of this study, NTP-CERHR (2004) noted that the analytical techniques used for serum and urine analysis of ethylene glycol may not have been adequately sensitive to detect a difference.

In rats exposed nose-only for 30 minutes to ^{14}C-labeled ethylene glycol vapor (32 mg/mg^3) or for 17 minutes to ^{14}C-ethylene glycol aerosol (184 mg/m^3) on gallium oxide particles, between 75 and 85% of

the deposited radiolabel was found to be distributed throughout the body regardless of the form of the compound (Marshall and Cheng 1983). In its review, NTP-CERHR (2004) estimated that 60–90% of the inhaled dose was absorbed in this study.

3.4.1.2 Oral Exposure

Indirect evidence of the oral absorption of ethylene glycol by humans is available from case reports of clinical symptoms in persons accidentally or intentionally ingesting ethylene glycol (Hewlett et al. 1986; Jacobsen et al. 1988; Robinson and McCoy 1989; Walton 1978). Direct evidence of absorption comes from measurements of the plasma concentration of ethylene glycol after acute poisoning (studies report levels ranging from 1 to 40 mmol/L; Hewlett et al. 1986; Jacobsen et al. 1988); however, because the amounts ingested in these events were generally unknown, and blood analyses were performed at varying times after exposure, the data are not useful for quantifying the rate or extent of oral absorption in humans.

In rats, ingested ethylene glycol is rapidly absorbed, usually reaching peak blood levels within 1 hour after single gavage doses of 150–20,000 mg/kg (Frantz et al. 1989, 1996a, 1996c; Pottenger et al. 2001; Winek et al. 1978). Absorption is equally rapid in other species, with peak blood levels reached within 1–3 hours after gavage exposure in mice, monkeys, and dogs (Frantz et al. 1991, 1996a, 1996b; Grauer et al. 1987; Hewlett et al. 1989; McChesney et al. 1971). In addition, available data suggest near complete absorption of ingested ethylene glycol in both rats and mice. After gavage doses of 10 and 1,000 mg/kg ^{14}C-ethylene glycol to both rats and mice, the areas under the ethylene glycol plasma concentration versus time curves were comparable to those observed with equivalent intravenous doses (Frantz et al. 1989, 1991, 1996a).

Results of one study suggest that pregnancy does not alter absorption kinetics in rats dosed once on Gd 10. The time course and peak plasma levels of ethylene glycol did not differ between pregnant and nonpregnant rats given 10 or 2,500 mg/kg by gavage (Pottenger et al. 2001).

3.4.1.3 Dermal Exposure

There are no data quantifying dermal absorption of ethylene glycol after *in vivo* human exposure.

In vivo studies with rats and mice suggest incomplete dermal absorption of ethylene glycol. In rats exposed to occluded dermal doses (applied to thoracic dorsal area after light clipping of fur) of 10 or

1,000 mg/kg ^{14}C-ethylene glycol or 1,000 mg/kg of a 50% solution of ^{14}C-ethylene glycol, measurement of radioactivity recovered in body tissues, excreta, and exhaled air suggested apparent absorption of 26–32% of the administered dose (Frantz et al. 1989, 1996b). In the same study, similar treatment of mice with 100 or 1,000 mg/kg ^{14}C-ethylene glycol or 1,000 mg/kg of 50% ^{14}C-ethylene glycol lead to apparent absorption estimates ranging from 60 to 84% (Frantz et al. 1991, 1996b).

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Data on the tissue distribution of ethylene glycol in humans exposed via inhalation are not available. Based on plasma concentrations of ethylene glycol in two volunteers who inhaled doses of 0.96 and 1.51 mg/kg, Carstens et al. (2003) estimated the volumes of distribution (Vd) to be 0.78 and 0.91 L/kg.

In rats inhaling ^{14}C-ethylene glycol vapor (32 mg/m^3 for 30 minutes) or aerosol (184 mg/mg^3 for 17 minutes), radioactivity was distributed quickly (Marshall and Cheng 1983). The authors estimated that 60% of ethylene glycol (in either form) was deposited in the respiratory tract, primarily in the nasal cavity, and 75–80% of the initial body burden was distributed throughout the body upon sacrifice immediately after exposure (Marshall and Cheng 1983).

3.4.2.2 Oral Exposure

After oral exposure, ethylene glycol is distributed throughout the body according to total body water. The apparent volume of distribution of ethylene glycol in humans exposed orally has been estimated to be 0.54–0.56 L/kg based on clearance data in two patients poisoned with ethylene glycol (Jacobsen et al. 1988). The ratios of urine to plasma ethylene glycol concentration in one patient were similar to those of ethanol, indicating distribution with total body water.

In rats, 6–22% of the radioactivity derived from single oral doses of 10 and 1,000 mg/kg of ^{14}C-ethylene glycol were recovered from body tissues and carcass (combined) 96 hours after exposure (Frantz et al. 1989, 1996b, 1996c); mice retained similar percentages (3–11%) in their tissues following single oral doses across the same range (Frantz et al. 1991, 1996b). Among the few tissues examined individually (liver, kidney, brain, fat, and lung), the highest radioactivity was found in the liver of both species (see Table 3-5). In two rhesus monkeys given single oral doses of about 1,100 mg/kg unlabeled ethylene glycol, the parent compound was evenly distributed throughout the tissues 4 hours after exposure; tissue

Table 3-5. Distribution of Radioactivity (Percent of Administered Dose) in Tissues 96 Hours After Oral or Percutaneous Exposure to ^{14}C-Ethylene Glycol

	Oral		Percutaneous		
	10 mg/kg	1,000 mg/kg	10 mg/kg (undiluted)	1,000 mg/kg (undiluted)	1,000 mg/kg (50% solution)
Female rats					
Liver	2.43±0.44[a]	0.74±0.22	0.69±0.38	0.47±0.07	0.48±0.11
Kidney	0.20±0.02	0.10±0.01	0.06±0.03	0.03±0.00	0.03±0.00
Brain	0.05±0.01	0.02±0.01	0.01±0.01	0.01±0.00	0.01±0.00
Lung	0.14±0.01	0.06±0.01	0.04±0.02	0.02±0.00	0.02±0.00
Carcass	9.58±1.24	4.20±0.13	2.24±1.25	1.39±0.18	1.70±0.35
Pelt	2.78±0.34	1.21±0.16	5.09±4.28	5.24±2.53	5.84±1.04
Male rats					
Liver	2.29±0.39	0.88± 0.17	1.06±0.10	0.53±0.09	0.39±0.09
Kidney	0.24±0.03	0.88±0.17	0.09±0.01	0.05± 0.01	0.03±0.01
Brain	0.04±0.00	0.01±0.00	0.02±0.00	0.01±0.00	0.01±0.00
Lung	0.13±0.02	0.06±0.01	0.04±0.01	0.02±0.01	0.01±0.00
Testis	0.12±0.02	0.06±0.01	0.05±0.00	0.03± 0.01	0.01±0.00
Carcass	11.60±1.09	4.97±0.52	3.39±0.72	2.10±0.48	1.34±0.20
Pelt	7.28±0.99	3.67±1.21	3.36±0.26	6.94±3.90	5.18±1.18
Female mice					
Liver	3.02±0.95	0.63±0.16	0.60±0.06	0.59±0.16	0.58±0.07
Kidney	0.30±0.13	0.11±0.01	0.06±0.01	0.07±0.02	0.06±0.02
Brain	0.09±0.04	0.05±0.00	0.02±0.01	0.02±0.01	0.02±0.01
Lung	0.11±0.05	0.05±0.01	0.02±0.00	0.28±0.01	0.02±0.01
Carcass/pelt	7.21±2.01	2.56±0.28	14.77±3.85	7.59±2.03	10.10±0.79

[a]Mean±standard deviation from groups of four animals.

Source: Frantz et al. 1996b, 1996c

to plasma concentration ratios ranged from 0.85 to 1.91 for the brain, heart, kidney, gastrointestinal tract, liver, lung, muscle, pancreas, and spleen (McChesney et al. 1971).

3.4.2.3 Dermal Exposure

Frantz et al. (1989, 1996b, 1996c) evaluated the distribution of a 10 or 1,000 mg/kg dose of undiluted ^{14}C-ethylene glycol or a 1,000 mg/kg dose of 50% aqueous ^{14}C-ethylene glycol applied dermally to rats under an occlusive bandage. Table 3-6 shows the disposition of radioactivity. The pelt contained the highest radioactivity (5–6% of applied dose) among the tissues examined (liver, kidney, brain, lung, pelt, and remaining carcass) (Frantz et al. 1989, 1996b). Similar experiments in mice at doses of 100 or 1,000 mg/kg undiluted ^{14}C-ethylene glycol or 1,000 mg/kg 50% aqueous solution of ^{14}C-ethylene glycol showed the highest radioactivity in the carcass and pelt combined (~8–15%) (Frantz et al. 1991, 1996b).

3.4.3 Metabolism

The metabolic pathway for ethylene glycol is shown in Figure 3-3. Asterisks indicate rate-limiting steps in the pathway. The metabolism of ethylene glycol was reviewed by NTP-CERHR (2004). Ethylene glycol is first converted to glycolaldehyde by nicotinamide adenine dinucleotide (NAD)-dependent alcohol dehydrogenase. Subsequent reduction of NAD results in the formation of lactic acid from pyruvate. Glycolaldehyde has a brief half-life and is rapidly converted to glycolic acid (and to a lesser extent glyoxal) by aldehyde dehydrogenase and aldehyde oxidase, respectively. Glycolic acid is oxidized to glyoxylic acid by glycolic acid oxidase or lactic dehydrogenase. Glyoxylic acid can be metabolized to formate, glycine, or malate, all of which may be further broken down to generate respiratory CO_2, or to oxalic acid, which is excreted in the urine. In excess, oxalic acid can form calcium oxalate crystals. Rate-limiting steps in the metabolism of ethylene glycol include the initial formation of glycolaldehyde and the conversion of glycolic acid to glyoxylic acid, both of which are saturable processes.

Both glycolic and oxalic acids are found in the blood and urine of unexposed individuals as a result of normal metabolism of proteins and carbohydrates (NTP-CERHR 2004). The ranges of background levels of glycolic acid are 0.0044–0.0329 mM (plasma) and 0.075–0.790 mM (urine) (NTP-CERHR 2004). For oxalic acid, the background ranges are 0.002–0.0233 mM (plasma) and 0.086–0.444 mM (urine) (NTP-CERHR 2004).

In two volunteers who inhaled ^{14}C-ethylene glycol for 4 hours, glycolic acid concentrations in the plasma peaked at about 4–5 hours after the commencement of exposure (Carstens et al. 2003). About 1% of the

Table 3-6. Radioactivity (Percent of Administered Dose) in Excreta 96 Hours After 6-Hour Occluded Percutaneous Exposure to ^{14}C-Ethylene Glycol

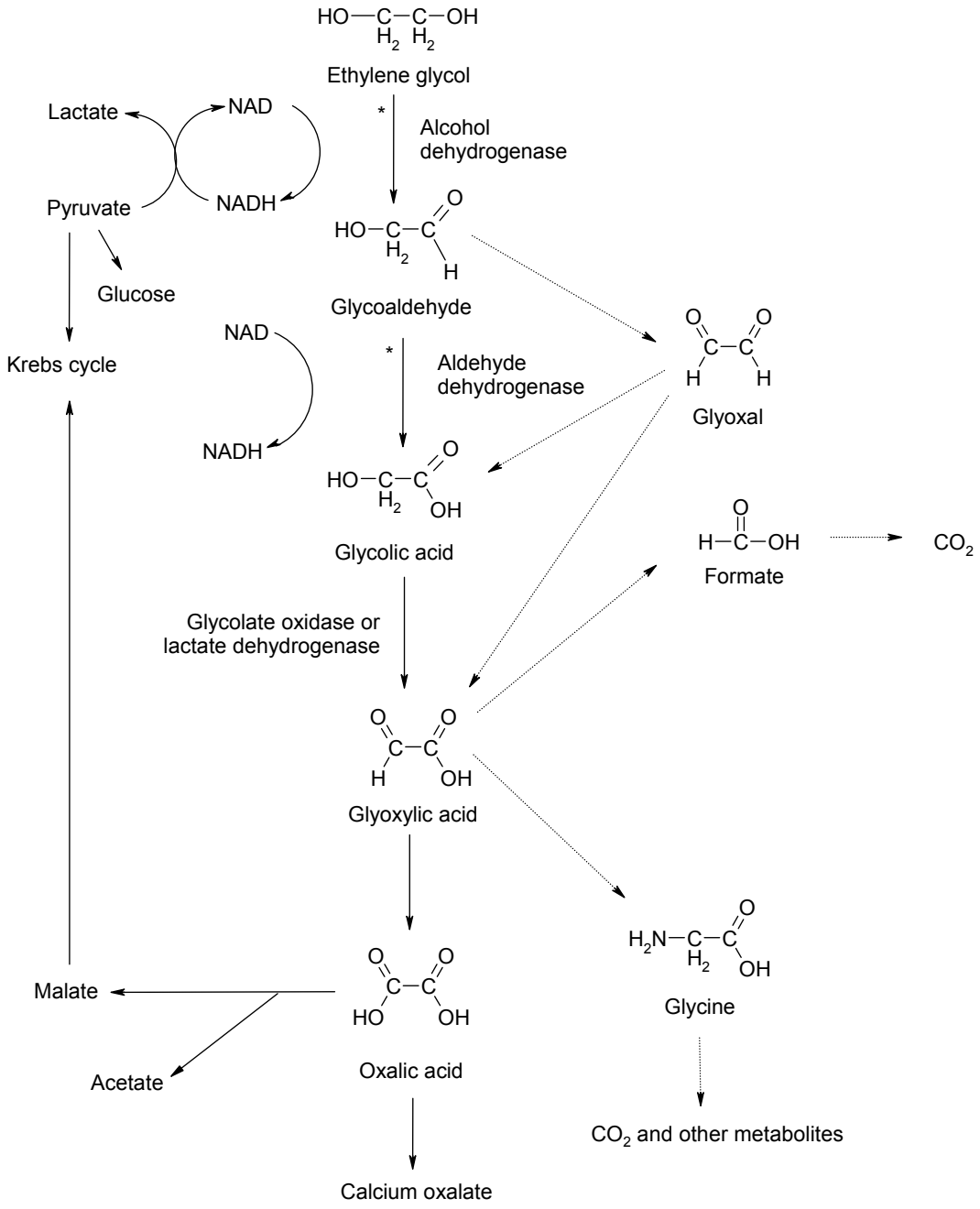
	Undiluted		50% Aqueous solution
	10 mg/kg	1,000 mg/kg	1,000 mg/kg
Female rats			
Expired CO_2	13.1±7.2[a]	11.4±1.8	9.3±1.9
Urine	8.2±4.7	7.6±0.9	4.4±0.7
Feces	1.1±0.7	1.4±1.0	0.5±0.2
Tissues	6.0±4.6	5.8±2.5	6.4±1.2
Carcass	2.2±1.2	1.4±0.2	1.7±0.4
Occlusion materials and skin	10.9±3.3	55.6±2.2	56.8±4.2
Total recovery[b]	42.4±19.2	84.7±2.0	82.7±4.5
Male rats			
Expired CO_2	14.0±1.1	14.4±4.4	5.9±2.0
Urine	6.7±0.8	8.1±2.5	4.6±1.4
Feces	1.1±0.5	0.6±0.1	0.6±0.1
Tissues	4.7±0.4	7.6±3.9	5.6±1.2
Carcass	3.4±0.7	2.1±0.5	1.3±0.2
Occlusion materials and skin	17.4±3.1	48.2±9.4	59.2±6.7
Total recovery[c]	48.9±5.0	83.8±4.1	81.2±3.8
Female mice			
Expired CO_2	10.4±2.0	15.9±4.8	10.4±3.2
Exhaled VOCs	34.0±5.9	33.1±8.6	20.9±2.6
Urine	6.7±2.8	12.3±5.6	5.4±1.6
Feces	6.1±2.4	7.5±1.7	6.4±2.4
Tissues	0.7±0.1	0.7±0.2	0.7±0.1
Carcass	14.8±3.8	7.6±2.0	10.0±0.8
Occlusion materials and skin	23.0±4.6	4.8±4.2	25.4±15.3
Total recovery[d]	99.5±9.4	89.2±3.9	85.2±6.7

[a]Mean±standard deviation from groups of four animals
[b]Cage wash accounted for 0.9–4%
[c]Cage wash accounted for 2–4%
[d]Cage wash accounted for 4–7%

CO_2 = carbon dioxide; VOCs = volatile organic compounds

Source: Frantz et al. 1996b, 1996c

Figure 3-3. Metabolic Pathway for Ethylene Glycol



*Rate-limiting steps

Source: NTP-CERHR 2004

estimated dose of 0.96–1.51 mg/kg was excreted in the urine as glycolic acid, and 0.08–0.28% was excreted as oxalic acid over 30 hours. Expired CO_2 was not measured in this study.

Plasma glycolate levels of 12.2 and 15.4 mmol/L were reported upon hospital admission of an infant female and an adult male, respectively, with ethylene glycol intoxication after oral exposure (Hewlett et al. 1986). The infant survived, while the adult male died, probably due to delayed treatment. In a case report of six adult male patients with ethylene glycol intoxication, one of whom died, plasma glycolate levels on admission ranged from 17.0 to 29.3 mmol/L (Jacobsen et al. 1984).

Glycolic acid was the major metabolite in the plasma of male rats exposed orally to single gavage doses of 10, 100, or 1,000 mg/kg ^{14}C-ethylene glycol (Frantz et al. 1989, 1996c). During the first 12 hours after dosing, no oxalate was detected in the plasma at any dose, but glyoxylate and glyoxal, as well as trace amounts of glycoaldehyde, were detected in plasma samples from the lower dose groups (100 and 1,000 mg/kg). In the 10 mg/kg group, glyoxylate levels exceeded glycolate levels throughout the 12 hours postdosing.

In rats given 2,000 mg/kg ethylene glycol by gavage, peak plasma levels of ethylene glycol occurred 2 hours after administration, while plasma glycolate levels peaked 6 hours after dosing (Hewlett et al. 1989). Dogs receiving 1,000 or 1,360 mg/kg ethylene glycol by gavage exhibited peak plasma ethylene glycol levels at 2 hours after dosing and peak plasma glycolate levels 4 hours after dosing (Hewlett et al. 1989).

Carney et al. (2001) showed that enzymes metabolizing glycolate are more quickly saturated with bolus subcutaneous dosing than with slow, continuous dosing (i.e., via infusion pump), leading to higher peak plasma glycolate levels (~3–10-fold higher) with bolus dosing. This study demonstrates the importance of dose rate on the dose at which glycolic acid metabolism is saturated.

In vivo studies in rats and mice show increasing urinary excretion (as a percent of dose) of glycolic acid and other metabolites with increasing dose, probably corresponding to saturation of glycolic acid metabolism. In male rats exposed to 10 or 1,000 mg/kg ^{14}C-ethylene glycol via gavage, most of the urinary radioactivity consisted of unmetabolized ethylene glycol at the low dose (>90% of urinary radioactivity) (Frantz et al. 1996c). At the high dose, glycolic acid comprised 25% of the urinary radioactivity in the first 12 hours after dosing and 37% during the following 12 hours. Oxalic acid was detected in the urine sample from the 12–24-hour interval, accounting for 7.4% of the urinary

radioactivity in that sample (Frantz et al. 1989, 1996c). The urinary metabolite pattern was similar in female rats treated at these doses (Frantz et al. 1989, 1996b). In mice exposed orally to 10, 100, 200, 400, or 1,000 mg/kg ^{14}C-ethylene glycol, the proportion of urinary radioactivity excreted as ethylene glycol or glycolate also varied with dose, with increasing excretion of glycolate at higher doses (Frantz et al. 1991, 1996b). No other metabolites were detected in mouse urine after oral doses.

In both rats and mice exposed percutaneously to 10 or 1,000 mg/kg (rats) or 100 or 1,000 mg/kg (mice) undiluted ^{14}C ethylene glycol, or 1,000 mg/kg 50% aqueous solution, most of the urinary radioactivity was excreted as ethylene glycol (Frantz et al. 1989, 1991, 1996b, 1996c). In rats, 87–100% of the urinary radioactivity apparently was parent compound, regardless of dermal dose. In mice, glycolate represented a greater proportion of urinary radioactivity after exposure to 1,000 mg/kg of 50% ethylene glycol (up to 20% in the 12–24-hour interval).

Urinary excretion of ethylene glycol and glycolate accounted for 20.7 and 4.5% (respectively) of a 2,000 mg/kg dose of ethylene glycol in rats over the course of 24 hours post-dosing (Hewlett et al. 1989). Male Porton rats receiving 1,000–1,110 mg/kg ethylene glycol in the drinking water for 21 days had urinary oxalate levels equivalent to 1.18% conversion of ethylene glycol to oxalate; rats given diets supplemented with 30 or 60% sucrose (administered to evaluate the role of carbohydrates in calcium oxalate crystal formation) excreted oxalate equivalent to 1.11 and 0.7% conversion of ethylene glycol, respectively (Rofe et al. 1986).

Corley and Soelberg (2005) evaluated levels of ethylene glycol, glycolic acid, and oxalate in the blood, urine, and/or kidneys of male Wistar rats treated with ethylene glycol doses up to 400 mg/kg/day for 12 months. In the kidneys, levels of glycolic acid and oxalate did not differ from controls at doses up to 150 mg/kg/day, but at ≥300 mg/kg/day, concentrations were substantially higher than controls and increased with dose. Levels of oxalate in the kidneys of rats exposed to 400 mg/kg/day averaged 18,800 µg/g, with large interindividual variability. Similar results were observed with glycolic acid in blood, with no difference from control at doses up to 150 mg/kg/day but 3.3-fold higher concentrations in rats dosed with 300 mg/kg/day. Ethylene glycol was excreted in the urine at levels proportional to dose across all doses tested. A disproportionate increase in urinary excretion of glycolic acid occurred at 300 mg/kg/day, suggesting that the metabolism of glycolic acid is saturated at doses between 150 and 300 mg/kg/day in male Wistar rats. Oxalate concentrations in blood and urine were similar across all doses, reflecting the low solubility of this compound in physiological fluids (Corley and Soelberg 2005).

In vitro data suggest that humans may metabolize glycolic acid at a higher rate than rats do. *In vitro* metabolism studies using liver homogenates from female humans and Sprague-Dawley rats generated Vmax/Km estimates of 2.16 and 0.68 L/hour/g for humans and rats, respectively (Corley et al. 2005a). Booth et al. (2004) reported Vmax/Km values of 0.43 and 0.28 L/hour/g (humans and rats, respectively) from a study using human and rat liver slices.

3.4.4 Elimination and Excretion

Little information is available on the elimination of ethylene glycol in humans; most of the elimination data are from humans accidentally poisoned and given therapeutic treatments to reduce the metabolism of ethylene glycol or extract it from the blood. In laboratory animals treated with ^{14}C-ethylene glycol, the primary routes of excretion are exhaled air and urine, regardless of the route of exposure. After oral exposure, saturation of metabolic pathways at higher doses leads to a shift in excretory pattern, with greater urinary excretion (and corresponding decreases in elimination via expired air) at higher doses.

3.4.4.1 Inhalation Exposure

Carstens et al. (2003) evaluated the urinary excretion of ethylene glycol and its two primary metabolites (glycolic and oxalic acids) in two volunteers who inhaled ^{14}C-ethylene glycol at doses estimated by the authors to be 0.96 and 1.51 mg/kg. Urinary excretion of ^{14}C-ethylene glycol up to 30 hours after exposure constituted 6.4–9.3% of the inhaled dose, while ^{13}C-glycolic acid and ^{13}C-oxalic acid together comprised 1–2% of the inhaled dose. However, the dose estimates are highly uncertain, as they were calculated by estimating the loss of ^{14}C-ethylene glycol from an inhalation vessel in which the compound was "warmed". Air concentrations to which the volunteers were exposed were not measured, and the warming temperature was not reported. The authors reported that ^{14}C-ethylene glycol was not detectable in exhaled air, but did not assess expiration of $^{14}CO_2$.

In rats, the major route of elimination for inhaled ethylene glycol is expiration of CO_2. Rats exposed for 30 minutes to ^{14}C-ethylene glycol vapor (32 mg/m^3) or for 17 minutes to ^{14}C-ethylene glycol aerosol (184 mg/m^3) excreted 63% (over 4 days) and 75% (over 6 days), respectively, of the initial body burden as $^{14}CO_2$ (Marshall and Cheng 1983). Urinary excretion constituted 20 and 12% of the initial body burden after vapor and aerosol exposures, respectively, while fecal excretion was 3% and 1% (Marshall and Cheng 1983).

3.4.4.2 Oral Exposure

In untreated adults, the serum half-life has been estimated to be between 3.0 and 8.4 hours (Jacobsen et al. 1988; Peterson et al. 1981). In a series of 19 patients, the mean half-life of ethylene glycol during a period without ADH inhibitor treatment and without dialysis was 8.6 hours, while elimination after fomepizole therapy was slower, with a half-life of 19.7 hours (Sivilotti et al. 2000). In another study, the half-life of ethylene glycol during fomepizole therapy was 11–14.75 hours (Baud et al. 1988). The approximate serum half-life of ethylene glycol was 1.5–3.0 hours in a child treated with hemodialysis and mannitol therapy (Rothman et al. 1986), and 2.7 hours in an adult male during hemodialysis and intravenous ethanol treatment (Cheng et al. 1987). As these half-lives indicate, the rate of ethylene glycol elimination is greatly increased by dialysis.

In laboratory animals, the elimination half-lives for ethylene glycol in the plasma have been estimated at 1.4–2.5 hours in rats given between 10 and 2,000 mg/kg; 0.3–1.1 hours in mice given doses between 10 and 1,000 mg/kg; 3.5 hours in dogs given 1,000–1,360 mg/kg; and 2.7–3.7 hours in monkeys given 1,110 mg/kg (Frantz et al. 1989, 1991, 1996a, 1996c; Hewlett et al. 1989; McChesney et al. 1971). The plasma elimination half-life for ethylene glycol was similar (1.4–1.7 hours) in pregnant rats treated with single oral doses of 10 or 2,500 mg/kg on Gd 10 (Pottenger et al. 2001). Data from intravenous administration of ethylene glycol show similar elimination half-lives (Frantz et al. 1989, 1991, 1996a, 1996c; Martis et al. 1982).

Frantz et al. (1989, 1991, 1996b, 1996c) treated rats and mice with single oral doses of ^{14}C-ethylene glycol between 10 and 1,000 mg/kg and measured radioactivity in exhaled air, excreta, tissues, and carcass up to 96 hours after exposure. Table 3-7 shows the disposition of radioactivity. In male and female rats, the major excretory routes were via CO_2 exhalation (27–48% of the administered radioactivity) and urinary elimination (21–43%); 2–4% was excreted via the feces (Frantz et al. 1989, 1996b, 1996c). Female mice showed a similar profile when exposed over the same dose range, exhaling 22–55% of the dose as CO_2 and 3–11% as exhaled volatile organic compounds (VOCs), while excreting 24–56% in the urine and 5–16% in the feces (Frantz et al. 1991, 1996b). In mice, the majority of the exhaled radioactivity was eliminated during the first 12 hours after dosing (Frantz et al. 1991, 1996b). Both mice and rats exhibited a dose-dependent shift in excretory patterns, as shown in the data in Table 3-7. An increase in urinary excretion of radioactivity was evident between 10 and 100 mg/kg in female mice, between 10 and 400 mg/kg in female rats, and between 800 and 1,000 mg/kg in male rats. In its review of these data, NTP-CERHR (2004) noted that the increased urinary excretion of radioactivity

Table 3-7. Radioactivity (Percent of Administered Dose) in Excreta 96 Hours After Oral or Exposure to ^{14}C-Ethylene Glycol

	10 mg/kg	400 mg/kg	600 mg/kg	800 mg/kg	1,000 mg/kg
Female rats					
Expired CO_2	47.9±0.8[a]	39.4±1.0	32.8±3.4	32.1±2.3	28.2±2.1
Urine	25.5±3.8	38.0±7.6	37.1±17.0	41.0±5.1	35.0±13.2
Feces	2.8±0.8	NA	NA	NA	4.4±3.4
Tissues	5.7±0.5	NA	NA	NA	2.2±0.4
Carcass	9.6±1.2	NA	NA	NA	4.2±0.1
Total recovery[b]	91.8±3.4	NA	NA	NA	83.3±3.5
Male rats					
Expired CO_2	42.2±0.3	38.77±0.85	34.00±1.5	30.12±2.13	27.3±1.4
Urine	26.2±2.1	20.52±9.44	25.78±10.25	26.69±6.41	42.7±7.1
Feces	2.9±0.6	NA	NA	NA	2.4±0.8
Tissues	10.1±1.1	NA	NA	NA	4.8±2.2
Carcass	11.6±1.1	NA	NA	NA	5.0±0.5
Total recovery[c]	93.4±2.1	NA	NA	NA	83.2±7.2
Female mice					
Expired CO_2	55.4±10.2	42.3±2.7	31.4±5.5	26.1±5.3	21.6±1.7
Exhaled VOCs	3.1±2.0	2.5±0.7	3.8±1.5	11.5±3.8	4.2±1.2
Urine	23.6±12.1	43.0±3.4	43.6±13.1	44.8±10.4	55.7±10.9
Feces	6.6±4.1	4.5±1.5	10.9±4.2	16.2±9.8	3.7±0.8
Tissues	3.6±1.1	2.3±0.9	1.3±0.3	1.1±0.7	0.8±0.2
Carcass	7.2±2.0	5.0±1.4	3.7±0.8	4.2±1.6	2.6±0.3
Total recovery[d]	102.1±12.9	100.1±1.6	96.4±3.8	106.7±7.6	94.1±6.6

[a]Mean±standard deviation from groups of four animals
[b]Cage wash accounted for 0.3–9%
[c]Cage wash accounted for 0.3–1%
[d]Cage wash accounted for 0.5–6%

CO_2 = carbon dioxide; NA = not applicable; VOCs = volatile organic compounds

Source: Frantz et al. 1996b, 1996c

probably resulted from saturation of the enzymes that metabolize glycolic acid, leading to increased excretion of this metabolite in the urine. Pottenger et al. (2001) provided data on urinary levels of ethylene glycol and glycolate in female rats exposed to doses of 10–2,500 mg/kg that confirmed the saturation of glycolic acid metabolism in the dose range between 150 and 500 mg/kg.

Monkeys given a single oral dose of 1 mL/kg ^{14}C-ethylene glycol (equivalent to 1,110 mg/kg) excreted about 24% of the administered dose as unchanged parent compound in the urine within 48 hours (McChesney et al. 1971). In dogs, approximately 50% of an oral dose of ethylene glycol (173 mmol/kg) was excreted via the urine within 72 hours after exposure (Grauer et al. 1987).

3.4.4.3 Dermal Exposure

Rats and mice were treated with dermal application of doses of 10–1,000 mg/kg undiluted ^{14}C-ethylene glycol or 1,000 mg/kg 50% aqueous solution of ^{14}C-ethylene glycol under occlusion for 6 hours, and radioactivity was measured in expired air, excreta, tissues, remaining carcass, and occlusion materials and skin (unabsorbed dose) for 96 hours after dosing (Frantz et al. 1989, 1991, 1996b, 1996c). Table 3-6 shows the disposition of radioactivity. In male and female rats, 6–14% of the administered dose was expired, while 4–8% was excreted in the urine, and ~1% was recovered from the feces (Frantz et al. 1989, 1996b, 1996c). In female mice treated similarly (except with a low dose of 100 mg/kg undiluted ethylene glycol), most of the administered dose was recovered as exhaled volatile organic compounds (21–34%) or CO_2 (10–16%) (Frantz et al. 1991, 1996b). Urine and feces each accounted for another 5–12% of the dose (Frantz et al. 1991, 1996b). No dose-related shift in excretory patterns was observed in either species, suggesting that metabolic pathways were not saturated under the slower absorption conditions observed with dermal exposure.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

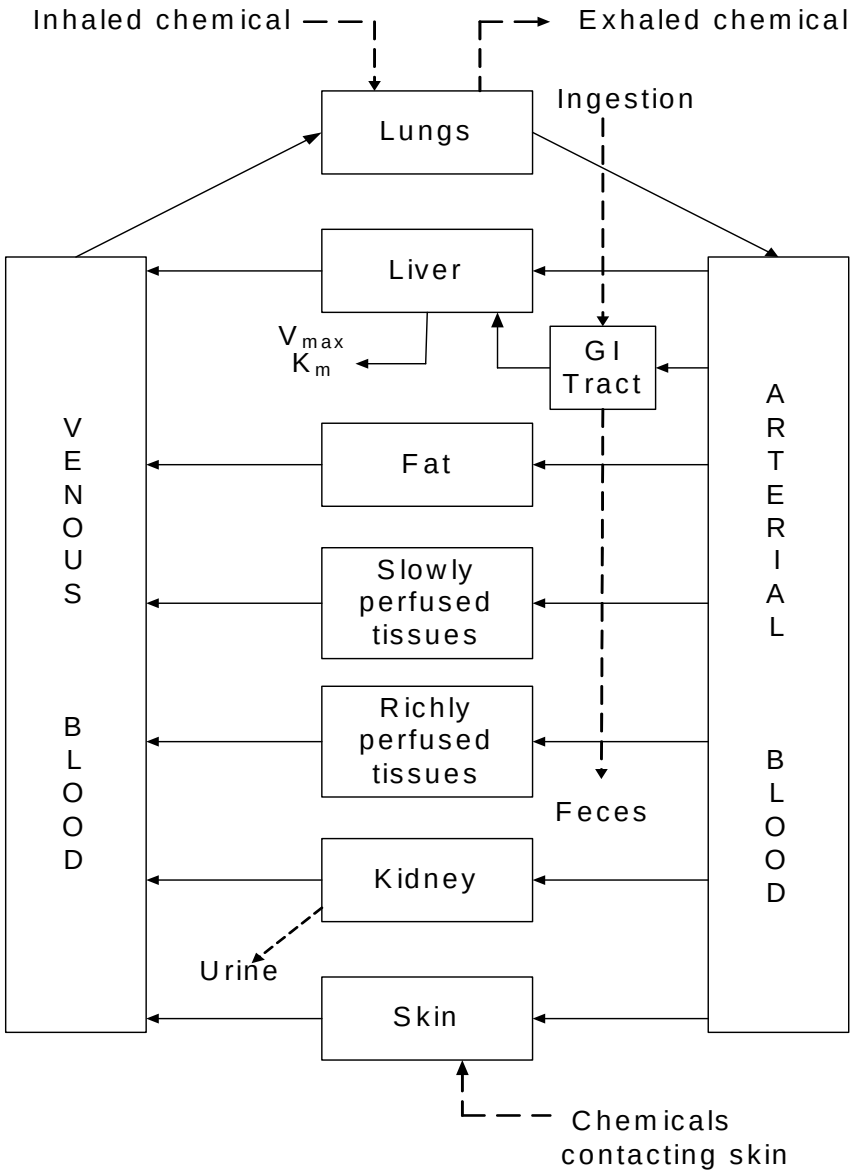
PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: adapted from Krishnan and Andersen 1994

If PBPK models for ethylene glycol exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

PBPK models are available for ethylene glycol and its intermediate metabolite, glycolic acid, in rats and humans (Corley et al. 2005a). The models include the inhalation, oral, dermal, intravenous, and subcutaneous routes of exposure. Models for ethylene glycol consist of eight compartments connected by blood flow (lungs, richly perfused tissues, poorly perfused tissues, fat, skin, gastrointestinal tract, liver, and kidney); models for glycolic acid have a similar structure except that the lung is included in the richly perfused tissue group. Gastrointestinal tract, lung, and skin were included separately in order to permit simulation of different exposure routes. Models for both compounds assume instantaneous dispersion of the compound through each compartment based on blood perfusion rates and partition coefficients. The models for ethylene glycol and glycolic acid are connected via a saturable metabolic route in the liver, and renal elimination of both compounds was modeled.

Physiological parameters used in the model are shown in Table 3-8. Tissue volumes were scaled to body weight; alveolar ventilation and cardiac output were scaled as $(\text{body weight})^{0.75}$; blood flows were scaled to cardiac output; and kidney parameters (glomerular filtration, tubule urine volume, and urine production) were scaled as a fraction of kidney weights. Partition coefficients used in the model are given in Table 3-9. Blood:air partition coefficients were measured *in vitro* using human and female Sprague-Dawley rat blood; tissue:blood coefficients were measured in rats, and human partition coefficients were assumed to equal those of rats.

A simplified metabolic pathway simulating metabolism of ethylene glycol to glycolic acid and from glycolic acid to glyoxylic acid (the rate-limiting steps) with saturable Michaelis-Menten kinetics was used in the model. Metabolic rate constants were estimated from *in vitro* data. Elimination via the kidneys was initially simulated as a first-order equation, but was modified to allow for reabsorption of glycolic acid in the renal tubules by a saturable Michaelis-Menten-like process in order to better predict elimination of this metabolite at low doses (<200 mg/kg). Table 3-9 shows the metabolic and renal elimination parameters used in the study.

The model was validated against several pharmacokinetic studies in rats and humans (Corley and McMartin 2005; Corley et al. 2005a). In the examples reported by Corley et al. (2005a), the model

Table 3-8. Physiological Parameters in PBPK Models for Ethylene Glycol

Parameter	SD rat	Human
Physiologic parameters		
Body weight (kg)	0.23	60
Tissue volumes (fraction of body weight)		
Blood	0.059	0.059
Liver	0.034	0.0314
Kidneys	0.007	0.0044
Lungs	0.005	0.0115
Gastrointestinal tract	0.05	0.034
Fat	0.07	0.231
Skin	0.19	0.051
Richly perfused	0.0423	0.0371
Slowly perfused	0.91, sum other tissues	
Flows (liter/hour/kg)		
Cardiac output	15	15
Alveolar ventilation	15	15
Blood flows (fraction of cardiac output)		
Liver	0.18	0.25
Gastrointestinal tract	0.15	0.21
Kidney	0.141	0.25
Fat	0.07	0.05
Skin	0.058	0.03
Richly perfused	1.0, sum other tissues	
Slowly perfused	0.17	0.17

Source: Corley et al. 2005a

Table 3-9. Biochemical Parameters in PBPK Models for Ethylene Glycol

	Ethylene glycol		Glycolic acid	
Parameter	SD rat	Human	SD Rat	Human
Absorption rate ($hour^{-1}$)				
Oral gavage	1–5	1	1	1
Subcutaneous	1	-	1	-
Intraperitoneal	1	-	-	-
Partition coefficients				
Blood:air	17,901	17,542	-	-
Blood:saline	1.14	1.14	3.36	3.36
Skin:saline	1.36	1.36	2.51	2.51
Skin:air	17,901	17,542	-	-
Liver:blood	0.96	0.96	0.97	0.97
Kidney:blood	1.22	1.22	1.40	1.40
Lung:blood	0.96	0.96	-	-
Fat:blood	0.64	0.64	1.09	1.09
Skin:blood	1.19	1.19	0.75	0.75
Gastrointestinal tract:blood	1.48	1.48	0.95	0.95
Richly perfused:blood	0.96	0.96	0.97	0.97
Slowly perfused:blood	0.67	0.67	0.70	0.70
Elimination (L/hour/kg)				
Urinary clearance	0.06	0.06	0.06 (alternate model)	0.06 (alternate model)

SD = Sprague-Dawley

Source: Corley et al. 2005a

predictions provided reasonably good fit to measured plasma concentrations of ethylene glycol and glycolic acid after oral exposure to ethylene glycol in female Sprague-Dawley rats and intraperitoneal exposure to male Wistar rats, although predictions of glycolic acid concentrations after low-dose (10 mg/kg) oral exposure were not as reliable. The authors suggested that differences in analytical methods used to measure glycolic acid in the dataset used to determine model parameters and the validation dataset may have contributed to the less reliable prediction after low-dose exposure. Validation against human data was complicated by the need to incorporate effects of therapeutic interventions on blood levels of ethylene glycol and glycolic acid in humans acutely poisoned with ethylene glycol. With modifications to simulate these effects, the model provided reasonably good predictions of blood levels reported in several clinical case reports over a broad range of oral doses (Corley and McMartin 2005).

Using the model for humans, Corley et al. (2005a) estimated that the threshold glycolic acid concentration for developmental effects in rodents (considered by the authors to be a peak of 2 mM) would only be reached in human females ingesting doses of 350 mg/kg (assuming a 58-kg female). However, the model was not calibrated to pregnancy, and it is not clear whether physiological and/or biochemical changes that could affect ethylene glycol toxicokinetics might occur during pregnancy (NTP-CERHR 2004). Furthermore, uncertainty in the glycolic acid saturation concentration in humans somewhat limits the usefulness of this model for predicting developmental toxicity in human embryos.

Data from a single study (Pottenger et al. 2001) suggested that pregnancy status did not affect the time course of ethylene glycol, glycolic acid, or oxalic acid pharmacokinetics in maternal blood and urine (including peak concentration, time of peak concentration, area under the concentration vs. time curve, or elimination half-time) when groups of pregnant and nonpregnant rats were treated by gavage with doses of 10 or 2,500 mg/kg ethylene glycol (pregnant rats treated on Gd 10). While Gd 10 is a sensitive time point for developmental effects, NTP-CERHR (2004) observed that pregnancy-related changes in metabolism would not be captured in this study due to the narrow exposure window. In their review, NTP-CERHR (2004) noted that there were no data to assess whether maternal levels of enzymes involved in ethylene glycol metabolism might change over the course of gestation. In addition, the study measured maternal, not fetal, levels of ethylene glycol and its metabolites. However, Corley et al. (2002) showed that levels of glycolic acid in rat embryos and extraembryonic fluid paralleled those of maternal blood, albeit at levels 1.4–4-fold higher than maternal levels. Slikker et al. (2004) reported that there are species-specific differences in the transfer of glycolic acid from maternal blood to conceptus. Likewise, fetal and/or placental differences in expression of enzymes metabolizing ethylene glycol and glycolic acid

over the course of gestation may affect local concentrations of glycolic acid to which the developing conceptus is exposed, yet little is known about these differences (NTP-CERHR 2004). In particular, there are no data on the ontogeny of glycolate oxidase (the enzyme that breaks down glycolic acid) expression in rodent or human embyros (NTP-CERHR 2004)

Additional data are needed to reduce uncertainty in the saturation concentration of glycolic acid in humans, and such data may alter the model predictions of peak glycolic acid concentrations in humans exposed to ethylene glycol. Although *in vitro* data suggest that humans may metabolize glycolic acid more efficiently than rats, based on comparisons of apparent Vmax/Km values obtained using liver homogenates and liver slices, there are no *in vivo* human data with which to predict the saturation point in humans (NTP-CERHR 2004).

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. No studies investigating the mechanism by which ethylene glycol is absorbed from the lung, gastrointestinal tract, or skin were located.

Distribution. As discussed in more detail in Section 3.4.2, there are limited data on the distribution of ethylene glycol after inhalation exposure. Studies in rats, mice, and monkeys, as well as limited data in humans, suggest that ethylene glycol is distributed according to total body water (Frantz et al. 1989, 1991, 1996b, 1996c; Jacobsen et al. 1988). There are no data on the sites of ethylene glycol metabolism or on the distribution of its primary metabolite (glycolic acid) in the body. The inverted yolk sac placenta, which develops in both mice and rats, tends to concentrate weak acids such as glycolic acid; neither humans nor rabbits develop a yolk sac placenta, and a preliminary study showed that glycolic acid does not concentrate in rabbit embryonic fluids (NTP-CERHR 2004). Corley et al. (2002) confirmed that the rat embryo and embryonic fluid concentrate glycolic acid, reaching levels roughly 2–4-fold higher than maternal blood. No additional data are available to characterize the mechanisms by which ethylene glycol is transported to the kidneys or developing fetus, the primary sites of toxic action.

Metabolism. As discussed in more detail in Section 3.4.3, ethylene glycol metabolism has been well characterized. Glycolic acid has been identified as the primary metabolite and putative developmental toxicant associated with ethylene glycol exposure, while a downstream metabolite (oxalate) is associated with renal toxicity. There are no data on the tissues most responsible for metabolism of ethylene glycol.

Two of the primary enzymes involved in ethylene glycol metabolism (alcohol dehydrogenase and aldehyde dehydrogenase) are also responsible for ethanol metabolism, and ethanol metabolism largely takes place in the liver. Thus, it is likely that the liver is also the primary site of ethylene glycol metabolism; however, other tissues, including the placenta, also produce these enzymes. Pharmacokinetic parameters (e.g., plasma half-life, area under the curve, and peak ethylene glycol concentration) are similar after both oral and intravenous exposure (Frantz et al. 1989, 1991, 1996a, 1996b, 1996c), indicating that a first-pass effect, if any, has a negligible effect on the toxicokinetics.

Excretion. As discussed in more detail in Section 3.4.4, studies in mice and rats of ethylene glycol excretion after oral, dermal, and intravenous exposure indicate that ethylene glycol is principally excreted as expired CO_2 and as both parent compound and glycolic acid in the urine (Frantz et al. 1989, 1991, 1996b, 1996c). At higher doses, oxalate was also excreted at measurable levels.

3.5.2 Mechanisms of Toxicity

There are three main effects responsible for the toxicity of ethylene glycol: increased osmolal gap, metabolic acidosis, and formation of calcium oxalate crystals. Several lines of evidence suggest that metabolites of ethylene glycol (Figure 3-3) are responsible for these effects. First, there is a latent period before the symptoms of acidosis appear; second, there is no correlation between observed toxicity and ethylene glycol blood concentration; and third, inhibition of ethylene glycol oxidation prevents toxicity (Jacobsen and McMartin 1986).

In the initial stages after ingestion (there are no reports of clinical toxicity in humans following inhalation or dermal exposure), the ethylene glycol concentration in extracellular fluids increases, leading to increased osmolality. This increased osmolality (hyperosmolarity) causes increased osmolal gap, one of the hallmarks of ethylene glycol intoxication. Osmolal gap is defined as a difference between the measured and calculated osmolality. Osmolality (calculated) can be estimated from the formula that takes into account normal serum concentrations of sodium, glucose, and BUN. This calculated osmolality is then compared to the serum osmolality measured following ethylene glycol ingestion; a difference >10 indicates an increased osmolal gap (Fligner et al. 1985). The increased osmolal gap is not specific to ethylene glycol intoxication and can occur when any unmeasured, osmotically active, low molecular weight solute (e.g., methanol or ethanol) is present in the serum. In dogs given oral doses of 10,743 mg/kg ethylene glycol, serum osmolality peaked (460 milliosmoles/kg) at 3–6 hours, and the osmolal gap peaked (134 milliosmoles/kg) at 3 hours, coinciding with peak serum ethylene glycol levels

at 3 hours (Grauer et al. 1984). In these animals, the anion gap was also significantly increased at 3 hours (19 Meq/L).

The second characteristic of ethylene glycol intoxication is metabolic acidosis. Ethylene glycol itself has low toxicity (Godolphin et al. 1980; Jacobsen and McMartin 1986), but it is metabolized to a variety of toxic metabolites such as glycolaldehyde, glycolic acid (glycolate), glyoxylic acid (glyoxylate), and oxalic acid (oxalate) (Jacobsen et al. 1988; Parry and Wallach 1974; Vale 1979; Wiener and Richardson 1988). In general, the accumulation of acids leads to acidosis, a state that is characterized by actual or relative decrease of alkali in body fluids in relation to the acid content. In the case of ethylene glycol, metabolic processes that follow ethylene glycol ingestion lead to the accumulation of glycolic and lactic acids resulting in metabolic acidosis. Glycolic acid is the most abundant ethylene glycol metabolite (Jacobsen et al. 1984). Following ingestion of high doses of ethylene glycol, glycolic acid tends to accumulate as a result of metabolic saturation. The accumulation of metabolites such as glycolic acid and oxalate, as well as lactic acid formed through the reduction of NAD, leads to an increased serum anion gap (the difference between the sum of the measured cations and anions) and metabolic acidosis, which are responsible for toxicity observed after ethylene glycol exposure. While lactate levels increase in some human cases up to 5–7 mmol (Jacobsen et al. 1984, 1988; Parry and Wallach 1974), glycolate levels range up to 20–25 mmol, thus accounting for a greater portion of the anion gap. Glycolic acid accounts for approximately 96% of the anion gap in ethylene glycol-poisoned patients (Gabow et al. 1986; Jacobsen et al. 1984). In dogs given oral doses of 10,743 mg/kg ethylene glycol, the anion gap was significantly increased at 3 hours (19 Meq/L) coinciding with peak serum ethylene glycol levels (Grauer et al. 1984). The maximum production of metabolites occurs 6–12 hours after ethylene glycol ingestion and coincides with development of respiratory and cardiovascular symptoms.

Nephrotoxicity and neurotoxicity can follow because oxalate can produce renal and brain damage as it chelates with calcium ions forming insoluble calcium oxalate monohydrate crystals, another characteristic of ethylene glycol poisoning (Jacobsen et al. 1988). Glycolic acid accumulation and metabolic acidosis do not contribute to renal toxicity, which is solely caused by oxalate crystal accumulation (Cruzan et al. 2004; Green et al. 2005). Oxalate crystal formation may lead to hypocalcemia and imbalance of serum divalent ion concentrations (Zeiss et al. 1989). Although the mechanism of ethylene glycol neurotoxicity is not completely understood, the available information on humans suggests that it occurs in two stages, an early one (30 minutes to 12 hours after exposure) and a late one (several days after exposure). The early-stage symptoms are due to the direct toxicity of ethylene glycol, while the late-stage neurotoxicity is due to metabolic acidosis caused by the accumulation of ethylene glycol metabolites, primarily glycolic

acid, which leads to metabolic acidosis. Additional evidence for this late neurotoxicity is crystalline deposits of calcium oxalate in the walls of small blood vessels found in the brain of humans who died of acute ethylene glycol poisoning (Froberg et al. 2006; Zeiss et al. 1989). Similar effects were observed in rats fed 2,500 mg/kg/day ethylene glycol for 13 weeks (Melnick 1984). The role of calcium in ethylene-glycol-induced neurotoxicity is not known but the formation of calcium oxalate crystals may cause perturbation of intracellular calcium homeostasis causing membrane abnormalities generally associated with cell injury and cell death. A generalized soft tissue mineralization that included the heart (vessels and muscle), lungs (interstitial), stomach, and vascular system occurred in male F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside 1982). These histopathological changes may be the result of altered calcium metabolism (Rajagopal et al. 1977). Other effects of ethylene glycol metabolites include inhibition of oxidative phosphorylation, respiration, glucose metabolism, protein synthesis, DNA replication, ribosomal ribonucleic acid (RNA) synthesis, central nervous system respiration, and serotonin metabolism (Vale 1979).

The presented data indicate that glycolic acid is the major toxic metabolite contributing to metabolic acidosis, which is a primary cause of systemic toxicity following exposure to ethylene glycol. Glycolic acid has also been identified as the proximate cause of developmental effects observed with ethylene glycol exposure (NTP-CERHR 2004; Slikker et al. 2004). A number of mechanistic studies have ruled out both ethylene glycol itself and other metabolites as the primary developmental toxicants, while metabolic acidosis was shown to interact with glycolic acid at high doses to enhance developmental effects. The available data suggest that peak concentrations in the range of 2–3 mM glycolic acid are necessary for developmental toxicity to occur in rodents (Carney et al. 2001; Corley et al. 2002; NTP-CERHR 2004; Slikker et al. 2004).

Klug et al. (2001) compared the effects of several ethylene glycol metabolites on rat whole embryos (Gd 9.5) in culture, observing that only glycolic acid affected embryonic development at metabolite concentrations observed in *in vivo* studies of ethylene glycol. Ethylene glycol and other metabolites did not affect development except at much higher concentrations than have been seen *in vivo*.

Using rat whole embryos (Gd 10) exposed to either ethylene glycol or glycolic acid for 46 hours *in vitro*, Carney et al. (1996) showed that concentrations up to 50 mM ethylene glycol did not cause morphological changes, while glycolic acid caused changes in the skeletal and craniofacial regions at concentrations of ≥12 mM. These changes are consistent with the dysmorphogenesis observed in rats after *in vivo* exposure to ethylene glycol. In the same study, the role of medium acidification in the

observed effects was investigated by comparing the effects of 12.5 mM glycolic acid (pH 6.7), 12.5 mM sodium glycolate (pH 7.4), and control medium (pH 7.4 or 6.7) on rat whole embryos in culture. The incidence of affected embryos was 67% in the glycolic acid group, 58% in the sodium glycolate group, 8% in the pH 6.7 controls, and 0% in the pH 7.4 controls. The authors concluded that glycolic acid was the primary developmental toxicant, and that medium acidification was a minor contributor to the observed effects.

In vivo studies have shown similar results. When glycolic acid was administered to CD rats via gavage on Gd 6–15, the observed effects on offspring were similar to those observed after ethylene glycol exposure (Munley et al. 1999). In an effort to determine the extent to which metabolic acidosis contributed to the developmental effects induced by glycolic acid, Carney et al. (1999) treated time-mated Sprague-Dawley rats with ethylene glycol (2,500 mg/kg) or glycolic acid (650 mg/kg) via gavage or sodium glycolate via subcutaneous injection on Gd 6–15. Metabolic acidosis was induced in both the ethylene glycol and glycolic acid groups, but not in the sodium glycolate treatment group. Upon sacrifice on Gd 21, fetal body weights were decreased and malformations were increased in all three groups, indicating that glycolate was capable of inducing effects in the absence of metabolic acidosis. The authors reported that developmental toxicity was enhanced by an interaction between metabolic acidosis and glycolate at high doses (Carney et al. 1999).

3.5.3 Animal-to-Human Extrapolations

Toxicokinetic and mechanistic data suggest that humans may be less sensitive than rodents to systemic and developmental effects of ingested ethylene glycol. *In vitro* studies by Corley et al. (2005a) and Booth et al. (2004) found that human liver tissue was more effective than liver tissue from rats and rabbits in metabolizing glycolic acid to glyoxylic acid, suggesting that humans are less likely to accumulate glycolic acid (the proximate developmental toxicant). In addition, NTP-CERHR (2004) reviewed preliminary data by Carney and coworkers indicating that the inverted yolk sac placenta, a stage in placental development that does not exist in humans or rabbits, tends to concentrate weak acids such as glycolic acid in the embryonic fluids. Corley et al. (2002) confirmed that the rat embryo and embryonic fluid concentrate glycolic acid, reaching levels roughly 2–4-fold higher than maternal blood. These data suggest enhanced sensitivity to ethylene glycol developmental effects in rodents compared with humans.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

Insufficient information is available to adequately assess the endocrine disruptor potential of ethylene glycol. No studies were located regarding endocrine disruption in humans after exposure to ethylene glycol.

No histopathological changes occurred in endocrine organs of rats or mice in acute-, intermediate- and chronic-duration oral studies of ethylene glycol. As discussed in the Endocrine Effects subsection of Section 3.2.2.2, histological examinations in these studies included the adrenals, pancreas, pituitary, thyroid, and/or parathyroids (Blood 1965; DePass et al. 1986a; Hong et al. 1988; Melnick 1984; NTP 1993; Robinson et al. 1990; Schladt et al. 1998; Woodside 1982). Assessments of endocrine function (e.g., hormone levels) were not conducted in these or other studies of ethylene glycol.

Reproductive toxicity studies showed that oral exposure to high doses of ethylene glycol affected fertility and fetal viability in mice and rats (Harris et al. 1992; Lamb et al. 1985; Morrissey et al. 1989; NTP 1986; Price et al. 1985; Schuler et al. 1984), and possibly male reproductive function in mice (Morrissey et al. 1989; NTP 1986) and gestational duration in rats (NTP 1988).

Ethylene glycol had no estrogenic or antiestrogenic activity in an *in vitro* MVLN cell-based transactivation assay (Freyberger and Schmuck 2005). MVLN cells constitutively express the estrogen receptor (ER) and are stably transfected with the luciferase reporter gene and the corresponding hormone responsive element derived from the *Xenopus* Vitellogenin A2 gene. Evaluations included cytotoxicity and luciferase gene expression in the absence and presence of estradiol stimulation, as well as ER-α binding affinity.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age

(Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Young children are susceptible to ethylene glycol poisoning through the accidental ingestion of antifreeze because it is a brightly colored, sweet tasting liquid that can be mistaken for a beverage (Leth and Gregersen 2005). Many ethylene glycol poisonings occur when an antifreeze bottle is in use or when antifreeze is not kept in its original container (e.g., if it is poured into a cup or soft drink bottle), because

children can ingest ethylene glycol from an accessible open container (EPA 2004b; Leth and Gregersen 2005). Children also may play in a puddle of antifreeze that has been spilled or drained onto the ground. Children and adolescents comprise a significant percentage of ethylene glycol acute intoxications from accidental or intentional ingestion. For example, in a total of 735 exposures voluntarily reported to U.S. poison control centers in 2003, 150 (20%) were younger than 19 years old and 84 (11%) were younger than 6 years old (Watson et al. 2004). Similarly, of 751 total exposures reported in 2005, 167 (22%) were ≤19 years old and 69 (9%) were younger than 6 years old (Lai et al. 2006). It has been reported that ingestion of as little as 10–15 mL ethylene glycol can be fatal in small children (White and Liebelt 2006).

A limited amount of information on health effects of ethylene glycol in children is available from several case reports of patients admitted to hospitals for treatment of acute oral poisoning. A 4-year-old girl (14 kg) who accidentally ingested an unknown amount of antifreeze containing 41% ethylene glycol vomited and was admitted to a hospital 4 hours later, where drowsiness, hypotonia, and metabolic acidosis subsequently developed (Harry et al. 1998). A 13-year-old girl (80 kg) who intentionally ingested approximately 4 fluid ounces of antifreeze (ethylene glycol concentration not reported) was brought to a hospital approximately 30 minutes after ingestion with no evidence of intoxication, but subsequently developed ataxia, dysarthria, metabolic acidosis, and oxalate crystals in the urine (Boyer et al. 2001). An 8-month-old boy (7.7 kg) who drank up to 120 mL ethylene glycol (95%) was taken to a hospital where he appeared lethargic; metabolic acidosis, increased osmolal gap, and oxalate crystals in the urine were detected 3–4 hours post-ingestion (Baum et al. 1999). Six children ranging in age from 22 months to 14 years were admitted to a hospital for treatment of ethylene glycol poisoning over a 4-year period (Caravati et al. 2004). Four of the children (7–13 years old, 22–50 kg) ingested between 30 and 120 mL (alleged doses) of antifreeze (ethylene glycol concentration not reported); the amounts ingested by the other two children were unknown. Presenting symptoms included dizziness, slurred speech, nausea, ataxia, and lethargy. Varying degrees of metabolic acidosis were also observed, but renal function was normal.

The effects in the pediatric patients summarized above are largely consistent with the first stage of ethylene glycol poisoning in adults (e.g., central nervous system depression, metabolic changes, gastrointestinal upset). Treatment with fomepizole (4-methylpyrazole), alone or in combination with other methods (see Section 3.11, Methods for Reducing Toxic Effects), generally mitigated the progression of the clinical course to the second and third stages of ethylene glycol poisoning (pronounced metabolic acidosis, cardiopulmonary compromise, and renal insufficiency) and led to full recovery. The case reports are consistent with an expectation that health effects in children and adults are similar.

Although there are no known differences in the toxicity of ethylene glycol between adults and children, there is no evidence to substantiate the presumption. There is no evidence to indicate that children are likely to be exposed to higher (or lower) amounts of ethylene glycol from everyday living, suggesting that children are perhaps equally at risk for non-accidental or non-intentional exposure and potential toxic side effects.

Information on the developmental toxicity of ethylene glycol is available from oral, inhalation, and dermal studies in rats, mice, and rabbits. Oral studies in animals have identified the developing fetus as the most sensitive target for acute-duration exposure to ethylene glycol. Gavage exposure of rats and mice to ethylene glycol during gestation results in a consistent pattern of developmental effects including reduced fetal body weight and increases in malformations, particularly axial skeletal malformations (Neeper-Bradley 1990; Neeper-Bradley et al. 1995; Price et al. 1985). No teratogenic effects were observed in rabbits orally exposed during gestation (Tyl et al. 1993). Results of inhalation developmental studies in rats and mice are generally consistent with the oral findings, but are confounded by concurrent oral exposure (Tyl 1985, 1988a; Tyl et al. 1995a, 1995b). A single study of dermal exposure to ethylene glycol in pregnant mice did not indicate developmental effects (Tyl 1988b; Tyl et al. 1995c).

Developmental effects of intermediate-duration oral exposure to ethylene glycol in animals include decreased body weight and kidney effects in offspring. In mice exposed via drinking water in a continuous breeding assay, pup body weights were reduced in both F_1 and F_2 generations (Morrissey et al. 1989; NTP 1986). Effects on postnatal kidney weight were observed in pups of rats exposed by gavage for 15 days during gestation (NTP 1988). In a three-generation dietary study in rats, no effects on gestation survival or pup body weight through postnatal day 21 were observed in F_1 or F_2 pups (DePass et al. 1986b). The available animal data are insufficient for determining whether postnatal developmental toxicity is a potential concern in exposed children. Effects of ethylene glycol on the immune and endocrine systems have not been adequately studied. Ethylene glycol did not induce dominant lethal mutations in orally-exposed rats (DePass et al. 1986b) and was consistently negative in *in vitro* genotoxicity assays in a variety of test systems, indicating that it is unlikely to affect DNA in parental germ cells.

No studies are available that describe potential differences in the toxicokinetics or the mechanism of action of ethylene glycol in children. As discussed in Section 3.5.2, Mechanisms of Toxicity, glycolic acid is the major toxic metabolite contributing to metabolic acidosis, which is a primary cause of systemic toxicity in children as well as adults following exposure to ethylene glycol. Glycolic acid has also been

identified as the proximate cause of the developmental effects in animals observed with ethylene glycol exposure (NTP-CERHR 2004; Slikker et al. 2004).

Ethylene glycol metabolism is known to involve alcohol dehydrogenase and aldehyde dehydrogenase, and may also involve cytochrome P450 isozymes (NTP-CERHR 2004). Polymorphisms in the genes encoding these enzymes may lead to wide variability in the production and elimination of glycolic acid and other metabolites in humans exposed to ethylene glycol, but data quantifying the range of variability are not currently available (NTP-CERHR 2004). In addition, fetal and/or placental differences in expression of these enzymes over the course of gestation will affect local concentrations of glycolic acid and other metabolites to which the developing conceptus is exposed, yet little is known about these differences (NTP-CERHR 2004).

A PBPK model for ethylene glycol in adult humans has been developed and has been used to estimate that the threshold glycolic acid concentration for developmental effects in rodents would only be reached in human females ingesting doses of 350 mg/kg (assuming a 58-kg female) (Corley et al. 2005a). However, the model was not calibrated to pregnancy, and it is not clear whether physiological and/or biochemical changes that could affect ethylene glycol toxicokinetics might occur during pregnancy. No models are available for children or lactating women. A PBPK model has also been developed for rats (Corley et al. 2005a), but there is no model for mice, which are more sensitive than rats to ethylene glycol developmental toxicity. Biomonitoring data for children, including levels of ethylene glycol in placental tissue, cord blood, neonatal blood, meconium fluid, or breast milk, have not been located.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and

interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to ethylene glycol are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by ethylene glycol are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Ethylene Glycol

The presence of parent compound in the blood and urine serves as the only biomarker of exposure that is specific to ethylene glycol. The half-life of ethylene glycol in plasma is estimated to be 3–7 hours in laboratory animals (Marshall 1982; Winek et al. 1978). Available human data indicate a similar half-life for ethylene glycol in human plasma (Eder et al. 1998). The elimination half-life of ethylene glycol in the urine of acutely intoxicated humans ranges from 3.0 to 8.4 hours (Jacobsen et al. 1988; Peterson et al. 1981). Based on the relatively short half-life in the blood and urine, the presence of parent compound would serve as a reliable biomarker of exposure only within the first day following exposure. Rapid methods for determining ethylene glycol in serum and urine are available for use in the clinical setting

(Aarstad et al. 1993; Blandford and Desjardins 1994), but may not always be readily available in emergency situations.

Other biomarkers of exposure are typically used in conjunction with serum and urinary ethylene glycol levels to assist in confirmation and quantitation of ethylene glycol intoxication. For example, levels of glycolic, lactic, and oxalic acid metabolites of ethylene glycol may be useful indicators of ethylene glycol-induced toxicity. However, these other biomarkers are not specific to ethylene glycol. As discussed in detail in Section 3.4, ethylene glycol is rapidly metabolized to glycolic acid, which accumulates in the blood and causes metabolic acidosis (Gabow et al. 1986; Jacobsen et al. 1984). Glycolic acid blood levels have been more closely correlated to clinical symptoms than ethylene glycol blood levels (Hewlett et al. 1986). Due to the rapid formation of glycolic acid in the body and its correlation to clinical symptoms of ethylene glycol poisoning, measurements of both parent compound and glycolic acid levels are important in diagnosis and treatment (Hess et al. 2004). Although glycolate is not a specific biomarker for ethylene glycol exposure (because it is an endogenous chemical that can also be obtained from the diet), an increase in plasma glycolate above the general background level (<1 mM) is a good indication of ethylene glycol exposure (McMartin 2007). It is particularly useful in situations where there is a lengthy period between exposure and blood sampling; in these situations, there is often no ethylene glycol in the plasma (due to its metabolism and elimination), whereas there still are elevated levels of glycolate. Only a few clinical toxicology laboratories routinely offer glycolic acid analyses (Hess et al. 2004; Pellegrino et al. 2006). Lactic acid may contribute to metabolic acidosis, whereas oxalic acid forms calcium oxalate crystals that are considered to be the cause of ethylene glycol-induced nephrotoxicity (Jacobsen and McMartin 1986; Moossavi et al. 2003; Pellegrino et al. 2006; Wiley 1999).

Metabolic acidosis with increased serum anion and osmolal gaps are suggestive of ethylene glycol poisoning, but do not provide a specific diagnosis. Serum anion gap is calculated from concentrations of sodium, chloride, and bicarbonate, is normally 12–16 mM, and is elevated after ethylene glycol ingestion (Chung and Tuso 1989; Factor and Lava 1987; Heckerling 1987; Hess et al. 2004; Spillane et al. 1991; Zeiss et al. 1989). The increase in the anion gap correlates with the elevation in plasma glycolate levels (Hess et al. 2004; Jacobsen et al. 1984). Osmolal gap represents the difference between the measured and calculated osmolalities and is also elevated during ethylene glycol intoxication. As unmetabolized ethylene glycol accounts for most of the osmolality gap, it is only raised in the initial stages of toxicity, and decreases later as anion gap increases due to metabolism of the parent compound to acidic intermediates (Hess et al. 2004; Leth and Gregersen 2005). The normal value for osmolal gap in humans is 10–15 mOsm/kg water (Egbert and Abraham 1999; Leth and Gregersen 2005; Scalley et al. 2002).

Normal osmolality gap does not exclude ethylene glycol poisoning, although an elevated osmolality gap is suggestive (Leth and Gregersen 2005).

The presence of calcium oxalate monohydrate crystals is an indicator of possible ethylene glycol intoxication, although not specific to ethylene glycol. The crystals can be found in renal tubules and/or urine after exposure to relatively large amounts of ethylene glycol (CDC 1987; Chung and Tuso 1989; Factor and Lava 1987; Godolphin et al. 1980; Heckerling 1987; Parry and Wallach 1974; Rothman et al. 1986; Siew et al. 1975a; Underwood and Bennett 1973).

3.8.2 Biomarkers Used to Characterize Effects Caused by Ethylene Glycol

Biomarkers of effects exist for ethylene glycol poisoning, but none are specific to ethylene glycol. These include clinical manifestations of nervous system, cardiopulmonary toxicity, renal toxicity, and laboratory findings of metabolic acidosis and calcium oxalate crystalluria.

Clinical manifestations progress in three main stages, although the course may vary between individuals. Signs of central nervous system toxicity appear within 0.5–12 hours following acute ingestion exposure and include ataxia, slurred speech, nystagmus, semiconsciousness, unresponsiveness, and somnolence that can culminate in convulsions and coma (CDC 1987; Cheng et al. 1987; Chung and Tuso 1989; Factor and Lava 1987; Hess et al. 2004; Leth and Gregersen 2005; Parry and Wallach 1974; Rothman et al. 1986; Spillane et al. 1991; Underwood and Bennett 1973; Zeiss et al. 1989). Neurological manifestations suggestive of cranial nerve damage, including facial paralysis and impaired vision, may appear in survivors as late as 1–2 weeks after an acute exposure to ethylene glycol (Chung and Tuso 1989; Factor and Lava 1987; Lewis et al. 1997; Mallya et al. 1986; Spillane et al. 1991). Cardiopulmonary manifestations, including respiratory distress and congestive heart failure, generally develop after 12–24 hours and usually in patients with coma; signs include tachycardia, dyspnea, tachypnea, hypertension, and pulmonary edema (Godolphin et al. 1980; Hess et al. 2004; Leth and Gregersen 2005; Parry and Wallach 1974; Siew et al. 1975a; Vale 1979; Zeiss et al. 1989). Renal failure occurs 24–72 hours after ingestion with clinical manifestations that include flank pain, hematuria, proteinuria, or anuria (Leth and Gregersen 2005).

Metabolic acidosis occurs approximately 12–24 hours following ethylene glycol ingestion, results from accumulation of acid metabolites (primarily glycolic acid), and is characterized by pronounced serum osmolal and anion gaps (Hess et al. 2004; Leth and Gregersen 2005). Serum osmolality is determined

from the concentrations of sodium, urea nitrogen, and glucose, and increased osmolality (osmolal gap) suggests the presence of unmeasured osmotically active substances such as ethylene glycol, methanol, ethanol, or acetone (Eder et al. 1998; Hoffman et al. 1993). As ethylene glycol is metabolized, the osmolal gap is decreased and the anion gap (the difference between the sum of the measured cations and anions) is increased (Jacobsen et al. 1988). Organic acids increase the anion gap; glycolic acid accounts for approximately 96% of the anion gap in ethylene glycol-poisoned patients (Gabow et al. 1986; Jacobsen et al. 1984).

Calcium oxalate crystals in the urine can appear 4–8 hours after ethylene glycol ingestion, and deposition of calcium oxalate monohydrate crystals in the renal tubules can subsequently result in nephropathy and eventual renal failure 24–72 hours after ingestion (CDC 1987; Chung and Tuso 1989; Factor and Lava 1987; Godolphin et al. 1980; Heckerling 1987; Jacobsen et al. 1988; Leth and Gregersen 2005; Parry and Wallach 1974; Rothman et al. 1986; Siew et al. 1975a; Underwood and Bennett 1973). Two forms of oxalate crystals may be found in the urine; the monohydrate form is an elongated crystal, and the anydrous form is octahedral (pyramid in shape). Renal toxicity can also be indicated by increased serum levels of BUN or creatinine (Grauer et al. 1987).

For more information on biomarkers for renal effects of chemicals see *ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage* (Agency for Toxic Substances and Disease Registry 1990) and for information on biomarkers for neurological effects see OTA (1990).

3.9 INTERACTIONS WITH OTHER CHEMICALS

Information regarding the influence of other chemicals on the toxicity of ethylene glycol comes from case studies describing treatment after accidental or intentional ingestion of ethylene glycol. The toxic effects of ethylene glycol result from its metabolic conversion by alcohol dehydrogenase into glycolic acid, which is further metabolized to oxalate. The formation of oxalate crystals is associated with renal toxicity encountered after exposure to ethylene glycol. Administration of ethanol, 4-methyl pyrazole, or 1,3-butanediol reduces or eliminates ethylene glycol toxicity. This is accomplished by the following mechanisms: (1) ethanol, which is also metabolized by alcohol dehydrogenase, competes with ethylene glycol for the enzyme, thus preventing the formation of potentially toxic ethylene glycol metabolites; (2) 4-methyl pyrazole inhibits the activity of alcohol dehydrogenase (Baud et al. 1987, 1988); and (3) 1,3-butanediol is also a competitive inhibitor of ethylene glycol biotransformation and reduces the formation of glycolic acid (Hewlett et al. 1983). Therefore, ethanol, 4-methyl pyrazole, and

1,3-butanediol reduce the toxicity of ethylene glycol by interacting with or inhibiting the activity of alcohol dehydrogenase, thus reducing the amount of glycolic acid and oxalate formed.

Magnesium and vitamin B_6 were found to affect the toxicity of ethylene glycol in animals. In rats, vitamin B_6 accelerates the oxidation of glyoxylate to carbon dioxide rather than to oxalate (Gershoff and Andrus 1962). Vitamin B_6 deficiency can cause inhibition of ethylene glycol's oxidation to carbon dioxide and thus cause an increase in ethylene glycol toxicity. Magnesium may prevent renal deposition of calcium oxalate by altering solvent characteristics of oxalate in urine (Browning 1965; Gershoff and Andrus 1962; Khan et al. 1993).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to ethylene glycol than will most persons exposed to the same level of ethylene glycol in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of ethylene glycol, or compromised function of organs affected by ethylene glycol. Populations who are at greater risk due to their unusually high exposure to ethylene glycol are discussed in Section 6.7, Populations with Potentially High Exposures.

Individuals deficient in vitamin B_6 could be more sensitive to toxic effects of ethylene glycol because vitamin B_6 may increase the accumulation of toxic metabolites (Browning 1965; Gershoff and Andrus 1962). Similarly, magnesium deficiency appears to encourage calcium oxalate deposition in the renal tubules, especially in the presence of high calcium levels (Ebisuno et al. 1987). Thus, individuals who are deficient in magnesium and/or ingest high levels of calcium may be more sensitive to the toxic effects of ethylene glycol.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to ethylene glycol. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to ethylene glycol. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to ethylene glycol:

Egbert PA, Abraham K. 1999. Ethylene glycol intoxication: Pathophysiology, diagnosis, and emergency management. ANNA J 26(3):295-300.

Ellenhorn MJ, Schonwald S, Ordog G, et al, eds. 1997. Ellenhorn's medical toxicology. Diagnosis and treatment of human poisoning. Baltimore, MD: Williams & Wilkins, 1152-1156.

Jolliff HA, Sivilotti MLA. 2004. Ethylene glycol. In: Dart RC, ed. Medical toxicology. 3rd. ed. Philadelphia, PA: Lippicott Williams & Wilkins, 1223-1230.

Mégarbane B, Borron SW, Baud FJ. 2005. Current recommendations for treatment of severe toxic alcohol poisonings. Intensive Care Med 31(2):189-195.

Scalley RD, Ferguson DR, Piccaro JC, et al. 2002. Treatment of ethylene glycol poisoning. Am Fam Physician 66(5):807-812.

Wiener SW. 2006. Toxic alcohols. In: Flomenbaum NE, Goldfrank LR, Hoffman RS, et al., eds. Goldfrank's toxicologic emergenices. New York, NY: McGraw-Hill Companies, Inc., 1447-1459.

White ML, Liebelt EL. 2006. Update on antidotes for pediatric poisoning. Pediatr Emerg Care 22(11):740-749.

3.11.1 Reducing Peak Absorption Following Exposure

No studies were found describing methods to reduce peak absorption of ethylene glycol after inhalation exposure. After oral exposure, nasogastric lavage may be of benefit in reducing absorption, but only if performed within 1–2 hours following ingestion (Barceloux et al. 1999; Egbert and Abraham 1999; Leth and Gregersen 2005; Wiley 1999). Activated charcoal is only partially effective at preventing gastrointestinal absorption of ethylene glycol because large amounts are needed to bind relatively small amounts of ethylene glycol, and the therapeutic window for this action is less than an hour (Scalley et al. 2002). The degree to which activated charcoal may lower absorption of ethylene glycol of may adsorb its toxic metabolites by entero-enteric dialysis is not known (Wiley 1999). Administration of syrup of ipecac for gastric emptying is strongly contraindicated due to the potential for altered mental status, seizures, and cardiac dysrhythmias, which may occur abruptly in ethylene glycol poisoning (Barceloux et al. 1999; Leth and Gregersen 2005; Wiley 1999). Dermal absorption can be minimized through washing the skin with soap to remove any existing ethylene glycol. Copious irrigation with water or saline can aid in ocular decontamination.

3.11.2 Reducing Body Burden

Clinical procedures for treating ethylene glycol poisoning focus on reduction of the body burden of ethylene glycol and its toxic metabolites, interference with toxic metabolite formation (which results in increased urinary excretion of parent compound), increased elimination of toxic metabolites produced, reduction of metabolic acidosis, and prevention of kidney failure. Procedures include administration of antidotes (ethanol or fomepizole), intravenous bicarbonate and hydration for profound acidemia, and hemodialysis for refractory acidosis (Barceloux et al. 1999; Egbert and Abraham 1999; Gardner et al. 2004; Leth and Gregersen 2005; Scalley et al. 2002).

Antidotes for ethylene glycol include the alcohol dehydrogenase inhibitors, ethanol and fomepizole, which act to decrease the alcohol dehydrogenase-catalyzed metabolism of ethylene glycol, thus effectively increasing the urinary excretion of ethylene glycol. Ethanol competes with ethylene glycol for alcohol dehydrogenase receptor sites and fomepizole acts as a potent inhibitor of alcohol dehydrogenase (Barceloux et al. 1999; Egbert and Abraham 1999; Gardner et al. 2004; Leth and Gregersen 2005). Antidotal therapy is indicated if ethylene glycol blood levels exceed 200 mg/L (Gardner et al. 2004). Hemodialysis (see below) may be avoided if patients are diagnosed and treated with fomepizole or ethanol early in the course of poisoning.

Fomepizole treatment has repeatedly been demonstrated to be a particularly effective therapy for ethylene glycol poisoning and is the preferred treatment/antidote in adults and children (Boyer et al. 2001; Caravati et al. 2004; Harry et al. 1998; Pizon and Brooks 2006; Scalley et al. 2002; White and Liebelt 2006). When compared with ethanol, the advantages of fomepizole include a slower rate of excretion by the kidneys, lack of central nervous system depression and hypoglycemia (which are major hazards of ethanol therapy in children), and easier maintenance of effective plasma levels (Scalley et al. 2002), indicating that ethanol should only be used if fomepizole is not available. The standard treatment regimen for fomepizole in adult and pediatric patients is an intravenous loading dose of 15 mg/kg followed by maintenance dosing of 10 mg/kg intravenous every 12 hours for four doses (Scalley et al. 2002; White and Liebelt 2006). Subsequent doses (if needed) are 15 mg/kg intravenous every 12 hours until plasma ethylene glycol level has been reduced below 20 mg/dL.

Intravenous fluid administration may be initiated early to increase urine output, which effectively increases the excretion of ethylene glycol and toxic metabolites such as glycolic and oxalic acids (Egbert and Abraham 1999). Sodium bicarbonate infusion is used to correct metabolic acidosis, increase

elimination of renal glycolic acid, and inhibit the precipitation of calcium oxalate crystals, although the latter benefit has not been demonstrated in clinical trials (Egbert and Abraham 1999; Leth and Gregersen 2005; Scalley et al. 2002).

Hemodialysis is indicated when initial serum ethylene glycol levels exceed 50 mg/dL or when ingestion of ethylene glycol results in refractory acidosis, deteriorating clinical status, or renal compromise (Egbert and Abraham 1999; Mégarbane et al. 2005; Scalley et al. 2002). Hemodialysis can effectively remove ethylene glycol and the acid metabolites, glycolic and oxalic acids, because they have low molecular weights and do not exhibit protein binding (Egbert and Abraham 1999). Hemodialysis is also effective in treating metabolic acidosis (Leth and Gregersen 2005; Scalley et al. 2002).

Thiamine (vitamin B_1) and pyroxidine (vitamin B_6) are co-factors for the metabolism of ethylene glycol. Thiamine is believed to reduce the formation of toxic oxalic acid by shifting glyoxylic acid metabolism to the less toxic α-hydroxy-β-ketoadipic acid (Egbert and Abraham 1999; Goldfrank et al. 2002). Pyroxidine, in the presence of magnesium, may promote the conversion of glyoxylic acid to glycine and benzoic acid, which also results in reduced toxic oxalic acid formation (Egbert and Abraham 1999; Gardner et al. 2004; Goldfrank et al. 2002; Leth and Gregersen 2005; Scalley et al. 2002). However, the efficacy of treatment with thiamine and pyroxidine has not been demonstrated in human cases of ethylene glycol poisoning.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

There are no documented methods for interfering with mechanisms of action for toxic effects of ethylene glycol and its potent metabolites. As described in Section 3.11.2, clinical procedures for treating ethylene glycol poisoning consist of measures focused on reduction of the body burden of parent compound and its metabolites that are responsible for ethylene glycol-induced adverse neurological, cardiovascular, metabolic, and renal effects.

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of ethylene glycol is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure

the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of ethylene glycol.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

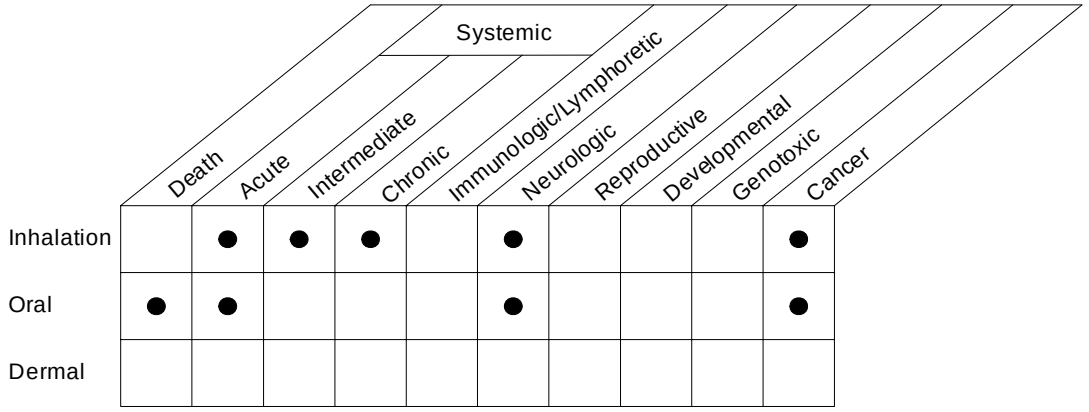
3.12.1 Existing Information on Health Effects of Ethylene Glycol

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to ethylene glycol are summarized in Figure 3-5. The purpose of this figure is to illustrate the existing information concerning the health effects of ethylene glycol. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

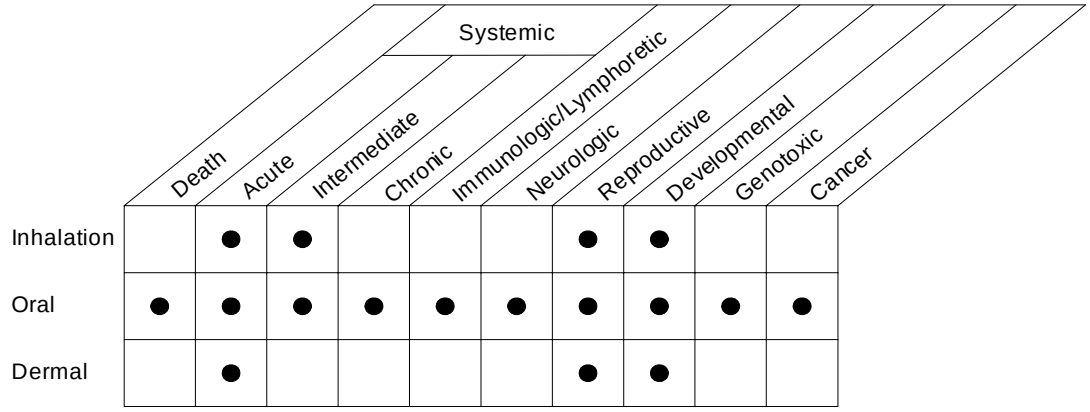
Information on the health effects of ethylene glycol inhalation in humans is limited to an experimental study of acute- to intermediate-duration exposure providing data on respiratory, renal, neurologic, and other systemic end points, an intermediate-duration study of kidney function in workers, and a chronic renal cancer mortality study in workers. Inhalation data in animals are also limited, consisting of three acute-duration developmental toxicity studies in rats and mice and an intermediate-duration systemic toxicity studies in rats, guinea pigs, rabbits, dogs, and monkeys.

Health effects data in orally exposed humans comprise numerous case reports of acute ingestion documenting the progression of neurologic, cardiovascular, renal, and other systemic effects. The health effects of ethylene glycol in orally exposed animals are generally well documented in acute-, intermediate-, and chronic-duration studies of systemic, reproductive, and developmental toxicity and

Figure 3-5. Existing Information on Health Effects of Ethylene Glycol



Human



Animal

● Existing Studies

carcinogenicity in rats and mice. A limited amount of information is available on the immunologic, lymphoreticular, and neurological effects of oral exposure in animals.

Information on the health effects of dermal exposure to ethylene glycol is essentially limited to two acute-duration studies in animals investigating skin and eye irritation in rabbits and developmental toxicity in mice.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. Information on the toxicity of acute-duration inhalation exposure to ethylene glycol is available from an experimental study in humans (Wills et al. 1974) and three developmental toxicity studies in rats and mice (Tyl 1988a; Tyl et al. 1995a, 1995b). In the human study, exposure to ethylene glycol aerosol at an average concentration of 23 mg/m^3 for 20–22 hours/day for 14 days was well-tolerated in 19 subjects with effects that were essentially limited to occasional complaints of mild upper respiratory tract irritation; there were no indications of renal or other systemic effects as shown by urinalysis and hematology, clinical chemistry, and neurobehavioral evaluations (Wills et al. 1974). Short-term (e.g., 15-minute), high-exposure sessions showed that respiratory tract irritation became common at approximately 140 mg/m^3. The developmental studies (Tyl 1988a; Tyl et al. 1995a, 1995b) are limited by confounding oral exposures, as discussed in data needs for Developmental Toxicity, but collectively suggest that 150 mg/m^3 is a conservative NOAEL for developmental toxicity in rats and kidney toxicity in mice. This concentration is similar to the 140 mg/m^3 LOAEL for respiratory tract irritation in humans (Wills et al. 1974). The human NOAEL of 23 mg/m^3 is a suitable basis for acute inhalation MRL derivation because it is based on evaluations for renal and other systemic effects as well as local irritation, and is within the NOAEL range for developmental toxicity in animals. Additional studies would help to better define the threshold for respiratory irritation in humans, particularly for acute exposures longer than several minutes, and confirm that the respiratory tract is the most sensitive target for acute exposure. These inhalation studies could also address the potential increased toxicity of ethylene glycol when it is heated (e.g., through its use as an automobile antifreeze/coolant).

Information on effects of acute-duration oral exposure to ethylene glycol is available from human case reports (Godolphin et al. 1980; Gordon and Hunter 1982; Hewlett et al. 1986; Jacobsen et al. 1984; Siew et al. 1975a; Zeiss et al. 1989), a 10-day drinking water study in rats (Robinson et al. 1990), a 4-day gavage study examining effects on hematology and reproductive organs (Hong et al. 1988), and developmental toxicity studies in mice, rats, and rabbits (Maronpot et al. 1983; Marr et al. 1992; Neeper-

Bradley et al. 1995; Price et al. 1985; Schuler et al. 1984; Tyl 1989; Tyl et al. 1993). The available human studies consist of clinical case reports of high-dose intentional or accidental ingestion of ethylene glycol, and thus, are not suitable for dose-response assessment or MRL consideration. The 4-day gavage study (Hong et al. 1988) identified bone marrow effects in mice at doses of 50–250 mg/kg/day; these included suppressed granulocyte-macrophage progenitor formation at ≥50 mg/kg/day, bone marrow hypocellularity at ≥100 mg/kg/day, and suppressed iron uptake in the bone marrow at 250 mg/kg/day. The biological significance of these effects is uncertain in light of the lack of supporting evidence for effects on bone marrow, spleen, or hematology in longer-duration studies of mice and rats exposed to much higher doses (DePass et al. 1986a; Gaunt et al. 1974; Melnick 1984; NTP 1993; Robinson et al. 1990; Schladt et al. 1998). Additional testing for acute bone marrow effects is needed to corroborate the findings of the Hong et al. (1988) study, clarify their biological significance, and confirm that bone marrow effects are not an appropriate basis for MRL derivation. The remaining animal studies collectively identify the developing fetus as the most sensitive target of acute oral exposure to ethylene glycol. Among the developmental toxicity studies, the study by Neeper-Bradley et al. (1995; Tyl 1989) in mice identified the lowest LOAEL (500 mg/kg/day for increased incidence of total malformations and bilateral extra rib 14), and dose-response data for these effects were used in deriving the acute oral MRL for ethylene glycol.

Information on the acute dermal toxicity of ethylene glycol is limited to one study in rabbits that found minimal skin and eye irritation following single applications (Clark et al. 1979) and one negative developmental toxicity study in mice exposed to 3,549 mg/kg/day for 6 hours/day on Gd 6–15 (Tyl 1988b; Tyl et al. 1995c). Additional dermal studies would be helpful in evaluating the potential for systemic toxicity by this route of exposure.

Intermediate-Duration Exposure. Information on the toxicity of intermediate-duration inhalation exposure to ethylene glycol is available from two studies in humans (Gérin et al. 1997; Wills et al. 1974) and one multiple species study in animals (Coon et al. 1970). In one of the human studies, health effects were assessed in 19 subjects who were exposed to ethylene glycol aerosol at an average concentration of 30 mg/m^3 for 20–22 hours/day for 30 days (Wills et al. 1974). Effects were essentially limited to occasional complaints of mild upper respiratory tract irritation; there were no indications of renal or other systemic effects as shown by urinalysis and hematology, clinical chemistry, and neurobehavioral evaluations. This study identified a NOAEL of 30 mg/m^3 for respiratory irritation and systemic effects for near-continuous exposure to ethylene glycol for 30 days. The other human study found no effects on kidney function in 33 male aviation workers who were intermittently exposed to ethylene glycol during

airplane de-icing operations during a 2-month winter period (Gérin et al. 1997), but identification of a NOAEL is precluded by inadequate monitoring data. In the animal study, rats, guinea pigs, and small numbers of rabbits, dogs, and monkeys were exposed to ethylene glycol aerosol in concentrations of 0, 10, or 57 mg/m^3 for 8 hours/day, 5 days/week for 6 weeks, or to 0 or 12 mg/m^3 continuously for 90 days (Coon et al. 1970). The 6-week intermittent exposure study identified a NOAEL of 57 mg/m^3 for kidney histopathology and other systemic effects in all species. Continuous exposure to 12 mg/m^3 for 90 days caused ocular irritation and/or mortality in rats, rabbits, and guinea pigs (Coon et al. 1970), but confidence in this LOAEL is low due to small numbers of animals and likely confounding by oral exposure from ingestion of aerosol deposited on the fur, and its relevance is unclear because there were no eye irritation or other effects in humans near-continuously exposed to 30 mg/m^3 for 30 days (Wills et al. 1974). The human NOAEL of 30 mg/m^3 is not a suitable basis for intermediate-duration MRL derivation due to insufficient information on renal and other possible systemic effects of exposures longer than 30 days. Although exposures as long as 90 days were conducted in the animal study, it was limited in scope (e.g., lacked sufficient numbers of animals and urinalysis) and likely confounded by oral exposure. A well-designed, intermediate-duration systemic toxicity study in animals is needed to provide a sufficient basis for inhalation MRL derivation.

Information on intermediate-duration oral toxicity of ethylene glycol essentially consists of several generally well-designed studies in rats (Cruzan et al. 2004; Gaunt et al. 1974; Melnick 1984; Robinson et al. 1990) and mice (Melnick 1984; NTP 1993). These studies consistently showed that the kidney is the predominant and most sensitive target of intermediate-duration oral exposure, and that renal toxicity varied with sex, species, and strain, with males more sensitive than females, rats more sensitive than mice, and Wistar rats more sensitive than other strains of rats. The 16-week studies of Cruzan et al. (2004) and Gaunt et al. (1974) were considered for intermediate oral MRL consideration because they provide dose-response data for the critical effect in the most sensitive species, strain, and sex (i.e., kidney lesions in male Wistar rats). The NOAEL and LOAEL values were 150 and 500 mg/kg/day in the Cruzan et al. (2004) study and 71 and 180 mg/kg/day in the Gaunt et al. (1974) study. Although Gaunt et al. (1974) identified a lower apparent LOAEL, this study is not suitable for MRL consideration because the animal care was questionable and the daily dose was not constant. Nearly all of the rats showed evidence of respiratory infection (pneumonia) and infectious salivary adenitis, either of which could have confounded the results. Chemical intake varied throughout the study because the amount of ethylene glycol in the diet was not adjusted for changing food consumption and body weight. The Cruzan et al. (2004) study does not have the limitations of the Gaunt et al. (1974) study, but the data set for the critical effect (crystal nephropathy) is not appropriate for BMD analysis because the incidences increased from

0% in rats exposed to ≤150 mg/kg/day to 100% at ≥500 mg/kg/day (i.e., at all higher doses). Basing the intermediate-duration MRL on the NOAEL of 150 mg/kg/day yields a value that is higher than the acute-duration oral MRL. Because available evidence indicates that the acute-duration oral MRL for ethylene should be protective for kidney effects following longer-term exposure, the acute-duration value was adopted for intermediate-duration exposure. An intermediate-duration oral toxicity study in male Wistar rats that provides dose-response data for kidney effects in the 150–300 mg/kg/day dose range is needed to provide a suitable basis for MRL derivation.

No information is available on the intermediate-duration dermal toxicity of ethylene glycol. Studies using the dermal route would be useful because absorption and systemic distribution of ethylene glycol has been shown in dermal toxicokinetic studies in rats and mice (Frantz et al. 1989, 1991, 1996b, 1996c).

Chronic-Duration Exposure and Cancer. Information on the health effects of chronic inhalation exposure to ethylene glycol is essentially limited to the negative results of an epidemiologic study on renal cancer mortality in humans (Bond et al. 1985). This study is not suitable for assessing chronic inhalation toxicity because it lacks noncancer end points, measured exposure concentrations, and other relevant information. Chronic testing in animals is needed to provide a basis for chronic inhalation MRL derivation and to adequately assess the potential for inhalation carcinogenicity.

Chronic oral effects of ethylene glycol have been evaluated in three studies in rats (Blood 1965; DePass et al. 1986a; Wilson et al. 2005) and two studies in mice (DePass et al. 1986a; NTP 1993) using dietary exposure. None of the studies provided evidence of carcinogenicity. The main target organs for toxicity were the kidneys in rats and liver in mice, and rats were more sensitive than mice. Effects in the rats included kidney lesions and mortality at ≥300 mg/kg/day in Wistar males (Wilson et al. 2005), kidney lesions at ≥375 mg/kg/day and mortality at 750 mg/kg/day in Sprague-Dawley males (Blood 1965), and kidney lesions and mortality at 1,000 mg/kg/day in F344 males (DePass et al. 1986a). The study in male Wistar rats (Wilson et al. 2005) is the most appropriate basis for chronic MRL derivation because it identified the lowest LOAEL (300 mg/kg/day) and is the only study providing information on effects of chronic exposure in Wistar rats, a strain shown to be approximately twice as sensitive as F344 rats to kidney toxicity in a 16-week study (Cruzan et al. 2004). The data set for the critical effect in this study, oxalate nephropathy is not appropriate for BMD analysis because the incidences increased from 0% in the rats exposed to ≤150 mg/kg/day to 92% at 300 mg/kg/day (next highest dose) and 100% at 400 mg/kg/day (highest dose). Basing the MRL on the NOAEL of 150 mg/kg/day yields an intermediate-duration MRL that is higher than the acute-duration oral MRL. It is against ATSDR policy to derive a

chronic-duration MRL that is higher than the acute-duration MRL, although available evidence indicates that the acute MRL should be protective for chronic kidney effects. A chronic oral study in male Wistar rats that provides dose-response data for kidney effects in the 150–300 mg/kg/day dose range is needed to provide an appropriate basis for chronic-duration MRL derivation. This study could also be used to confirm the lack of carcinogenicity in the available studies.

Genotoxicity. Human genotoxicity data were not located for ethylene glycol. A single *in vivo* study was located in which ethylene glycol did not produce dominant lethality in orally-exposed rats (DePass et al. 1986b). Available *in vitro* assays in a variety of test systems consistently provide negative results for genotoxicity (Abbondandolo et al. 1980; Clark et al. 1979; Griffiths 1979, 1981; Hastwell et al. 2006; Kubo et al. 2002; McCann et al. 1975; McCarroll et al. 1981; McGregor et al. 1991; Miller et al. 2005; Pfeiffer and Dunkelberg 1980; Storer et al. 1996; Zeiger et al. 1987). Additional *in vivo* animal studies could be conducted to more completely assess the genotoxicity of ethylene glycol, although available data do not indicate that the compound is of genotoxicity concern.

Reproductive Toxicity. Studies have not addressed the reproductive toxicity of ethylene glycol in humans. Reproductive testing in animals includes three multigeneration studies (one in rats and two in mice) and several shorter studies (15–20 days in rats and mice) by the oral route (DePass et al. 1986b; Harris et al. 1992; Lamb et al. 1985; Morrissey et al. 1989; NTP 1986. 1988). The only effect in rats was an increase in gestational duration, whereas fertility and fetal viability were affected in mice. Mice also showed some changes in sperm parameters, as well as testicular and epididymal lesions (Morrissey et al. 1989; NTP 1986); however, the incidence of testicular effects was high in the control group, so the relationship to ethylene glycol exposure is uncertain. Additional reproductive testing may not be needed because several multigeneration studies have been conducted, most studies suggest that reproductive effects occur at higher doses than developmental effects, and there are no toxicokinetic data suggesting that reproductive effects would be route-specific.

Developmental Toxicity. Studies have not addressed the developmental toxicity of ethylene glycol in humans. The developmental toxicity of oral exposure to ethylene glycol has been studied in rats, mice and rabbits over a wide range of doses (DePass et al. 1986b; Harris et al. 1992; Lamb et al. 1985; Maronpot et al. 1983; Marr et al. 1992; Morrissey et al. 1989; Neeper-Bradley 1990; Neeper-Bradley et al. 1995; NTP 1986; Price et al. 1985; Schuler et al. 1984; Tyl 1989; Tyl et al. 1993), indicating that further evaluation of the developmental toxicity of orally-administered ethylene glycol toxicity may not be warranted. The most sensitive indicator of developmental toxicity appears to be an increased

incidence of malformations, primarily skeletal malformations, in both mice and rats. Available data suggest that malformations appear in mice at lower doses than those which cause malformations in rats. As indicated in the discussion of data needs for Acute-Duration Exposure, the acute oral MRL for ethylene glycol is based on developmental effects in mice exposed to ethylene glycol daily by gavage on Gd 6–15 (Neeper-Bradley et al. 1995; Tyl 1989). An uncertainty in the acute-duration oral MRL that may need to be addressed stems from the use of gavage administration in the MRL study. Bolus doses from gavage administration lead to higher peak blood concentrations of glycolic acid (the proximate developmental toxicant) than occur with slower dose-rates associated with environmentally-relevant exposures (Carney et al. 2001; Corley et al. 2002; NTP-CERHR 2004). Because the MRL study used gavage administration, the dose at which effects were observed is likely lower than would be observed with non-bolus dosing.

Developmental toxicity has also been assessed in rats and mice by the inhalation route. Results of the inhalation developmental studies are generally consistent with the oral findings, but are confounded by concurrent oral exposure via ingestion of aerosolized ethylene glycol on the fur of exposed animals (Tyl 1985, 1988a; Tyl et al. 1995a, 1995b). The studies included a nose-only inhalation study in mice aimed at reducing the confounding oral exposure, but these animals had exposure by ingestion of ethylene glycol deposited on the face (Tyl 1988a; Tyl et al. 1995a). Additionally, stress from restraint in the nose-only exposure study may have contributed to the developmental effects observed with ethylene glycol (NTP-CERHR 2004), which were similar in nature to effects observed in a study of restrained nose-only exposure to water vapor (Tyl et al. 1994). Because of the confounding oral exposure in both the whole-body and nose-only studies, as well as the confounding effect of stress due to restraint in the nose-only study, additional testing is needed to adequately evaluate developmental effect levels from inhalation exposure to ethylene glycol. Given the problems of oral exposure from deposition of ethylene glycol on the fur, the feasibility of conducting an adequate inhalation study is unclear.

A single well-designed study of dermal gestational exposure to ethylene glycol found no developmental toxicity in mice (Tyl 1988b; Tyl et al. 1995c). Additional dermal testing could confirm the apparent low potential for developmental toxicity by this route of exposure.

Immunological and Lymphoreticular Effects. A limited amount of information on immunological and lymphoreticular effects of ethylene glycol is available from oral studies in animals. There were no histopathological alterations in the spleen, lymph nodes, or thymus, or consistent changes in leukocyte counts in rats or mice in acute-, intermediate-, and chronic-duration oral studies of ethylene

glycol (Blood 1965; DePass et al. 1986a; Gaunt et al. 1974; Hong et al. 1988; Melnick 1984; NTP 1993; Robinson et al. 1990; Schladt et al. 1998). Immune responses were suppressed in mice administered a single 12,000 mg/kg (0.8 LD_{50}) gavage dose of ethylene glycol; effects included increased mortality from *E. coli*-induced infection, decreased number of spleen colony-forming units, decreased numbers of antibody-producing cells in spleen to sheep erythrocytes and Vi-agglutinin, decreased activity of natural killer cells, decreased antibody-dependent cytotoxicity of splenocytes to sheep erythrocytes, and decreased delayed-type hypersensitivity to sheep erythrocytes (Zabrodskii and Germanchuk 2000; Zabrodskii et al. 2003). Other dose levels were not tested in this study. Although the findings indicate that a very high single dose of ethylene glycol was immunotoxic in mice, no studies of immune function following repeated acute exposure (e.g., 14 days) or intermediate- or chronic-duration exposure have been conducted. Comprehensive immunological testing that includes a range of dose levels and repeated exposures is needed to adequately assess the immunotoxic potential of ethylene glycol.

Neurological Effects. Information on the neurotoxicity of inhaled ethylene glycol is essentially limited to a human study in which subjects were exposed to ethylene glycol aerosol for 20–22 hours/day for up to 30 days (Wills et al. 1974). No effects were seen in electroencephalographs or a battery of psychological tests conducted after 14 and 30 days of exposure (23 and 30 mg/m^3, respectively); the tests evaluated simple reaction time, reaction time with discrimination, visual-motor coordination, depth perception, and mental ability (encoding and subtraction accuracy). There were no clear clinical signs of neurotoxicity; slight headache and backache were occasional complaints, but incidence and frequency were not reported. No human or animal studies were located that provide information on neurological effects of dermal exposure.

The neurotoxicity of acute oral exposure to large doses of ethylene glycol is well characterized in humans. Adverse neurological reactions are among the first symptoms to appear in human ethylene glycol poisoning. These symptoms are attributable directly to unmetabolized ethylene glycol, resemble ethanol intoxication, occur within 30 minutes to 12 hours after exposure, and include ataxia, disorientation, restlessness, slurred speech, and somnolence, progressing to convulsions and coma (Cheng et al. 1987; Factor and Lava 1987; Gordon and Hunter 1982; Robinson and McCoy 1989; Vale 1979; Woolf et al. 1992). Some evidence exists that damage to the cranial nerves may occur much later in the toxic process, especially if supportive therapy is delayed (Chung and Tuso 1989; Factor and Lava 1987; Lewis et al. 1997; Mallya et al. 1986; Spillane et al. 1991).

Clinical signs of neurotoxicity similar to those in humans summarized above occurred in rats, dogs, and cats following administration of large oral bolus doses of ethylene glycol (Beckett and Shields 1971; Clark et al. 1979; Dial et al. 1994; Grauer et al. 1987; Penumarthy and Oehme 1975). No clinical signs of neurotoxicity or histopathological changes in brain, spinal cord, or peripheral nerve tissue were observed in rats or mice exposed to ethylene glycol in the diet or drinking water in acute-, intermediate-, or chronic-duration studies (Blood 1965; DePass et al. 1986a; Gaunt et al. 1974; Melnick 1984; NTP 1993; Robinson et al. 1990; Schladt et al. 1998). Tests of neurobehavioral function have not been conducted in orally-exposed animals. Although there were no effects on neurobehavioral function in humans exposed by inhalation (Wills et al. 1974), neurobehavioral testing in orally-exposed animals is needed to adequately assess the neurotoxic potential of lower doses of ethylene glycol.

Epidemiological and Human Dosimetry Studies. A limited amount of epidemiological data on ethylene glycol is available from two studies of workers mainly exposed by inhalation with possible secondary exposure by the dermal route. One of these occupational studies evaluated kidney function in a small number of aviation workers who were intermittently exposed to ethylene glycol during airplane de-icing operations over a 2-month winter period (Gérin et al. 1997). Personal exposures to ethylene glycol vapor and aerosol were measured, but most samples were below the detection limit and average levels were not reported. This study found no indication of renal impairment based on a limited number of urinary end points (albumin, β-*N*-acetyl-glucosaminidase, β-2-microglobulin, and retinol-binding protein). The other study assessed renal cancer mortality in 1,666 chemical plant employees and found no increase in a small number of workers exposed to unmeasured levels of ethylene glycol (Bond et al. 1985). Epidemiological studies of orally-exposed humans are not available, although numerous clinical case reports of intentional or accidental ingestion have documented neurological, renal, and other effects of high acute doses of ethylene glycol. The available information suggests that ethylene glycol is likely to cause effects in humans similar to those found in animals. Additional epidemiological studies investigating dose-response relationships between ethylene glycol exposure and likely target organ toxicity would be useful. Potential study populations include individuals exposed through dermal contact with ethylene glycol-containing automobile antifreeze and individuals who live near hazardous waste sites, industrial facilities where ethylene glycol is produced or used, or areas where ethylene glycol-based de-icing formulations are used and may be exposed through dermal contact with contaminated soil or water, inhalation of ethylene glycol vapor or mist, or ingestion of contaminated groundwater. Additionally, occupational exposure through inhalation of ethylene glycol vapor or mist and dermal contact is expected for individuals involved in airport de-icing spray operations. Background exposure of the general population is not expected to be important because ethylene glycol is rapidly degraded in air,

water, and soil, and available monitoring data indicate that it is only found near areas of release (Atkinson 1989; Battersby and Wilson 1989; Conway et al. 1983; Kameya et al. 1995; McGahey and Bouwer 1992; Revitt and Worrall 2003; Schoenberg et al. 2001; Staples et al. 2001).

Biomarkers of Exposure and Effect.

Exposure. The only biomarker of exposure that is specific to ethylene glycol is parent compound in the blood and urine. Based on the relatively short half-life of ethylene glycol in the blood and urine (Eder et al. 1998; Jacobsen et al. 1988; Peterson et al. 1981), parent compound would likely be detectable only within a few hours to 1 day following acute ingestion. Rapid methods for determining ethylene glycol in serum and urine are available for use in the clinical setting (Aarstad et al. 1993; Blandford and Desjardins 1994), but are often not readily available in emergency situations.

Other identified biomarkers of exposure are not specific to ethylene glycol. They include ethylene glycol metabolites such as glycolic, lactic, and oxalic acids in blood and/or urine; and calcium oxalate monohydrate crystals in renal tubules and/or urine. However, increased blood glycolate above normal human background levels is strongly indicative of ethylene glycol exposure and is often used for clinical diagnosis or confirmation.

Based on available information regarding the toxicokinetics of ethylene glycol and its metabolites, and available methods for identifying parent compound and metabolites in body fluids, it appears that ethylene glycol poisoning can be adequately diagnosed in most cases. Additional studies to assess additional potential biomarkers of exposure for ethylene glycol do not appear necessary at this time.

Effect. Biomarkers of effects exist for ethylene glycol poisoning, but none are specific to ethylene glycol. These include clinical manifestations of central nervous system, cardiopulmonary, and renal toxicity, and laboratory findings of metabolic acidosis and calcium oxalate crystalluria. Clinical manifestations progress in three main stages. Signs of central nervous system toxicity appear within 0.5–12 hours following acute ingestion, although manifestations suggestive of cranial nerve damage may appear as late as 1–2 weeks after exposure (CDC 1987; Cheng et al. 1987; Chung and Tuso 1989; Factor and Lava 1987; Hess et al. 2004; Leth and Gregersen 2005; Lewis et al. 1997; Mallya et al. 1986; Parry and Wallach 1974; Rothman et al. 1986; Spillane et al. 1991; Underwood and Bennett 1973; Zeiss et al. 1989). Cardiopulmonary manifestations generally develop after 12–24 hours and renal failure occurs after 24–72 hours (Godolphin et al. 1980; Hess et al. 2004; Leth and Gregersen 2005; Parry and Wallach

1974; Siew et al. 1975a; Vale 1979; Zeiss et al. 1989). Ethylene glycol-induced metabolic acidosis occurs approximately 12–24 hours following ingestion and is characterized by pronounced serum osmolal and anion gaps (Eder et al. 1998; Hess et al. 2004; Hoffman et al. 1993; Gabow et al. 1986; Jacobsen et al. 1984; Leth and Gregersen 2005). Calcium oxalate crystals in the urine can appear 4–8 hours after ethylene glycol ingestion, and deposition of crystals in the renal tubules can subsequently result in nephropathy and eventual renal failure 24–72 hours after ingestion (CDC 1987; Chung and Tuso 1989; Factor and Lava 1987; Godolphin et al. 1980; Heckerling 1987; Jacobsen et al. 1988; Leth and Gregersen 2005; Parry and Wallach 1974; Rothman et al. 1986; Siew et al. 1975a; Underwood and Bennett 1973). Based on the well-characterized sequence of events and toxicity targets of ethylene glycol poisoning, studies for additional biomarkers of effect for ethylene glycol do not appear necessary.

Absorption, Distribution, Metabolism, and Excretion. Additional data are needed to quantify the absorption, distribution, metabolism, and excretion of inhaled ethylene glycol across relevant concentration ranges in humans and animals. Only one study evaluating toxicokinetic data in humans exposed via inhalation was identified (Carstens et al. 2003). However, this study is limited by inadequate definition of exposure levels and a lack of data on expired CO_2. Similarly, one study of the toxicokinetics of inhaled ethylene glycol in animals used only a single vapor and a single aerosol concentration (Marshall and Cheng 1983).

Although the human oral absorption of ethylene glycol has not been quantitatively characterized, case reports indicate that humans can absorb toxicologically significant amounts of this compound by the oral route (Godolphin et al. 1980; Gordon and Hunter 1982; Hewlett et al. 1986; Jacobsen et al. 1984, 1988; Karlson-Stiber and Persson 1992; Litovitz et al. 1990, 1991; Peterson et al. 1981; Siew et al. 1975a; Walton 1978; Zeiss et al. 1989). In addition, several case reports (Cheng et al. 1987; Hewlett et al. 1986; Jacobsen et al. 1984, 1988; Peterson et al. 1981; Rothman et al. 1986) provide data on plasma levels of ethylene glycol or glycolate in humans acutely poisoned with ethylene glycol. While the tissue levels of ethylene glycol in humans exposed orally have not been studied, information on the volume of distribution and urine-to-plasma concentration ratios suggest distribution with total body water (Jacobsen et al. 1988). Data on the distribution of radioactivity in mice, rats, and monkeys exposed orally support this finding (Frantz et al. 1989, 1991, 1996b, 1996c; McChesney et al. 1971).

The *in vivo* metabolism of ethylene glycol has been thoroughly studied in rats and mice exposed via intravenous, oral, and dermal routes (Frantz et al. 1989, 1991, 1996b, 1996c). Data on plasma and urinary metabolites from these studies support the widely accepted metabolic scheme for ethylene glycol.

Because metabolites of ethylene glycol (glycolic and oxalic acids) have been identified as the probable proximate toxicants (for both renal and developmental effects), additional data on the specific isozymes responsible for metabolizing ethylene glycol and glycolic acid, inter-individual variability in metabolic parameters (e.g., polymorphisms in genes encoding these isozymes), and developmental ontogeny of these isozymes are needed to better characterize species differences and identify sensitive subpopulations. In addition, further information is needed on species differences in metabolic rates and saturation points, as available data provide inadequate information on the relative sensitivity of humans and laboratory rodents.

Because most human exposure has been associated with acute accidental or intentional poisoning incidents, there are few data on the elimination kinetics of ethylene glycol after oral exposure in humans. Most of the available estimates of plasma elimination half-lives have been confounded by concurrent therapeutic treatments such as ethanol administration or hemodialysis that modify elimination kinetics. Elimination of orally-administered ethylene glycol across a broad dose range has been thoroughly studied in rats and mice (Frantz et al. 1989, 1991, 1996b, 1996c), and to a more limited extent in monkeys (McChesney et al. 1971).

No data describing the kinetics of *in vivo* human dermal exposure were found in the literature. The *in vitro* permeability of human skin to ethylene glycol has been studied, with widely varying results. Using full-thickness cadaver skin, Loden (1986) estimated a percutanous absorption rate of 118 μg/cm^2/hour with a steady-state concentration of 0.97 mg/cm^2, while Driver et al. (1993) estimated absorption rates of 0.09–0.25 μg/cm^2/hour for three different skin samples. Although the absorption, distribution, metabolism, and elimination of ethylene glycol administered dermally has been thoroughly studied in rats and mice (Frantz et al. 1989, 1991, 1996b, 1996c), acute *in vivo* studies in humans are needed to better characterize the toxicokinetics for this route of exposure.

All of the toxicokinetic data in humans and animals were collected after acute exposures to ethylene glycol; there are no data on toxicokinetics after intermediate- or chronic-duration exposures. Intermediate- and chronic-duration data are needed in order to adequately assess absorption, metabolism, and elimination with prolonged exposure. Studies with heated ethylene glycol would be useful due to the potential increased toxicity of ethylene glycol when it is heated (e.g., through its use as an automobile antifreeze/coolant).

Comparative Toxicokinetics. Species differences in *in vivo* toxicokinetics are not well characterized. While there are high quality toxicokinetic data comparing absorption, distribution, metabolism, and excretion in mice and rats (Frantz et al. 1989, 1991, 1996a, 1996b, 1996c), available data in other species (Hewlett et al. 1989; McChesney et al. 1971) are more limited; in many cases, only single dose levels were used, the numbers of animals per dose were small, and mass balance information was incomplete. Available data in humans are limited to acute, high-dose exposures, with toxicokinetic data often confounded by the effects of therapeutic interventions.

Using a PBPK model for humans, Corley et al. (2005a) estimated that the threshold glycolic acid concentration for developmental effects in rodents (considered by the authors to be a peak of 2 mM) would only be reached in human females ingesting doses of 350 mg/kg (assuming a 58-kg female). However, the model was not calibrated to pregnancy, and it is not clear whether physiological and/or biochemical changes that could affect ethylene glycol toxicokinetics might occur during pregnancy.

Slikker et al. (2004) reported that there are species-specific differences in the transfer of glycolic acid, the primary metabolite and putative developmental toxicant associated with ethylene glycol exposure, from maternal blood to conceptus. NTP-CERHR (2004) noted that the inverted yolk sac placenta that develops in both mice and rats tends to concentrate weak acids including glycolic acid; neither humans nor rabbits develop a yolk sac placenta. A preliminary study by Carney and coworkers (2001) showed that glycolic acid does not concentrate in rabbit embryonic fluids, while Corley et al. (2002) have shown in rats that glycolic acid is consistently higher in the conceptus compared to the maternal blood. In addition, fetal and/or placental differences in expression of enzymes metabolizing ethylene glycol and glycolic acid over the course of gestation will affect local concentrations of glycolic acid to which the developing conceptus is exposed, yet little is known about species differences in the ontogeny of these enzymes (NTP-CERHR 2004).

Additional data are needed to reduce uncertainty in the saturation concentration of glycolic acid in humans. Although *in vitro* data suggested that humans may metabolize glycolic acid more efficiently than rats (Corley et al. 2005a; Booth et al. 2004), there are no *in vivo* human data to confirm this observation.

Methods for Reducing Toxic Effects. No studies were found describing methods to reduce peak absorption of ethylene glycol after inhalation exposure. After oral exposure, gastric lavage and possibly activated charcoal may be of benefit in reducing absorption, but only if performed within 1–2 hours

following ingestion (Barceloux et al. 1999; Egbert and Abraham 1999; Leth and Gregersen 2005). Dermal absorption can be minimized through washing the skin with soap to remove any existing ethylene glycol. Copious irrigation with water or saline can aid in ocular decontamination.

Clinical procedures for treating ethylene glycol poisoning focus on reducing the body burden of ethylene glycol and its toxic metabolites, interference with toxic metabolite formation (which results in increased urinary excretion of parent compound), increased elimination of toxic metabolites produced, reduction of metabolic acidosis, and prevention of kidney failure. Procedures include administration of antidotes (ethanol or fomepizole), intravenous bicarbonate and hydration for profound acidemia, and hemodialysis for refractory acidosis (Barceloux et al. 1999; Egbert and Abraham 1999; Gardner et al. 2004; Leth and Gregersen 2005; Scalley et al. 2002).

Antidotes for ethylene glycol include the alcohol dehydrogenase inhibitors, ethanol and fomepizole, which act to decrease the alcohol dehydrogenase-catalyzed metabolism of ethylene glycol, thus effectively increasing the urinary excretion of ethylene glycol (Barceloux et al. 1999; Egbert and Abraham 1999; Gardner et al. 2004; Leth and Gregersen 2005).

Intravenous fluid administration may be initiated early to increase urine output, which effectively increases the excretion of ethylene glycol and toxic metabolites such as glycolic and oxalic acids (Egbert and Abraham 1999). Sodium bicarbonate infusion is used to correct metabolic acidosis, increase elimination of renal glycolic acid, and inhibit the precipitation of calcium oxalate crystals (Egbert and Abraham 1999; Leth and Gregersen 2005; Scalley et al. 2002).

Hemodialysis can effectively remove ethylene glycol and the acid metabolites, glycolic and oxalic acids, because they have low molecular weights and do not exhibit protein binding (Egbert and Abraham 1999).

Thiamine (vitamin B_1) and pyroxidine (vitamin B_6) are co-factors for the metabolism of ethylene glycol and may reduce toxicity by assisting in the formation of relatively nontoxic metabolites (Egbert and Abraham 1999; Gardner et al. 2004; Goldfrank et al. 2002; Leth and Gregersen 2005; Scalley et al. 2002). However the efficacy of treatment with thiamine and pyroxidine has not been demonstrated in human cases of ethylene glycol poisoning.

There are no documented methods for interfering with mechanisms of action for toxic effects of ethylene glycol and its potent metabolites.

Additional information that might be useful in treating ethylene glycol poisoning include studies designed to identify additional methods to reduce the body burden of ethylene glycol and its toxic metabolites and studies designed to elucidate methods for interfering with mechanisms of action.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

A limited amount of information on health effects of ethylene glycol in children is available from several case reports of patients admitted to hospitals for treatment of acute oral poisoning (Baum et al. 1999; Boyer et al. 2001; Caravati et al. 2004; Harry et al. 1998). The effects in these pediatric patients were largely consistent with the first stage of ethylene glycol poisoning in adults (e.g., central nervous system depression, metabolic changes, gastrointestinal upset). Treatment with fomepizole (4-methylpyrazole), alone or in combination with other methods, generally mitigated the progression of the clinical course to the second and third stages of ethylene glycol poisoning (pronounced metabolic acidosis, cardiopulmonary compromise, and renal insufficiency) and led to full recovery. The case reports are consistent with an expectation that health effects in children and adults are similar. Although there are no known differences in the toxicity of ethylene glycol between adults and children, there is no evidence to substantiate the presumption. There is no evidence to indicate that children are likely to be exposed to higher or lower amounts of ethylene glycol from everyday living, suggesting that children are perhaps equally at risk for non-accidental/non-intentional acute oral exposure and potential toxic side effects. Information is lacking on the toxicity of longer duration exposures in children, as well as on developmental effects in children.

No studies are available that describe potential differences in the toxicokinetics or the mechanism of action of ethylene glycol in children. Glycolic acid is the major toxic metabolite contributing to metabolic acidosis, which is a primary cause of systemic toxicity in children as well as adults following exposure to ethylene glycol. Glycolic acid has also been identified as the proximate cause of the developmental effects in animals observed with ethylene glycol exposure (NTP-CERHR 2004; Slikker et al. 2004).

Limited mechanistic information suggests that humans may be less sensitive than rodents to the developmental effects of ethylene glycol. Two *in vitro* studies (Booth et al. 2004; Corley et al. 2005a)

suggested that humans metabolize glycolic acid more efficiently than rats, although the data supporting the glycolic acid metabolic rate in humans are limited (NTP-CERHR 2004). Additionally, NTP-CERHR (2004) reviewed preliminary data indicating that the inverted yolk sac placenta, a stage in placental development that occurs in rats and mice but does not exist in humans or rabbits, tends to concentrate weak acids such as glycolic acid in the embryonic fluids. These data suggest enhanced sensitivity to ethylene glycol developmental effects in rodents compared with humans, although NTP-CERHR (2004) characterized the available data as inconclusive.

Ethylene glycol metabolism is known to involve alcohol dehydrogenase and aldehyde dehydrogenase, and may also involve cytochrome P450 isozymes (NTP-CERHR 2004). Polymorphisms in the genes encoding these enzymes may lead to wide variability in the production and elimination of glycolic acid and other metabolites in humans exposed to ethylene glycol, but data quantifying the range of variability are not currently available (NTP-CERHR 2004). In addition, fetal and/or placental differences in expression of these enzymes over the course of gestation will affect local concentrations of glycolic acid and other metabolites to which the developing conceptus is exposed, yet little is known about these differences (NTP-CERHR 2004).

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

No relevant health effects studies for ethylene glycol were located in the Federal Research in Progress database (FEDRIP 2007).

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4. CHEMICAL AND PHYSICAL INFORMATION

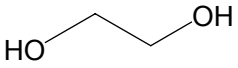
4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of ethylene glycol is located in Table 4-1. This information includes synonyms, chemical formula and structure, and identification numbers.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of ethylene glycol is located in Table 4-2.

Table 4-1. Chemical Identity of Ethylene Glycol[a]

Characteristic	Information
Chemical name	Ethylene glycol
Synonyms and trade names	1,2-Dihydroxyethane; 1,2-ethandiol; 1,2-ethane-diol; 2-hydroxyethanol; ethylene alcohol; ethylene dihydrate; glycol; monoethylene glycol; MEG; Lutrol-9; Dowtherm Sr 1; Fridex; Norkool; Ramp; Tescol; Ucar 17
Chemical formula	$C_2H_6O_2$
Chemical structure	
Identification numbers:	
CAS registry	107-21-1
NIOSH RTECS	KW2975000[b]
EPA hazardous waste	No data
DOT/UN/NA/IMDG shipping	No data
HSDB	5012
NCI	C00920

[a]All information obtained from HSDB 2007 except where noted.
[b]RTECS 2007

CAS = Chemical Abstracts Service; DOT/UN/NA/IMDG = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-2. Physical and Chemical Properties of Ethylene Glycol[a]

Property	Ethylene glycol
Molecular weight	62.07
Color	Clear, colorless[b]
Physical state	Liquid[b]
Melting point	-12.69 °C[c]
Boiling point	197.3 °C[c]
Density:	
at 20 °C (g/cm^3)	1.1135[d]
Vapor density	2.14 (air=1)
Odor	Odorless
Odor threshold	No data
Solubility:	
Water at 20 °C	Miscible with water
Organic solvent(s)	Soluble in lower aliphatic alcohols, glycerol, acetic acid, acetone; slightly soluble in ether; practically insoluble in benzene, chlorinated hydrocarbons, petroleum ether, oils.
Partition coefficients:	
Log K_{ow}	-1.36[e]
K_{oc}	1 (estimated)
Vapor pressure at 25 °C	0.089 mm Hg (extrapolated)[f]
Henry's law constant at 25 °C	$6x10^{-8}$ atm-m^3/mole[g]
Autoignition temperature	398 °C
Flashpoint	127 °C[h]
Explosive limits	3.20–53%[i]
Conversion factors	1 ppm = 2.58 mg/m^3 1 mg/m^3 = 0.39 ppm

[a]All information obtained from HSDB 2007, except where noted.
[b]Lewis 2001
[c]Lide 2005
[d]O'Neil et al. 2001
[e]Hansch et al. 1995
[f]AIChE 1995
[g]Hine and Mookerjee 1975
[h]Forkner et al. 2004
[i]Rebsdat and Mayer 2005

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5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.1 PRODUCTION

Commercial production of ethylene glycol in the United States began in 1925 (McClelland and Rector 1951; Miller 1966). The reported U.S. production volume was 2.189 million pounds (993 metric tons) during that year (McClelland and Rector 1951). Large-scale commercial use of ethylene glycol as an antifreeze began in 1930; this use led to a strong demand for ethylene glycol, especially during and after World War II (Brown et al. 1980; McClelland and Rector 1951). Production volume information is not available for this period. By 1950, the U.S. production volume had risen to 510 million pounds (230,000 metric tons) (Brown et al. 1980; McClelland and Rector 1951). Production continued to rise steadily, reaching approximately 900,000 metric tons by 1968 (Brown et al. 1980). Ethylene glycol production rose more dramatically in late 1960s and early 1970s due to the additional demand for use of this substance in the manufacturing of polyester fiber and film (Brown et al. 1980; CMR 1972, 1975). Production in 1970 was approximately 1,400,000 metric tons (Brown et al. 1980). Production fluctuated between 1,500,000 and 1,800,000 metric tons over the next 18 years, reaching 1,820,000 metric tons by 1978 (Brown et al. 1980).

Production volume information is not available for years following 1978; however, production capacity data for the 1980s, 1990s, and early 2000s have been located. Total reported U.S. production capacities were 2,638,000 metric tons during 1981, 2,820,000 metric tons during 1990, 3,727,000 metric tons during 2001, and 3,402,000 metric tons during 2006 (CMR 1981, 1990, 2001; SRI 2006). The growth of ethylene glycol production capacity over these decades has been attributed to the demand for this substance in the manufacture of plastics, especially polyethylene terephthalate (PET) resin bottles (CMR 1981, 1987, 1984, 1990, 1993, 1998, 2001, 2004). The companies that produced ethylene glycol in the United States, their production sites, and their annual capacities during 2006 (the most recent year for which figures are available) are shown in Table 5-1 (SRI 2006).

Table 5-2 summarizes the number of facilities in each state that manufactured or processed ethylene glycol in 2005, the ranges of maximum amounts on site, if reported, and the activities and uses as reported in the Toxics Release Inventory (TRI) (TRI05 2007). The data listed in this table should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list.

The first method used in the commercial production of ethylene glycol in the United States was the conversion of chlorohydrin to ethylene oxide and subsequent hydrolysis to ethylene glycol (Forkner et al.

Table 5-1. Companies that Produce Ethylene Glycol in the United States and Annual Capacities During 2006

Company	Location	Capacity (million pounds/year)	Capacity (metric tons)
The Dow Chemical Company	Plaquemine, Louisiana	550	249,500
	Seadrift, Texas	625	283,600
	Taft, Louisiana	1,700	771,300
Eastman Chemical Company			
Voridian Division	Longview, Texas	230	104,400
Equistar Chemicals, LP	Bayport, Texas	580	263,100
Formosa Plastics Corporation, USA	Point Comfort, Texas	693	314,400
Huntsman LLC			
Huntsman Performance Products	Port Neches, Texas	560	254,100
Old World Industries, Inc.	Pasadena, Texas	695	315,300
PD Glycol	Beaumont, Texas	790	358,400
Shell Chemical Company	Geismar Louisiana	1,075	487,700
Total		7,498	3,402,000

Source: SRI 2006

Table 5-2. Facilities that Produce, Process, or Use Ethylene Glycol

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AK	8	10,000	9,999,999	2, 3, 4, 7, 9, 12
AL	69	0	99,999,999	1, 2, 3, 6, 7, 8, 9, 10, 11, 12, 13, 14
AR	42	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
AZ	32	0	999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
CA	159	0	999,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14
CO	42	0	49,999,999	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12
CT	21	1,000	999,999	1, 5, 6, 7, 8, 9, 11, 12
DE	27	0	999,999	1, 5, 6, 7, 8, 10, 11, 12, 13
FL	62	0	49,999,999	2, 3, 4, 6, 7, 8, 9, 10, 11, 12
GA	99	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
HI	2	10,000	999,999	12
IA	74	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
ID	27	0	999,999	1, 2, 3, 5, 7, 8, 10, 11, 12
IL	147	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
IN	105	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
KS	58	0	999,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
KY	83	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14
LA	106	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
MA	64	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
MD	45	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
ME	18	1,000	999,999	1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 14
MI	134	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MN	65	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MO	108	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
MS	49	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
MT	18	1,000	999,999	2, 3, 4, 7, 8, 9, 10, 11, 12
NC	136	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
ND	10	1,000	9,999,999	7, 8, 9, 10, 11, 12
NE	24	0	9,999,999	2, 3, 4, 7, 8, 9, 10, 11, 12
NH	21	0	99,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
NJ	152	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
NM	25	100	999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
NV	15	0	999,999	1, 2, 3, 4, 5, 6, 7, 11, 12, 13
NY	82	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
OH	134	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
OK	54	0	9,999,999	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12
OR	44	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
PA	114	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13

Table 5-2. Facilities that Produce, Process, or Use Ethylene Glycol

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
PR	47	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
RI	31	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
SC	104	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
SD	5	1,000	99,999	7, 8, 11, 12
TN	108	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
TX	251	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
UT	30	100	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14
VA	98	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
VT	8	0	99,999	11, 12
WA	52	0	99,999,999	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 14
WI	92	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
WV	82	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
WY	14	100	9,999,999	2, 3, 4, 7, 9, 10, 11, 12

[a]Post office state abbreviations used
[b]Amounts on site reported by facilities in each state
[c]Activities/Uses:

1. Produce
2. Import
3. Onsite use/processing
4. Sale/Distribution
5. Byproduct
6. Impurity
7. Reactant
8. Formulation Component
9. Article Component
10. Repackaging
11. Chemical Processing Aid
12. Manufacturing Aid
13. Ancillary/Other Uses
14. Process Impurity

Source: TRI05 2007 (Data are from 2005)

2004; Rebsdat and Mayer 2005). A second method was introduced in 1937, the direct oxidation of ethylene to ethylene oxide followed by hydrolysis to ethylene glycol (Brown et al. 1980; Forkner et al. 2004). This soon became the primary method for the production of ethylene glycol and is currently the only method used in the United States (Brown et al. 1980; Forkner et al. 2004; Rebsdat and Mayer 2005).

Other methods that have been used to manufacture ethylene glycol include the direct oxidation of ethylene and synthesis from carbon monoxide, methanol, hydrogen, and formaldehyde (Forkner et al. 2004; Rebsdat and Mayer 2005). The methanol and formaldehyde used in the latter method is obtained from syngas, which is originally obtained from coal.

Ethylene oxide is converted to ethylene glycol through uncatalyzed neutral hydrolysis (pH 6–10) in the presence of a large excess of water at high temperatures and pressures (Forkner et al. 2004; Rebsdat and Mayer 2005). Selectivity of ethylene glycol is 89–91% in this process. The primary byproduct is diethylene glycol with higher glycols such as triethylene and tetraethylene glycols formed in smaller amounts. The product mixture is fed through a series of evaporators to remove the water and then through vacuum distillation for separation and refinement of the individual glycols.

5.2 IMPORT/EXPORT

Both U.S. imports and exports of ethylene glycol have increased since the 1970s. Annual ethylene glycol imports rose from 29,300 metric tons in 1977 to 289,000 metric tons in 2006, while annual exports rose from 56,800 metric tons in 1978 to 573,000 metric tons in 2006 (HSDB 2007; ITA 2007). From 2000 to 2006, the average annual U.S. import and export quantities were 317,000 and 556,000 metric tons, respectively (ITA 2007). Annual U.S. ethylene glycol import and export quantities reported for different years are listed in Table 5-3. Over 70% of the ethylene glycol imported into the United States during 2006 was imported from Saudi Arabia (114,846 metric tons) and Canada (93,669 metric tons) (ITA 2007).

5.3 USE

Ethylene glycol has been used in a wide variety of industrial applications because of its unique chemical and physical properties. Ethylene glycol dissolves in water and is miscible in alcohol and acetone, has the capacity to hold large amounts of heat before boiling, and lowers the freezing point of water (Lewis 2001; O'Neil et al. 2001; Rebsdat and Mayer 2005). In addition, ethylene glycol is hygroscopic (has the ability to absorb twice its weight in water), is suitable for use as an industrial humectant (drying agent), and

Table 5-3. U.S. Ethylene Glycol Imports and Exports by Year in Metric Tons

Year	Imports	Exports	Reference
1977	29,300	NA	HSDB 2007
1978	NA	56,800	HSDB 2007
1982	17,000	NA	HSDB 2007
1983	NA	31,600	HSDB 2007
1985	130,000	270,000	HSDB 2007
1986	156,980	NA	CMR 1987
1989	167,869	NA	CMR 1990
1994	239,000	423,000	CMR 1996
1997	172,000	898,000	CMR 1998
2000	287,000	534,000	ITA 2007
2001	332,000	334,000	ITA 2007
2002	363,000	527,000	ITA 2007
2003	316,000	660,000	ITA 2007
2004	277,000	724,000	ITA 2007
2005	351,000	540,000	ITA 2007
2006	289,000	573,000	ITA 2007

NA = not available

possesses excellent solvent properties (Forkner et al. 2004; Lewis 2001; O'Neil et al. 2001). Approximately 35% of all ethylene glycol produced is used to make PET solid-state resins, 26% is used in antifreeze, 24% is used to make polyester fibers, 4% is used to make polyester film, 3% is used in PET chip resin exports, and 8% is used in surface coatings, polyester and alkyd resins, chemical intermediates, and other miscellaneous industrial applications (CMR 2004).

Ethylene glycol plays an essential role in the transportation industry, where it is used as an ingredient in hydraulic brake fluids, as the major component in automotive antifreeze/coolant, and as a component of de-icing fluids for aircraft, runways, and taxiways (Forkner et al. 2004; Lewis 2001; O'Neil et al. 2001; Rebsdat and Mayer 2005). Another important industrial use for ethylene glycol is as an intermediate in the synthesis of esters, ethers, and resinous products, particularly polyester fibers and resins (O'Neil et al. 2001; Rowe and Wolf 1982). As a solvent, ethylene glycol is used in the paint and plastic industries in the formulation of printers' inks, stamp pad inks, and inks for ball point pens, and as a softening agent in cellophane (O'Neil et al. 2001). Ethylene glycol has also been used as a stabilizer for soy bean foam used in fire extinguishers and in the manufacture of explosives, plasticizers, elastomers, and synthetic waxes (Lewis 2001; O'Neil et al. 2001). According to Browning (1965), small amounts of ethylene glycol have been used in pharmaceutical preparations (components of skin lotions and powders, and as a substitute for glycerin); more recent information describing this use has not been located.

5.4 DISPOSAL

Two promising methods for the complete (>99%) destruction of ethylene glycol in waste water are ultraviolet (UV) light-catalyzed oxidation and supercritical oxidation. In the UV light-catalyzed oxidation method, ethylene glycol-containing waste water in the presence of 10% hydrogen peroxide is oxidized by UV irradiation (200–250 nm) with light from a mercury lamp (DOE 1993b). The UV/hydrogen peroxide undergoes photochemical decomposition to produce OH radicals that are strong oxidants capable of oxidizing most organic compounds stepwise to complete mineralization (e.g., carbon dioxide and water). In the supercritical water oxidation method, the waste water is subjected to oxidation at >550 °C and 4,000 psi pressure with a residence time of <30 seconds (DOE 1993a).

A new technology, *in situ* vitrification (a thermal treatment technology) (Drajun 1991), has shown potential for the remediation of soil contaminated with ethylene glycol. During the *in situ* vitrification process, contaminated soil is transformed into silicate glass using large amounts of electrical energy and a crystalline product similar to obsidian is formed. Another novel approach involving an encapsulated

biooxidation method proposes that sodium percarbonate encapsulated in polyvinylidene chloride be inserted in subsurface soil by a method called hydraulic fracturing. Oxygen slowly released from the encapsulated sodium percarbonate increases the number of glycol-degrading organisms. This method is expected to remediate soils contaminated with glycols via enhanced aerobic biodegradation in subsurface soils (Vesper et al. 1994).

Distillation of used automobile and heavy duty engine coolant under reduced pressure has been assessed to be an acceptable technology for recycling ethylene glycol in terms of economic potential, waste reduction potential, and product quality that meets both American Society for Testing and Materials (ASTM) and SAE standards (EPA 1993d).

6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

Ethylene glycol has been identified in at least 37 of the 1,689 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2007). However, the number of sites evaluated for ethylene glycol is not known. The frequency of these sites can be seen in Figure 6-1.

Ethylene glycol is released to the environment in manufacturing and processing waste streams and as the result of disposal of industrial and consumer products containing this compound. The major sources of release are from the disposal of used antifreeze and the use of de-icing solutions at airports. When released to the environment, ethylene glycol is expected to partition to surface water and groundwater. Because of its high solubility in water and lack of adsorption or partitioning into soils, ethylene glycol will have a high mobility in soil and potential to leach into groundwater. Volatilization from moist soil and water surfaces is not expected to be important based on its Henry's law constant.

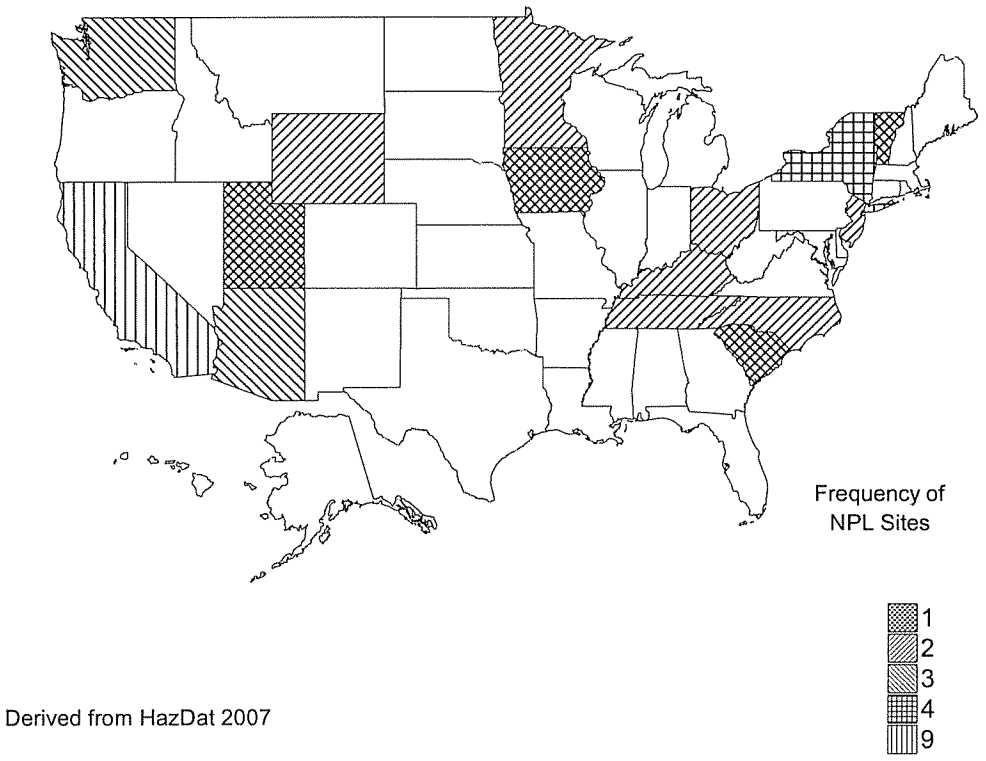
Ethylene glycol degrades rapidly in all environmental media and it does not bioaccumulate. Biodegradation is the most important transformation process in water and soil. Based on available data, ethylene glycol is biodegraded in soil and water under both aerobic and anaerobic conditions within a day to a few weeks. Aerosols or vapors released to the atmosphere readily undergo photochemical oxidation with an estimated half-life of 1.4 days. Background concentrations of ethylene glycol in air, water, soil, and sediment are not available. Reported ethylene glycol vapor concentrations up to 22 mg/m^3 have been measured in the air surrounding airport de-icing operations.

The most important routes of exposure to ethylene glycol for members of the general population are dermal contact with ethylene glycol-based antifreeze and intentional or accidental oral exposures. In occupational settings, workers are exposed via dermal contact and possibly inhalation during activities involving the heating or spray application of fluids containing this compound.

6.2 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ 10 or more full-time

Figure 6-1. Frequency of NPL Sites with Ethylene Glycol Contamination



employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005).

6.2.1 Air

Estimated releases of 2.9 million pounds (1,300 metric tons) of ethylene glycol to the atmosphere from 1,487 domestic manufacturing and processing facilities in 2005, accounted for about 29% of the estimated total environmental releases from facilities required to report to the TRI (TRI05 2007). These releases are summarized in Table 6-1.

During the application of de-icing solutions to aircraft, an estimated 49–80% of de-icing solutions containing ethylene glycol are released on airport runway aprons. The remainder is retained on the aircraft or is immediately dispersed to the air (Sills and Blakeslee 1992).

Ethylene glycol has been identified in air samples collected at 2 of the 37 NPL hazardous waste sites where it was detected in some environmental media (HazDat 2007).

6.2.2 Water

Estimated releases of 0.54 million pounds (250 metric tons) of ethylene glycol to surface water from 1,487 domestic manufacturing and processing facilities in 2005, accounted for about 6% of the estimated total environmental releases from facilities required to report to the TRI (TRI05 2007).

Ethylene glycol is released to surface waters in waste water from production and processing facilities, from spills, in runoff (e.g., through the use of the compounds as de-icing fluids), and in the disposal of used antifreeze (Christian and Moorehead 1985; EPA 2000; Ware 1988). Ethylene and propylene glycol concentrations up to 19,000 mg/L (ppm) were detected in storm water runoff at the Salt Lake City Airport

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Ethylene Glycol[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AK	3	49	No data	0	0	0	49	0	49
AL	28	21,793	5	0	125,255	3,996	146,803	4,246	151,049
AR	9	10,109	3,936	9,149	50	0	23,244	0	23,244
AZ	17	17,285	No data	0	11,005	0	28,290	0	28,290
CA	90	78,185	15	1,021	91,829	39	151,955	19,134	171,088
CO	16	6,418	1,534	0	5	440	7,957	440	8,397
CT	8	485	0	0	11	0	485	11	496
DE	5	265	441	0	0	250	706	250	956
FL	32	13,489	0	1,780	3,647	0	15,359	3,557	18,916
GA	56	13,540	1,255	0	12,250	273	20,558	6,760	27,318
IA	31	17,944	630	0	1,973	19,150	18,574	21,123	39,697
ID	3	256	0	0	11,983	0	11,989	250	12,239
IL	91	66,545	6,094	0	49,449	2,470	72,644	51,914	124,558
IN	51	21,299	12,805	2,200	184,206	816	37,709	183,617	221,326
KS	15	17,961	No data	0	0	0	17,961	0	17,961
KY	32	12,727	4,689	0	10,926	0	26,414	1,928	28,342
LA	48	66,510	8,395	17,293	151,332	0	232,626	10,904	243,530
MA	24	13,882	0	0	569	2,682	13,882	3,251	17,133
MD	20	6,699	0	0	775	1,528	6,699	2,303	9,002
ME	1	1,584	No data	0	250	0	1,584	250	1,834
MI	66	30,954	470	0	193,543	25,652	35,641	214,978	250,618
MN	30	23,317	6,323	0	2	9,435	29,640	9,437	39,077
MO	48	6,234	0	0	62	325	6,296	325	6,621
MS	10	70,756	24,005	0	1,040	0	94,766	1,035	95,801
MT	7	2,838	1,210	0	37,778	263	41,798	291	42,088
NC	59	426,179	19,421	0	49,957	20,065	456,210	59,412	515,622
ND	5	0	0	0	0	2,898	0	2,898	2,898
NE	9	6,952	0	0	2,471	1,358	7,023	3,758	10,781
NH	2	250	No data	0	0	0	250	0	250
NJ	49	21,757	2,579	0	2,648	3,491	24,438	6,037	30,475
NM	9	2,157	10	2,737	0	2,953	2,167	5,690	7,857
NV	4	6	No data	0	194,638	0	194,644	0	194,644
NY	28	3,262	22,390	0	2	44,200	25,652	44,202	69,854
OH	95	70,121	16,725	490,427	30,028	8,515	582,488	33,328	615,816
OK	26	39,376	0	7,204	79,654	73	126,220	87	126,307
OR	18	8,406	5	0	0	13	8,411	13	8,424
PA	73	19,316	4,701	18,000	10,464	87,549	43,540	96,490	140,030

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Ethylene Glycol[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
PR	16	26,960	0	0	164,831	0	26,960	164,831	191,791
RI	9	1,129	0	0	130	0	1,129	130	1,259
SC	49	537,162	43,890	0	4,290	25,449	581,051	29,739	610,790
SD	1	0	No data	0	0	0	No data	0	0
TN	28	185,878	246,538	0	141,436	260	442,297	131,815	574,112
TX	154	259,642	110,634	1,297,195	104,527	38,215	1,360,388	449,824	1,810,213
UT	8	20,792	No data	0	0	212	20,792	212	21,004
VA	24	403,344	350	0	9,286	0	412,960	20	412,980
VT	1	0	256	0	208	176	256	384	640
WA	17	18,203	0	0	444	24,236	18,203	24,680	42,883
WI	44	130,007	3,922	0	1,290	1,985	133,935	3,270	137,205
WV	15	149,596	698	0	40,247	371	181,663	9,249	190,912
WY	3	5	No data	0	250	0	5	250	255
Total	1,487	2,851,624	543,926	1,847,006	1,724,741	329,338	5,694,311	1,602,323	7,296,634

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other on-site landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI05 2007 (Data are from 2005)

in Utah, and airport runoff was found to contain up to 3,100 mg/L (ppm) at the Toronto International Airport in Canada and up to 5,050 mg/L (ppm) at the Denver Airport in Colorado (Sills and Blakeslee 1992). Ethylene glycol was detected, but not quantified, in effluents from a chemical plant in Brandenburg, Kentucky (EPA 1976).

EPA (2000) estimated that 21 million gallons of aircraft deicing fluid (including both ethylene and propylene glycol-based fluids) are discharged to surface waters per year in the United States with an additional 2 million gallons discharged to publicly owned treatment works (POTWs). These releases are expected to decrease as source reduction technologies and recycling/recovery systems are improved. Airports that have updated equipment and collection systems have achieved a 70% collection efficiency on average.

Ethylene glycol that is released onto the ground when used in aircraft de-icing fluid may contaminate nearby groundwater (Corsi et al. 2001a). Groundwater samples collected from a perched water table at the Ottawa Airport in Canada contained 415 mg/L (ppm) of ethylene glycol (Sills and Blakeslee 1992).

Ethylene glycol has been identified in surface water and groundwater samples collected at 1 and 7 of the 37 NPL hazardous waste sites, respectively, where it was detected in some environmental media (HazDat 2007).

6.2.3 Soil

Estimated releases of 1.7 million pounds (780 metric tons) of ethylene glycol to soils from 1,487 domestic manufacturing and processing facilities in 2005, accounted for about 17% of the estimated total environmental releases from facilities required to report to the TRI (TRI05 2007). An additional 1.9 million pounds (840 metric tons), constituting about 19% of the total environmental emissions, were released via underground injection (TRI05 2007). These releases are summarized in Table 6-1.

The major sources of ethylene glycol release to soil are from the disposal of used antifreeze fluids and de-icing fluids containing this compound (EPA 1979, 1987a; Ware 1988). Ethylene glycol may also be released to soil via natural processes associated with the metabolism of ethylene by plants (Blomstrom and Beyer 1980).

Ethylene glycol has been identified in soil and sediment samples collected at 2 and 1 of the 37 NPL hazardous waste sites, respectively, where it was detected in some environmental media (HazDat 2007).

6.3 ENVIRONMENTAL FATE

6.3.1 Transport and Partitioning

Ethylene glycol has a low vapor pressure (0.089 mm Hg at 25 °C) and is miscible with water (see Table 4-2). If released to the atmosphere (e.g., as vapors generated at elevated temperatures), ethylene glycol should exist almost entirely in the vapor phase (Eisenreich et al. 1981). The high solubility of ethylene glycol in water ensures that at least partial removal of the compound will occur by wet deposition. The low Henry's law constant value for this compound ($6.00x10^{-8}$ atm-m^3/mole, see Table 4-2) suggests that ethylene glycol released to surface water will not partition to the atmosphere via volatilization (Simmons et al. 1976; Thomas 1990). Ethylene glycol is not expected to adsorb to sediment or soil particulates based on an estimated K_{oc} value of 1 (see Table 4-2). Based on the low K_{oc} value (see Table 4-2), ethylene glycol is expected to have a very high mobility in soil and could leach into groundwater (Swann et al. 1983).

The low octanol/water partition coefficient (K_{ow}) value of -1.36 (see Table 4-2) suggests that bioconcentration and biomagnification of ethylene glycol are not likely to occur. Laboratory testing with this compound confirms insignificant bioconcentration in fish (Freitag et al. 1985). The bioconcentration factor (BCF) for ethylene glycol in fish (Golden ide) was 10 after 3 days of exposure.

Ethylene glycol is expected to be highly mobile, particularly in moist soils, and it may leach into groundwater upon release to surface soils. In laboratory studies, ethylene glycol was found to percolate rapidly through soil columns with little or no adsorption (LA DOTD 1989; Løkke 1984); however, rapid biodegradation is expected to limit the extent of leaching through soil (see Section 6.3.2.3). The compound may also volatilize from dry surface soils (EPA 1979, 1987a; Hine and Mookerjee 1975). In dry soils, ethylene glycol liquid can enter the soil system and travel through the porous media before contacting free water. Amoozegar et al. (1986) reported that in dry soils (<1% water) the rate of ethylene glycol movement was the slowest of 6 organic liquids tested (toluene, xylene, kerosene, acetone, and isopropyl alcohol).

6.3.2 Transformation and Degradation

6.3.2.1 Air

Ethylene glycol released to the atmosphere is expected to undergo rapid photochemical oxidation via reaction with hydroxyl radicals. The half-life for the photochemical oxidation of ethylene glycol is 1.4 days calculated using a measured reaction rate constant of $7.7x10^{-12}$ cm^3/molecule-second at 25 °C, assuming a 12-hour day and an average day-light atmospheric hydroxyl radical concentration of $1.5x10^6$ radicals/cm^3 (Atkinson 1989; EPA 1993e). Direct photolysis of ethylene glycol is not expected since alcohols do not absorb UV light at environmental wavelengths (above 295 nm) (Boethling and Mackay 2000).

6.3.2.2 Water

Biodegradation is the most important transformation process for ethylene glycol in surface waters under both aerobic and anaerobic conditions (Staples et al. 2001). In screening tests using aerobic sewage sludge inocula, the incubation time for the majority of the biodegradation of ethylene glycol to take place ranges from within a day to a few weeks (Bridie et al. 1979; Conway et al. 1983; Ettinger 1956; Heukelekian and Rand 1955; Lamb and Jenkins 1952; Means and Anderson 1981; Pitter 1976; Price et al. 1974; Slave et al. 1974; Wagner 1976; Young et al. 1968). Evans and David (1974) performed river die-away tests in which ethylene glycol, added to river water at concentrations ≤10 mg/L (ppm), was completely biodegraded after 3 days at 20 °C and after 14 days at 8 °C.

Other reports of biotransformation of ethylene glycol include anaerobic metabolism (Battersby and Wilson 1989; Bieszkiewicz et al. 1979; Dwyer and Tiedje 1983; Hovious et al. 1973; Kameya et al. 1995; Watson and Jones 1977). Based on available data, ethylene glycol appears to be completely degraded within 1–2 weeks under anaerobic conditions (Battersby and Wilson 1989; Dwyer and Tiedje 1983; Kameya et al. 1995). Schoenberg et al. (2001) measured first-order biodegradation rate constants of 3.5–5.2 per day for ethylene glycol-based deicing fluids under anaerobic conditions, corresponding to half-lives of <1 day. Dwyer and Tiedje (1983) proposed that the methanogenic degradation pathway proceeds through formation of ethanol followed by acetate.

Waste water carrying ethylene glycol can be purified using the activated sludge method provided the concentration of ethylene glycol does not exceed 1,000 mg/L (ppm) (Bieszkiewicz et al. 1979). Similar results were observed for the degradation of ethylene glycol in groundwater (McGahey and Bouwer

1992). At an initial substrate concentration of 111 mg/L (ppm), naturally occurring microorganisms in groundwater biodegraded ethylene glycol with a calculated half-life of <1 day following a lag phase of <3 days.

Ethylene glycol is not expected to undergo significant abiotic transformation in surface waters via hydrolysis or oxidation (EPA 1979; Harris 1990). Glycols are resistant to hydrolysis (Harris 1990). Ethylene glycol is not expected to undergo direct photolysis in sunlit waters since alcohols do not absorb UV light at environmental wavelengths (above 295 nm) (Boethling and Mackay 2000). However, indirect photolysis of ethylene glycol sorbed to goethite (a common natural constituent of surface water sediments) by near ultraviolet radiation (300–400 nm) has been demonstrated in the laboratory. Formaldehyde and glycolaldehyde were detected as degradation products (Cunningham et al. 1985).

6.3.2.3 Sediment and Soil

Biodegradation under both aerobic and anaerobic conditions is also the most important transformation process for ethylene glycol in soils, with half-lives similar to or less than those measured in surface waters (EPA 1987a).

The rate of biodegradation of ethylene glycol in simulated subsurface soils is dependent on substrate concentrations, soil types, and ambient soil temperatures (McGahey and Bouwer 1992). Greater than 95% removal was consistently accomplished in <5 days and 7 days at ethylene glycol concentrations of 100 and 1,000 ppm, respectively; however, substrate concentrations of 10,000 ppm showed negligible loss of ethylene glycol. The rate of degradation was higher in soils with high organic matter. A doubling in the degradation rate was also observed with a 10 °C increase in soil temperature. McGahey and Bouwer (1992) concluded that microorganisms naturally occurring in soils and groundwater are effective in biodegrading ethylene glycol with the half-life ranging from 0.2 to 0.9 days. Approximately 23–26% of ethylene glycol at 2.25 ppm was biodegraded in anaerobic sandy till soil grab sample tests run for 86 and 140 days (Løkke 1984).

Klecka et al. (1993) studied the biodegradation of aircraft de-icing fluids in soils adjacent to airport runways at various ethylene glycol concentrations and at various temperatures ranging from -2 to 25 °C. Generally, the rate of biodegradation of ethylene glycol was faster in soils with low glycol concentrations, high organic carbon content, and higher ambient soil temperatures. Ethylene glycol present in soils at concentrations <6,000 mg/kg (ppm) biodegraded at an average rate of 3.0 mg/kg (ppm) soil/day at -2 °C,

at 19.7 mg/kg (ppm) soil/day at 8 °C, and at an average rate of 66.3 mg/kg (ppm) soil/day at 25 °C (Klecka et al. 1993). Based on these results, biodegradation is expected to play a major role in removing ethylene glycol residues from soils adjacent to airport runways and taxiways. Revitt and Worrall (2003) measured first-order biodegradation rate constants of 0.064 day^{-1} at 1 °C, 0.082 day^{-1} at 4 °C, and 0.091 day^{-1} at 8 °C for an ethylene glycol-diethylene glycol de-icing formulation on paving blocks taken from an airport. These rates correspond to half-lives of 10.8, 8.5, and 7.6 days, respectively.

As in surface waters, abiotic transformation of ethylene glycol in soil is not expected to be a significant process (EPA 1987a).

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to ethylene glycol depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of ethylene glycol in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on ethylene glycol levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for monitoring ethylene glycol in a variety of environmental media are detailed in Chapter 7.

6.4.1 Air

Ethylene glycol was detected in ambient air samples, at time-weighted average (TWA) concentrations of <0.05–0.33 mg/m^3 as aerosol and <0.05–10.4 mg/m^3 as vapor, following spray application of de-icing fluids containing 50% solutions of the compound to the surfaces of bridges. The ambient air samples were collected above the sprayed bridges (LA DOTD 1989). Ethylene glycol was detected as a vapor above 2.5 mg/m^3 in 18 out of 154 air samples collected during de-icing operations at a Montreal airport (Gérin et al. 1997). The maximum concentration reported was 22.0 mg/m^3. Three of the air samples contained ethylene glycol as a mist at concentrations of 76, 91, and 190 mg/m^3.

Ethylene glycol was identified in air samples collected in four new manufactured houses and seven site-built houses (Hodgson et al. 2000). The range and geometric mean of ethylene glycol concentrations were <9.2–43.8 and 17.9 mg/m^3, respectively, in the manufactured houses and 20.7–491 and 2.7 mg/m^3, respectively, in the site-built houses. Latex paint was indicated as a source for ethylene glycol emissions in the new houses.

Background concentrations of ethylene glycol in ambient air are not available.

6.4.2 Water

Monitoring data from several contractor and airport authority reports reveal that storm water runoff from airports may contain several hundred to several thousand mg/L (ppm) glycols (Sills and Blakeslee 1992). Ethylene and propylene glycol levels up to 19,000 mg/L (ppm) were detected in storm water from the Salt Lake City International Airport. The concentration of ethylene glycol in runoff from runway apron areas at the Toronto International Airport ranged from 75.0 mg/L to 3,100 mg/L (ppm) and was up to 70 mg/L (ppm) in a stream that received runoff from the airport. Ethylene glycol concentrations ranged from 2 to 660 mg/L (ppm) and from 5 to 170 mg/L (ppm) in streams flowing through Winnipeg International Airport and St. John's International Airport (Newfoundland), respectively (Environment Canada 2000). Ethylene glycol concentrations were less than the detection limit (25 mg/L) in Etobicoke Creek, which receives storm water from Lester B. Pearson International Airport in Toronto. The concentration of ethylene glycol in storm water runoff from Stapleton International Airport in Denver, Colorado ranged from near zero to 5,050 mg/L (ppm) (Sills and Blakeslee 1992). Although the potential for groundwater contamination is quite low for many airports with predominantly heavy soil, the movement of glycols through unsaturated silty sand can be potentially high (Sills and Blakeslee 1992). Thus, although ethylene glycol was not detected even in shallow soils at the edge of the runway at the Stapleton International Airport, the groundwater in the perched water table at Ottawa International Airport in Canada, which contained sandy soil, was found to contain ethylene glycol at levels up to 415 mg/L (ppm). Peak concentrations occurred in June and declined to nondetectable levels by the fall.

No information was found regarding background concentrations of ethylene glycol in surface water, groundwater, or drinking water.

6.4.3 Sediment and Soil

No information was found regarding the concentrations of ethylene glycol in soil or sediment.

6.4.4 Other Environmental Media

Ethylene glycol has been found to migrate into a number of foods from regenerated cellulose films containing triethylene glycol and polyethylene glycol as softening agents. Ethylene glycol was detected

in fruit cakes at 27–34 mg/kg (ppm) after 84–336 days of storage, in meat pies at <10 mg/kg (ppm) after 3–7 days of storage, in toffee at <10–22 mg/kg (ppm) after 168–450 days of storage, in madeira cake at <10–22 mg/kg (ppm) after 21–28 days storage, and in boiled sweets at 14–34 mg/kg (ppm) after 168–450 days storage (Castle et al. 1988a). According to Kashtock and Breder (1980), ethylene glycol can migrate into food simulants from polyethylene terephthalate (PET) bottles used in the packaging of carbonated beverages. The compound was detected at a concentration of about 100 ppb (0.1 ppm) in a 3% acetic acid solution used as a food simulant after 6 months of storage at 32 °C (Kashtock and Breder 1980). These authors stated that the source of ethylene glycol in this food simulant is the small amount of unreacted ethylene glycol in the polyethylene terephthalate polymer. More recent information regarding levels of ethylene glycol in food is not available.

Ethylene glycol has been identified in negligible amounts in the water-soluble component of cigarette smoke (Schumacher et al. 1977).

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Ethylene glycol concentrations in blood, urine, tissue, or breast milk are not available for the general population in the United States. The most common route of human exposure to ethylene glycol for members of the general population is dermal contact with ethylene glycol-based automobile antifreeze. However, intentional or accidental ingestion of antifreeze is the most serious type of exposure, resulting in thousands of ethylene glycol poisonings including several deaths reported each year in the United States (Fraser 2002; Leth and Gregersen 2005).

Exposure to ethylene glycol through consumption of foods or drinks stored in plastics made from this chemical may be possible if the plastic contains unreacted ethylene glycol that can migrate into the food (Kashtock and Breder 1980). However, current levels of ethylene glycol in food have not been located; therefore, evidence is not available to indicate that this as an important route of human exposure to ethylene glycol.

Background concentrations of ethylene glycol in air, surface water, groundwater, drinking water, soil, and sediment are not available. Ethylene glycol is not expected to be found in the environment away from areas where it is released since this substance is degraded within days to a few weeks in air, water, and soil. Therefore, inhalation of ambient air, ingestion of drinking water, and dermal contact with water or soil are not expected to be important routes of exposure of the general population to ethylene glycol.

Contact with the skin and eyes is the most likely route of worker exposure to ethylene glycol. Inhalation may be an important route of human exposure under occupational conditions where the compound is heated or if mists are generated by heat or violent agitation (Rowe and Wolf 1982). Individuals involved in airport de-icing operations are exposed to ethylene glycol through inhalation since the de-icing formulation is sprayed through the air, generating ethylene glycol vapor and mist.

Gérin et al. (1997) measured urinary ethylene glycol concentrations for 33 aviation workers exposed to de-icing fluid over a winter period of 2 months. Ethylene glycol was detected above 2.5 mg/m^3 (limit of quantification) as vapor in only 18 out of 154 air samples collected during spray operations. Reported concentrations ranged from 0.9 to 22 mg/m^3. Ethylene glycol was also detected as a mist in three of the air samples with concentrations of 76, 91, and 190 mg/m^3. Ethylene glycol quantities in post-shift and next-morning urine samples exceeded 5 mmol/mol creatinine in 16 of the 33 workers (5.2–129 mmol/mol creatinine). Exposure was greatest for basket operators and coordinators. Air samples taken from the breathing zones of workers applying de-icing fluids (50% ethylene glycol) to bridge surfaces contained the compound at concentrations of <0.05–2.33 mg/m^3 as aerosols and <0.05–3.37 mg/m^3 as vapors (LA DOTD 1989).

Laitinen et al. (1995) measured urinary ethylene glycol concentrations in 10 car mechanics frequently exposed to ethylene glycol and a control group of 10 male office workers. Levels were noticeably higher in the urine of the mechanics. Ethylene glycol and oxalic acid concentrations were 7.3 and 47 mmol/mol creatinine, respectively, in the urine of the car mechanics and 1.7 and 36 mmol/mol creatinine, respectively, in the urine of the controls. Ethylene glycol concentrations in the air of the mechanics' environment were negligible. Letzel et al. (2000) reported mean and median ethylene glycol concentrations of 0.31 and 0.23 mg/L, respectively, measured in the urine of 16 individuals living in Germany who had no known occupational exposure to glycols.

The National Occupational Exposure Survey (NOES) conducted by NIOSH during 1981–1983 estimated that 1.5 million workers are potentially exposed to ethylene glycol each year (NIOSH 1990).

6.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in Section 3.7, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

Ethylene glycol antifreeze is a brightly-colored liquid and can be confused for a beverage, especially when it is not kept in its original container (Leth and Gregersen 2005). The sweet taste of ethylene glycol adds to this attraction. For this reason, some states have mandated that the bittering agent, denatonium benzoate, be added to ethylene glycol antifreeze formulations (Hogue 2006). Although the presence of a bittering agent is expected to deter children from accidentally ingesting ethylene glycol-based antifreeze, caution should still be used. Harry et al. (1998) reported a case of accidental ingestion of ethylene glycol antifreeze by a 4-year-old child even though the formulation contained denatonium benzoate.

Many ethylene glycol poisonings occur when an antifreeze bottle is in use (EPA 2004b; Leth and Gregersen 2005). Children may touch or ingest ethylene glycol from an opened container of antifreeze that an adult has placed within reach. Children may also play with a puddle of antifreeze that has been spilled onto the ground.

Children who live near facilities that manufacture or use ethylene glycol may be exposed to this substance through contact with contaminated soil or water. Children living near airports where ethylene glycol is used in de-icing operations may be exposed through ingestion of contaminated groundwater or inhalation of ethylene glycol vapor.

Biomonitoring data for children, including levels of ethylene glycol measured in breast milk, neonatal blood, cord blood, and meconium fluid have not been located.

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Workers in industries involved in the manufacture or use of products containing high concentrations of ethylene glycol (e.g., antifreeze, coolants, de-icing fluids, brakes fluids, solvents) may be exposed to concentrations of the compound at levels higher than the general population, particularly in operations involving heating or spraying of these materials.

Members of the general population who currently have potentially high exposures to ethylene glycol include individuals living near sites where ethylene glycol is manufactured or used and individuals living near waste disposal sites contaminated with ethylene glycol. Persons living near airports where large amounts of ethylene glycol are used for de-icing of aircraft or near hazardous waste sites are potentially at greater risk of exposure, particularly from consumption of contaminated groundwater.

6.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of ethylene glycol is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of ethylene glycol.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.8.1 Identification of Data Needs

Physical and Chemical Properties. As seen in Table 4-2, the relevant physical and chemical properties of ethylene glycol are known (AIChE 1995; Forkner et al. 2004; Hansch et al. 1995; Hine and Mookerjee 1975; HSDB 2007; Lewis 2001; Lide 2005; O'Neil et al. 2001; Rebsdat and Mayer 2005) and predicting the environmental fate and transport of ethylene glycol based on these properties is possible. No further information is needed.

Production, Import/Export, Use, Release, and Disposal. According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information for 2005, became available in May of 2007. This database is updated yearly and should provide a list of industrial production facilities and emissions.

Production, Import/Export, Use, Release, and Disposal. Knowledge of production and use data for a chemical is important in predicting its potential for environmental contamination and human exposure. Both recent and historical production data are available for ethylene glycol (Brown et al. 1980; CMR 1981, 1984, 1987, 1990, 1993, 1998, 2001, 2004; Forkner et al. 2004; McClelland and Rector 1951; Miller 1966; Rebsdat and Mayer 2005; SRI 2006). Similarly, data on the import/export volumes for ethylene glycol for the last several years are available (HSDB 2007; ITA 2007). Information on the various uses of this compound is also available (Browning 1965; CMR 2004; Forkner et al. 2004; Lewis 2001; O'Neil et al. 2001; Rebsdat and Mayer 2005; SRI 2006). Ethylene glycol enters the environment primarily during its use as a component of automotive antifreeze/coolants, as a de-icing fluid for aircraft, and as an intermediate in the synthesis of polyester fibers (Klecka et al. 1993; Lewis 2001; O'Neil et al. 2001; Rowe and Wolf 1982). Major sources of ethylene glycol releases to soils are from the disposal of used antifreeze and de-icing solutions in hazardous waste sites (EPA 1979, 1987a; Ware 1988). Information regarding the disposal of ethylene glycol-containing waste waters (DOE 1993a, 1993b) and for remediation of ethylene glycol contaminated soils (Drajun 1991; Vesper et al. 1994) is available.

Environmental Fate. Information regarding the fate of ethylene glycol in the air is available that suggests that the compound would be primarily found in the vapor phase and would likely be removed from the atmosphere via wet deposition (Eisenreich et al. 1981; EPA 1979). Ethylene glycol undergoes rapid photochemical oxidation via reaction with hydroxyl radicals with a half-life of 1.4 hours (Atkinson 1989; EPA 1993e). Because of its high solubility in water, the compound is expected to be transported primarily in aqueous media (EPA 1979) and will not partition to the atmosphere via volatilization from water (Thomas 1990). Adsorption to sediment or soil particles is not expected to be significant based on the low K_{oc} value; therefore, ethylene glycol is expected to have a high mobility in soil and the potential to leach into groundwater (Swann et al. 1983). Ethylene glycol is degraded in both water (Battersby and Wilson 1989; Bieszkiewicz et al. 1979; Bridie et al. 1979; Caskey and Taber 1981; Dwyer and Tiedje 1983; Evans and David 1974) and soil (Klecka et al. 1993; McGahey and Bouwer 1992; Revitt and Worrall 2003) primarily by biodegradation. Based on available data, ethylene glycol is biodegraded

under both aerobic and anaerobic conditions from within a day to a few weeks. No additional information on degradation of ethylene glycol in air, water or soil is required.

Bioavailability from Environmental Media. Available information regarding the rate of ethylene glycol absorption following inhalation, oral, or dermal contact has been discussed in the Toxicokinetics section (see Section 3.4). Although no data on ethylene glycol's bioavailability from contaminated air are available, the bioavailability from inhalation exposure is expected to be high because ethylene glycol is likely to be present in the vapor phase (Eisenreich et al. 1981) and not in the particulate phase in the adsorbed state. Similarly, no data on the bioavailability of ethylene glycol from water, soil, or plant material are available; however, ethylene glycol is miscible in water and does not adsorb readily to soil. Ethylene glycol, therefore, is expected to be readily bioavailable from soil and water. Information on the bioavailability of ethylene glycol from actual environmental media needs further development.

Food Chain Bioaccumulation. Based on its low K_{oc} value, ethylene glycol is not expected to bioconcentrate in aquatic food chains. Freitag et al. (1985) reported a BCF value of 10 measured in fish. Information is also lacking regarding the biomagnification potential of ethylene glycol through aquatic food chains, although biomagnification is probably a minor process because of the rapid degradation rate for the chemical in aquatic systems. No further information on the bioconcentration or biomagnification potential of ethylene glycol is needed.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of ethylene glycol in contaminated media at hazardous waste sites are needed so that the information obtained on levels of ethylene in the environment can be used in combination with the known body burden of ethylene glycol to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Information on the number of hazardous waste sites on the NPL at which ethylene glycol was detected in air, surface water, groundwater, soil, and sediment is available (HazDat 2007). However, more specific information, such as concentrations in these media, is needed to give a better indication of the potential for human exposure to ethylene glycol in areas near hazardous waste sites.

No information was located on the concentration of ethylene glycol in ambient air. Time weighted average concentrations of the compound as both an aerosol and a vapor were measured following the spray application of de-icing fluids containing ethylene glycol on a bridge (LA DOTD 1989) and at an

airport (Gérin et al. 1997). These data are not general enough to estimate inhalation exposure to ethylene glycol for the general population in the United States. No data on the level of ethylene glycol in drinking water were located, although ethylene glycol has been detected at up to 415 mg/L (ppm) in groundwater in the vicinity of an airport (Sills and Blakeslee 1992). No information on the background levels of ethylene glycol in soil, surface water, or groundwater was located. Additional information regarding the levels of ethylene glycol in ambient air, drinking water, surface water, groundwater, and soil is needed. Some older data on ethylene glycol levels in foods, particularly those stored in cellulose films or in PET bottles are available (Castle et al. 1988a; Kashtock and Breder 1980). Additional quantitative information on current levels of ethylene glycol in various environmental media and levels of contamination in foods would be helpful in assessing the health risks to the general population and in occupational settings.

Exposure Levels in Humans. Little quantitative information on ethylene glycol levels in various human tissues and body fluids of a control population, populations near hazardous waste sites, or occupationally exposed groups in the United States is available. Most information is available for oral exposures derived from intentional or accidental poisonings (Gabow et al. 1986; Hewlett et al. 1986; Jacobsen et al. 1988; Parry and Wallach 1974; Robinson and McCoy 1989; Vale 1979; Wiener and Richardson 1988). Some information is available on plasma glycolate levels for poisoning victims admitted to a hospital (Jacobsen et al. (1984), and on urine and other tissues (Cheng et al. 1987; Rothman et al. 1986; Winek et al. 1978). Data are needed on the levels of ethylene glycol and its metabolites in body tissues and fluids especially from dermal and inhalation studies. Two studies have been located that report urinary concentrations for airport de-icing workers and car mechanics (Gérin et al. 1997; Laitinen et al. 1995). Additional information on control populations, populations living in the vicinity of hazardous waste sites, and those who are occupationally exposed to ethylene glycol is needed.

This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. Information regarding the background exposure of children to ethylene glycol is not available. Body burden studies measuring the levels of ethylene glycol in the blood, urine, and body tissue of children would be helpful. Studies measuring the levels of this chemical in the neonatal blood, cord blood, and meconium fluid of infants would also be helpful. Levels of ethylene glycol in breastmilk have not been located. Children are considered to be more susceptible to accidental ingestion of ethylene glycol than adults (Leth and Gregersen 2005). Information is needed regarding how effective the use of a bittering agent has been in deterring accidental ingestion of ethylene glycol by children.

Child health data needs relating to susceptibility are discussed in Section 3.12.2, Identification of Data Needs: Children's Susceptibility.

Exposure Registries. No exposure registries for ethylene glycol were located. This substance is not currently one of the compounds for which a sub-registry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for sub-registries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

6.8.2 Ongoing Studies

Ongoing studies related to the potential for human exposure to ethylene glycol were not located.

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7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring ethylene glycol, its metabolites, and other biomarkers of exposure and effect to ethylene glycol. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

7.1 BIOLOGICAL MATERIALS

Table 7-1 is a summary of some of the most commonly used methods reported in the literature for detecting ethylene glycol in biological samples. The primary method for measuring ethylene glycol in biological samples is derivatization followed by gas chromatography (GC) using either a flame ionization detector (FID) or mass spectrometry (MS) for quantification. GC is the preferred analytical method because of the ease of sample preparation and the accuracy of the quantification of sample concentrations. Alkali flame ionization detectors have also been used for ethylene glycol analysis and give a response ratio of 3:1 compared with FID (Bogusz et al. 1986).

Sample preparation for GC is important and proceeds through several steps: acidification, esterification, and extraction into an organic solvent. The use of internal standards is necessary for quantification.

Detection of ethylene glycol in biological samples using GC with either FID or MS is very sensitive, with detection limits ranging from sub to low ppm. The coefficient of variation (CV) varies with the concentration of glycol used but typically ranges from 0.4 to 27% and is usually <10%. In GC procedures, the glycols and their acid metabolites are derivatized to form esters in order to facilitate quantitative elution from the chromatographic columns (see Table 7-1). Yao and Porter (1996) and Porter et al. (1999) have developed a procedure for the simultaneous determination of ethylene glycol and glycolic acid in human serum. The entire procedure can be completed in <2 hours. Simple and rapid methods are also available for the quantitation of the glycols in urine, serum, or deproteinated whole blood. These methods use direct sample injection without prior solvent extraction and derivatization

Table 7-1. Analytical Methods for Determining Ethylene Glycol in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human plasma	Deproteinization with acetic acid; vortex; centrifugation; supernatant spiked with internal standard; reaction with butyl-boronic acid; neutralize with NH_4OH, extraction with dichloromethane; concentration.	HRGC/MS	5 ppm	94–106	Giachetti et al. 1989
Human serum	Internal standard (in acetonitrile) added to sample; centrifugation to remove protein precipitate; esterification with butylboronic acid and 2,2-dimethoxypropane; neutralization with NH_4OH in acetonitrile.	HRGC/FID	NR	95	Smith 1984
Human serum (ethylene glycol and glycolic acid)	Addition of internal standard (in acetonitrile) followed by centrifugation. Supernate is treated with 2,2-dimethoxy-propane/dimethylformamide. Volume is then reduced, but not to dryness.	GC/MS	10 ppm	91.1% (ethylene glycol); 77.6% (glycolic acid)	Porter et al. 1999
Human serum	Deprotonization of samples via ultrafiltration followed by addition of internal standard to the filtrate and injection into the GC.	CCGC	10 ppm	NR	Williams et al. 2000
Human serum and urine	Internal standard added; centrifugation; derivatization with phenylboronate in methanol.	HRGC/FID	1.0 ppm	89–98	Houzé et al. 1993
Human blood/tissue	Anhydrous Na_2SO_4 ground with sample; derivatization with *n*-butylboronic acid in acetone containing internal standard; centrifugation or filtration.	GC/FID/AFID	NR	70	Bogusz et al. 1986

Table 7-1. Analytical Methods for Determining Ethylene Glycol in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human serum (glycolic acid)	Colorimetric: precipitation of protein with trichloroacetic acid followed by centrifugation, addition of chromotropic acid, heating, and dilution; gas chromatographic: addition of internal standard and acetone followed by centrifugation, addition of NaOH, evaporation to dryness, and formation of methyl ester.	Absorbance at 580 nm or GC/FID as appropriate	1.0 mmol/L (60 ppm, w/v) for both methods; 3–6% RSD	NR	Fraser and MacNeil 1993
Humans serum (glycolic acid)	Extraction from salted, acidified serum using methyl ethyl ketone followed by removal of organic phase and evaporation to dryness and derivatization with PNBDI.	HPLC/UV	0.05 mmol/L (3 ppm, w/v); 1% RSD	NR	Hewlett et al. 1986
Urine	Acidification; extraction with $CHCl_3$; concentration; TLC.	TLC	NR	NR	Riley et al. 1982
Urine (sodium fluorescein)	Untreated samples read in borosilicate tubes.	Fluorescence (Wood's lamps)	NR	NR	Winter et al. 1990
Dog urine (glycolic acid)	Dilution; NaCl addition and acidification; extraction in MEK; evaporation; dissolution of residue in ethylacetate; derivatization with PNBDI.	HPLC/UV	1–2 ng	96	Hewlett et al. 1983
Human plasma, urine (oxalate)	Heparinized blood deproteinated by addition of acetonitrile and phosphate buffer (pH=7), centrifugation, removal of solvent and evaporation to dryness; derivatization as for urine; urine acidified and derivatized using 1,2-diaminobenzene, adjustment of pH to 5–6, centrifugation.	HPLC/UV	Plasma: 0.15 mg/L (ppm, w/v); 7.5% RSD; urine: 0.5 mg/L (ppm, w/v); 5% RSD.	85	Brega et al. 1992
Kidney tissue dog (hippurate)	Tissue ground with acidic methanol; filtration; concentration; spot on 254 nm TLC plate.	TLC	NR	NR	Riley et al. 1982

Table 7-1. Analytical Methods for Determining Ethylene Glycol in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human tissue	Samples extraction in HPLC grade water for 24 hours; filtration of supernatant.	HPLC/RI	5 ppm	98 at 1 mg/mL (1,000 ppm)	Wu and Malinin 1987

AFID = alkali flame ionization detector; ATP = adenosine triphosphate; $CHCl_3$ = chloroform; CH_3OH = methanol; CCGC = Capillary column gas chromatography; ECD = electron capture detector; EG = ethylene glycol; FID = flame ionization detector; GC = gas chromatography; HCl = hydrochloric acid; $HClO_4$ = chloroform; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; KOH = potassium hydroxide; MEK = methylethyl ketone; $MgSO_4$ = magnesium sulfate; MS = mass spectrometry; NaCl = sodium chloride; NAD = nicotinamide adenine dinucleotide; Na_2SO_4 = sodium sulfate; NH_4OH = ammonium hydroxide; NR = not reported; PG = propylene glycol; PNBDI = O-*p*-nitrobenzyl-N,N'-diisopropylisourea; RSD = relative standard deviation; RI = refractive index detector; TLC = thin-layer chromatography; UV = ultraviolet detector; w/v = weight:volume

(Aarstad et al. 1993; Edinboro et al. 1993; Jonsson et al. 1989). However, such methods, particularly those that use packed columns may misidentify propionic acid (found in patients with methylmalonic acidemia) as ethylene glycol (Shoemaker et al. 1992).

High performance liquid chromatography (HPLC) has also been used to identify ethylene glycol and its metabolites such as glycolate (Hewlett et al. 1983, 1986), hippurate (Riley et al. 1982), and oxalate (Brega et al. 1992) in biological samples, particularly blood and urine. Positive results may be confirmed with GC/MS. This makes GC/MS the preferred method since the HPLC step can be omitted. However, HPLC methods to measure plasma levels of glycolate have been used to aid in diagnosis and treatment of ethylene glycol poisoning (Hewlett et al. 1986; Jacobsen et al. 1988). Gas chromatographic and colorimetric methods for quantification of glycolate have been presented (Fraser and MacNeil 1993).

Microscopy can be used to identify metabolic products of ethylene glycol. Scanning electron microscopy (SEM) at 20 kilovolts will detect crystals of calcite, calcium oxalate monohydrate, and calcium oxalate dihydrate in kidney tissue (Siew et al. 1975b). Phase-contrast polarization and light microscopy X-ray powder diffraction may be used to identify hippuric acid crystals in urine (Foit et al. 1985).

The use of ethylene oxide to sterilize tissue for transplantation may result in the formation of ethylene glycol when ethylene oxide is in prolonged contact with tissue. To quantify the formation of ethylene glycol in tissue, an HPLC method using a differential refractive index detector has been developed. The HPLC system can be used to detect ppm levels of ethylene glycol with a sensitivity of $2x10^{-6}$ refractive index unit full scale. This procedure has three advantages: (1) requires only 4 minutes for analysis, (2) simple sample preparation, and (3) good reproducibility (Wu and Malinin 1987).

Techniques using GC and various detection systems to detect and quantify ethylene glycol in human blood have been developed for use in hospital laboratories to assist in the diagnosis of ethylene glycol poisoning (caused by drinking antifreeze containing ethylene glycol). These techniques are quite rapid, usually <30 minutes, and do not require elaborate sample preparation. The specific techniques used for each analytical method are listed in the table if that information was provided by the author(s).

An alternative method, developed in a hospital, for detecting ethylene glycol in blood is the use of the DuPont *Automated Clinical Analyzer* triglyceride assay pack. This enzymatic method, while relatively simple, cannot be used when the triglyceride concentration of the serum exceeds 12 g/L and requires that positive results for ethylene glycol be confirmed using another method (Ochs et al. 1988; Ryder et al.

1986). The enzymatic method has been modified to eliminate some of the interference problems present in the earlier methods (Blandford and Desjardins 1994).

Thin-layer chromatography (TLC) with a chloroform solvent has been used to detect ethylene glycol and its metabolites in urine or renal tissue (Riley et al. 1982). Metabolites of ethylene glycol in the blood may be detected by analytical isotachophoresis using a system equipped with both a conductivity detector and an ultraviolet detector. Blood and serum samples should not have been previously treated with oxalate, citrate, or ethylene diamine tetracetic acid. This technique may be of value when ethylene glycol poisoning is suspected but sufficient time has elapsed for metabolism of the compound to have occurred (Ovrebo et al. 1987). A simple and rapid colorimetric method that uses chromatropic acid has been proposed for the quantitation of glycolic acid, the major toxic metabolite of ethylene glycol (Fraser and MacNeil 1993).

No information was located on detecting ethylene glycol in feces, adipose tissue, or human milk.

7.2 ENVIRONMENTAL SAMPLES

As with biological samples, GC is the major technique used to determine ethylene glycol concentrations in environmental samples whether in air, water, food, drugs, or other substances. Capillary gas chromatography with FID or ECD, possibly followed by MS, generally gives good quantitative results down to the ppm range with recovery usually >80%. The determination of ethylene glycol in air requires adsorption onto a surface and subsequent extraction. Water samples may be analyzed without preparation (EPA 1995a, 1995b). Detection of ethylene glycol in foods and drugs may be accomplished by chromatography of the sample; for substances with a high fat content, extraction with hexane may be used to remove the fat. Table 7-2 is a summary of some of the most commonly used methods reported in the literature for detecting ethylene glycol in environmental samples. The specific techniques used for each analytical method are listed in the table if that information was provided by the author(s).

Air sampling for ethylene glycol is performed by adsorption onto a resin column such as Amberlite XAD-2. Although activated charcoal filters have some utility, recovery is greater with the Amberlite, and it is the preferred adsorption medium. Ethylene glycol is then solvent-extracted with recovery of 98%. If activated charcoal is used for adsorption, 5% methanol in dichloromethane is the best solvent with maximum recovery of 84% (Andersson et al. 1982, 1984). An alternative method for sampling ethylene glycol involves passage of air through a glass fiber filter with a silica gel tube. Ethylene glycol is then

Table 7-2. Analytical Methods for Determining Ethylene Glycol in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Sample collection on glass fiber filters and silica gel; extraction with methanol; ultrasonic bath (Method 5523).	GC/FID	0.05 ppm	93.4–101	NIOSH 1996
Air	Sample adsorbed on Amberlite® XAD-2 with personal sampling pump; extraction with diethyl ether.	GC/FID	NR	75–98	Andersson et al. 1982
Water	Direct injection (Method 8015C).	GC/FID	NR	NR	EPA 2000
Water	Direct injection (Method 8430).	GC/FTIR	NR	NR	EPA 1996
Plastics	Sample extraction from plastic with carbon disulfide.	GC/FID	16.5 ng	58–61	Muzeni 1985
Plastics	Sample extraction with solvent of ethylacetate-water-methanol.	GC/FID	2 ppm	NR	De Rudder et al. 1986
Ground tobacco	Extraction with anhydrous methanol.	GC/FID	NR	NR	AOAC 1990b
Aqueous solution	Sample concentration, then dilution with water; concentration with helium gas; redilution.	GC/FID	50 ppb	97–103	Kashtock and Breder 1980
Beer	Addition of ammonium sulfate and extract with ethyl acetate.	HRGC/FID	0.73 ppm	88	Williamson and Iverson 1993
Food	Addition of hot water to sample to obtain slurry; extraction with hexane; precipitation of sugars with calcium hydroxide; concentration; derivatization with BSTFA.	HRGC/FID; GC/MS	10 ppm	78–107	Castle et al. 1988b
Anchovies	Extraction with methanol and concentration.	HRGC/MS/MS (PICI)	12.5 ppb	NR	Matusik et al. 1993

BSTFA = bis(trimethylsilyl)trifluoroacetamide; FID = flame ionization; GC = gas chromatography; HRGC = high resolution gas chromatography; MS = mass spectrometry; MS/MS = tandem mass spectrometry; PICI = positive ion chemical ionization

extracted in a 2-propanol:water solvent mixture and injected into the gas chromatograph (Tucker and Deye 1981). A similar version of this method is the NIOSH-approved method for the determination of ethylene glycol in occupational air (see Table 7-2).

A portable, automated, photoionization gas chromatograph has been used to detect ethylene glycol in air samples in industrial facilities at levels as low as 0.05 ppm (Adams and Collins 1988).

Ethylene glycol may be detected by a colorimetric reaction with 3-methyl-2-benzothiazolinone hydrazone hydrochloride after oxidation of the glycols to the corresponding aldehydes with acidified permanganate. The solution is read at 630 nm in a spectrophotometer. This method may be used for ethylene glycol in water (Evans and Dennis 1973) or to detect ethylene oxide in air (Kring et al. 1984); however, this method is not quantitative and is relatively insensitive compared with GC/MS.

The migration of ethylene glycol from plastics into solution can be studied with GC. Sample preparation methods include extraction in hydrochloric acid (Ball 1984), distilled water (Spitz and Weinberger 1971), carbon disulfide (Muzeni 1985), dimethylformamide (Danielson et al. 1990), and a mixture of ethyl acetate, water, and methanol (De Rudder et al. 1986). Other methods for detecting ethylene glycol in industrial products include HPLC (Aboul-Enein and Islam 1989) and a periodate flow-through ion-selective electrode (Diamandis et al. 1980).

The presence of ethylene glycol in foods packaged with plastic films containing the compounds has been studied, as have ethylene glycol levels in drugs sterilized with ethylene oxide. Sample preparation is important because procedures vary depending on the fat content of the food sample. Foods with low fat content can be extracted with ethyl acetate, derivatized to a trimethylsilyl ether, and then injected into the gas chromatograph. For foods with a high fat content, hexane is used as the defatting agent prior to derivatization. Quantifying ethylene glycol in wines requires no preparation of the samples prior to analysis (Kaiser and Rieder 1987; Klaus and Fischer 1987). Drugs in aqueous solutions may be analyzed directly, water insoluble drugs should be extracted in water, and ointments may be dissolved in hexane and then extracted with water. Recovery is between 80 and 114%, with detection limits in the low-ppm range (Hartman and Bowman 1977; Manius 1979). Although the use of TLC (Ballarin 1980) has been recommended, it has been superseded by GC.

No information was located on techniques for detecting and analyzing ethylene glycol in soil.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of ethylene glycol is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of ethylene glycol.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Methods are available for the determination of ethylene glycol in blood, tissue, and urine (Bogusz et al. 1986; Giachetti et al. 1989; Houzé et al. 1993; Riley et al. 1982; Smith 1984; Wu and Malinin 1987) with sensitivities in the low and sub-ppm range. Methods are also available for metabolites of ethylene glycol (glycolic acid, oxalic acid) in blood and urine (Brega et al. 1992; Fraser and MacNeil 1993; Hewlett et al. 1986) with sensitivities as low as 3 ppm for glycolic acid and of 0.15 ppm for oxalic acid. Glycolic acid was identified in Chapter 3 as a sensitive biomarker of exposure to ethylene glycol. These methods seem to be adequate for the measurement of ethylene glycol and its metabolites in the human population.

Effect. Serum concentrations of blood urea nitrogen (BUN) or creatinine can serve as indicators of renal toxicity induced by exposure to ethylene glycol, but these are not specific for ethylene glycol intoxication (Grauer et al. 1987).

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for the determination of ethylene glycol have been reported for air (Andersson et al. 1982; NIOSH 1996), water or aqueous solutions (EPA 1996, 2000; Kashtock and Breder 1980), and

foods (Castle et al. 1988b; Matusik et al. 1993; Williamson and Iverson 1993). Methods have also been developed for the determination of glycols that leach from plastics (De Rudder et al. 1986; Muzeni 1985) and can end up in foods stored in containers made from the plastics.

The MRL for inhalation exposure to ethylene glycol is 0.5 ppm and thus requires a method level of detection (LOD) of 0.5 ppm. This value is below the LODs of the methods reported. Although it should be possible to increase the sampling volumes and increase the sensitivities of the methods, this would need to be shown to be free of problems. Oral MRLs have been established to be 2 mg/kg/day for acute exposure and 0.2 mg/kg/day for chronic exposure. Assuming a 70-kg individual and oral intakes of either 2 L/day of water or 2 kg/day of food, analytical methods would need sensitivities <70 ppm in water or food. The methods reported for aqueous solutions (LOD=50 ppb, Kashtock and Breder 1980), beer (LOD=0.73 ppm, Williamson and Iverson 1993), and foods (LOD=10 ppm, Castle et al. 1988b; LOD=12.5 ppb, Matusik et al. 1993) should be adequate to measure acute exposure. However, chronic exposure requires method LODs of approximately 7 ppm and only the methods of Kashtock and Breder (1980) for aqueous solutions, Williamson and Iverson (1993) for beer, and Matuslik et al. (1993) for anchovies can meet this requirement. The applicability of these methods to other beverages and foods has not been demonstrated. Thus, additional methods should be developed and validated for ethylene glycol in other beverages and foods at concentrations relevant to the chronic oral MRL.

7.3.2 Ongoing Studies

Ongoing studies related to the potential for human exposure to ethylene glycol were not located.

8. REGULATIONS AND ADVISORIES

The international and national regulations and guidelines regarding ethylene glycol in air, water, and other media are summarized in Table 8-1.

An MRL of 2 mg/m^3 has been derived for acute-duration inhalation exposure (14 days or less) to ethylene glycol. The MRL is based on a NOAEL of 23 mg/m^3 for respiratory tract irritation and systemic toxicity in humans (Wills et al. 1974), which was divided by an uncertainty factor of 10 (for human variability).

An MRL of 0.8 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to ethylene glycol. BMD dose modeling was conducted using developmental toxicity data in mice (total malformations and a skeletal variation) (Neeper-Bradley et al. 1995; Tyl 1989). The resulting $BMDL_{10}$ of 76 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to derive an MRL of 0.8 mg/kg/day for acute-duration oral exposure to ethylene glycol.

The acute-duration oral MRL of 0.8 mg/kg/day has been adopted for intermediate-duration oral exposure (15–364 days) to ethylene glycol. The critical effect for intermediate-duration exposure is kidney lesions in male Wistar rats (Cruzan et al. 2004). Basing the intermediate-duration MRL on the NOAEL for this effect yielded a value that is higher than the acute-duration MRL. It is against ATSDR policy to derive an intermediate-duration MRL that is higher than the acute-duration MRL. Because available evidence indicates that the acute-duration MRL should be protective for kidney effects following longer-term exposure, the acute-duration value of 0.8 mg/kg/day was adopted for intermediate-duration exposure.

EPA (IRIS 2007) assigned ethylene glycol an oral reference dose (RfD) of 2.0 mg/kg/day with an uncertainty factor of 100 (10 for interspecies extrapolation and 10 for differences in individual human sensitivity) based on kidney toxicity in rats (DePass et al. 1986a).

EPA has not derived an inhalation reference concentration (RfC) for ethylene glycol.

Neither the International Agency for Research on Cancer (IARC) nor the EPA has classified ethylene glycol for human carcinogenicity (IARC 2006; IRIS 2007). The American Conference of Governmental Industrial Hygienists (ACGIH) has classified ethylene glycol as an A4 carcinogen (not classifiable as a human carcinogen) (ACGIH 2006). The National Toxicology Program (NTP) has not classified ethylene

Table 8-1. Regulations and Guidelines Applicable to Ethylene Glycol

Agency	Description	Information	Reference
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification	No data	IARC 2006
WHO	Air quality guidelines	No data	WHO 2000a
	Drinking water quality guidelines	No data	WHO 2004
NATIONAL			
Regulations and Guidelines:			
a. Air			
ACGIH	TLV (8-hour TWA)	No data	ACGIH 2006
	TLV-ceiling (aerosol only)	100 mg/m^3	
EPA	The Second List of AEGL priority chemicals for guideline development[a]	Yes	EPA 2007a
	Hazardous air pollutant	Yes	EPA 2007b 42 USC 7412
NIOSH	REL (10-hour TWA)	Not established[b]	NIOSH 2005
	IDLH	No data	
OSHA	PEL (8-hour TWA) for general industry	No data	OSHA 2006 29 CFR 1910.1000
b. Water			
EPA	Drinking water standards and health advisories		EPA 2006b
	1-Day health advisory for a 10-kg child	20 mg/L	
	10-Day health advisory for a 10-kg child	6 mg/L	
	DWEL	70 mg/L	
	Lifetime	14 mg/L	
	10^{-4} Cancer risk	No data	
	National primary drinking water standards	No data	EPA 2003
c. Food			
EPA	Inert ingredients in pesticide products	List 3[c]	EPA 2004a
	Exempt from the requirement of a tolerance	When used in foliar applications to peanut plants	EPA 2007f 40 CFR 180.1040
FDA	Substance for use only as components of adhesives	Yes	FDA 2006 21 CFR 175
d. Other			
ACGIH	Carcinogenicity classification	A4[d]	ACGIH 2006

Table 8-1. Regulations and Guidelines Applicable to Ethylene Glycol

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
CPSC	Designated a hazardous substance under Section 3(b) of the Federal Hazardous Substances Act and requires special labeling	Ethylene glycol and mixtures containing 10% or more by weight of ethylene glycol[e]	CPSC 2007
EPA	Carcinogenicity classification	No data	IRIS 2007
	RfC	No data	
	RfD	2 mg/kg/day	
	Master Testing List	Yes[f]	EPA 2007c
	Superfund, emergency planning, and community right-to-know		
	Designated CERCLA hazardous substance	Yes[g]	EPA 2007d 40 CFR 302.4
	Reportable quantity	5,000 pounds	
	Effective date of toxic chemical release reporting	01/01/87	EPA 2007e 40 CFR 372.65
NTP	Carcinogenicity classification	No data[h]	NTP 2005

[a]The Second List of AEGL priority chemicals is a composite of 371 priority chemicals from numerous priority lists of acutely toxic chemicals and represents the selection of chemicals for AEGL development by the NAC/AEGL (EPA 2007a).
[b]NIOSH has not established a REL for ethylene glycol under the “Proposed Rule on Air Contaminants" (29 CFR 1910, Docket No. H-020) in which NIOSH questioned whether the OSHA PEL for ethylene glycol (ceiling 50 ppm) was adequate enough to protect workers from potential health hazards (NIOSH 2005).
[c]List 3: Inerts of unknown toxicity are placed on this list if there was no basis for listing it on any of the other lists. EPA continues to evaluate ethylene glycol as additional information becomes available, to determine if reclassification to List 1, 2, or 4 is appropriate (EPA 2004a).
[d]A4: not classifiable as a human carcinogen
[e]Ethylene glycol requires special labeling with the word “warning”' and the statement “harmful or fatal if swallowed” because ethylene glycol and mixtures containing 10% or more by weight of ethylene glycol are commonly marketed, stored, and used in a manner that increases the possibility of accidental ingestion (CPSC 2007).
[f]Ethylene glycol was recommended to the MTL by the U.S. EPA’s Office of Air and Radiation on the basis that it is a hazardous air pollutant. Ethylene glycol was added to the MTL in 1995 and EPA is initiating development of a testing action via TSCA Section 4 FRM, a TSCA Section 4 ECA, or a VTA (EPA 2007c). The testing needs indicated for health effects include acute, subchronic toxicity/90 day, neurotoxicity, and immunotoxicity studies.
[g]Designated CERCLA hazardous substance pursuant to Section 112 of the Clean Air Act.
[h]Ethylene glycol has undergone the following testing at NTP: toxicology, carcinogenesis, reproductive, developmental, and genetic toxicity studies (NTP 2007).

ACGIH = American Conference of Governmental Industrial Hygienists; AEGL = acute exposure guideline levels; CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; CPSC = Consumer Product Safety Commission; DWEL = drinking water equivalent level; ECA = Enforceable Consent Agreement; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; FRM = Final Rule-Making; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; MTL = Master Testing List; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; STEL = short-term expoure limit; TLV = threshold limit values; TSCA = Toxic Substances Control Act; TWA = time-weighted average; USC = United States Code; VTA = Voluntary Testing Agreement; WHO = World Health Organization

glycol for human carcinogenicity, but it appears on the Testing Status of Agents at NTP and has undergone standard toxicology, carcinogenesis, reproductive, developmental, and genetic toxicity studies (NTP 2007).

OSHA (2006) and NIOSH (2005) have not established a permissible exposure limit (PEL) or a recommended exposure limit (REL) for ethylene glycol. Under the "Proposed Rule on Air Contaminants" (29 CFR 1910, Docket No. H-020), NIOSH questioned whether the OSHA PEL for ethylene glycol (ceiling 50 ppm) was adequate to protect workers from potential health hazards (NIOSH 2005). ACGIH (2006) has established a 100 mg/m^3 ceiling limit for ethylene glycol.

EPA (2007b) has designated ethylene glycol as a hazardous air pollutant (HAP) under the Clean Air Act (CAA). Ethylene glycol is on the list of chemicals appearing in "Toxic Chemicals Subject to Section 313 of the Emergency Planning and Community Right-to-Know Act of 1986" and has been assigned a reportable quantity (RQ) limit of 5,000 pounds (EPA 2007d). The RQ represents the amount of a designated hazardous substance which, when released to the environment, must be reported to the appropriate authority.

Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), ethylene glycol is exempt from tolerances for residues when used in foliar applications to peanut plants (EPA 2007f).

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10. GLOSSARY

Absorption—The taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD)—Usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a BMD_{10} would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

Benchmark Dose Model—A statistical dose-response model applied to either experimental toxicological or epidemiological data to calculate a BMD.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility.

Cancer Effect Level (CEL)—The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-controlled study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without outcome.

Case Report—Describes a single individual with a particular disease or exposure. These may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Describes the experience of a small number of individuals with the same disease or exposure. These may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure—Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome. At least one exposed group is compared to one unexposed group.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at one point in time.

Data Needs—Substance-specific informational needs that if met would reduce the uncertainties of human health assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the adverse effects.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurs. The terms, as used here, include malformations and variations, altered growth, and *in utero* death.

Environmental Protection Agency (EPA) Health Advisory—An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Epidemiology—Refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one half of a quantity of a chemical from the body or environmental media.

Immediately Dangerous to Life or Health (IDLH)—The maximum environmental concentration of a contaminant from which one could escape within 30 minutes without any escape-impairing symptoms or irreversible health effects.

Immunologic Toxicity—The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

Immunological Effects—Functional changes in the immune response.

Incidence—The ratio of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

Mortality—Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a chemical.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An OR of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Organophosphate or Organophosphorus Compound—A phosphorus-containing organic compound and especially a pesticide that acts by inhibiting cholinesterase.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) allowable exposure level in workplace air averaged over an 8-hour shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests.

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic end points. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a

variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

q_1*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually µg/L for water, mg/kg/day for food, and µg/m^3 for air).

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the no-observed-adverse-effect level (NOAEL, from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a chemical.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—The American Conference of Governmental Industrial Hygienists (ACGIH) maximum concentration to which workers can be exposed for up to 15 minutes continually. No more than four excursions are allowed per day, and there must be at least 60 minutes between exposure periods. The daily Threshold Limit Value-Time Weighted Average (TLV-TWA) may not be exceeded.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

Time-Weighted Average (TWA)—An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose$_{(50)}$ (TD$_{50}$)—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Toxicokinetic—The absorption, distribution, and elimination of toxic compounds in the living organism.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis, 3 being the approximate logarithmic average of 10 and 1.

Xenobiotic—Any chemical that is foreign to the biological system.

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of

the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Environmental Medicine, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology and Environmental Medicine, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-32, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name(s): Ethylene glycol
CAS number(s): 107-21-1
Date: August 2007
Profile status: Final Draft Pre-Public Comment
Route: [X] Inhalation [ ] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Key to figure: 2
Species: Human

MRL: 2 [ ] mg/kg/day [ ] ppm [X] mg/m^3

Reference: Wills JH, Coulston F, Harris ES, et al. 1974. Inhalation of aerosolized ethylene glycol by man. Clin Toxicol 7(5):463-476.

Experimental design: Health effects were assessed in 19 male prisoners who voluntarily were exposed to ethylene glycol aerosol for 20–22 hours/day for 30 days. The diameter of the aerosol droplets ranged from 1 to 5 μm. Mean daily and mean weekly concentrations during the first 14 days of the study ranged from 0.8 to 44.8 and from 17 to 29 mg/m^3, respectively. Mean daily and mean weekly concentrations during the entire 30-day exposure period were 0.8–67 and 17–49 mg/m^3, respectively. The average mean weekly exposure was 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30. The average exposure levels did not include brief periods in which the concentration was intentionally raised to higher levels to assess acute responses. A control group consisted of 14 male prisoners; 10 of these men were never exposed to ethylene glycol, whereas the remaining 4 men had been exposed to a mean concentration of 37 mg/m^3 for 20–22 hours/day for 7 days during the week that preceded the start of the study. Subjective responses (symptoms) were monitored throughout the study. During the last 10 days of the study, the concentration of ethylene glycol was occasionally intentionally increased to various high levels (up to 308 mg/m^3) when the volunteers left the exposure chamber during meals; subjective responses to short exposures to the high concentrations were assessed when they reentered the chamber. Complete physical examinations that included slit-lamp, electrocardiographic, and electroencephalographic studies, and a battery of psychological tests designed to reveal effects on simple reaction time, reaction time with discrimination, visual-motor coordination, depth perception, and mental ability (encoding and subtraction accuracy), were conducted on all subjects pre-exposure and after 14 and 30 days of exposure. Blood samples were collected on days 0, 1, 3, 5, 8, 12, 19, 22, 26, and 29 for evaluation of hematology, clinical chemistry (including blood urea nitrogen, serum creatinine, and liver enzymes), and ethylene glycol concentration. Urine was evaluated daily for oxalate crystals, erythrocytes, and ethylene glycol, and twice weekly for volume, specific gravity, color, clarity, pH, amino acid nitrogen, and creatinine.

Effects noted in study and corresponding doses: Concentrations of ethylene glycol in the blood and urine were similar in the exposed and control groups. The near-continuous exposure levels (average 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30) were tolerated with effects that were limited to occasional complaints of upper respiratory tract irritation, slight headache, and low backache (incidences and other information not reported). The short-term, high-exposure sessions showed that the irritation became common at approximately 140 mg/m^3, and tolerated for only 15 minutes at 188 mg/m^3, 2 minutes at 244 mg/m^3, and one or two breaths at 308 mg/m^3. Based on these results and those of other trials, the investigators concluded that concentrations of about ≥200 mg/m^3 were intolerable due to strong irritation of the upper respiratory tract that included a burning sensation in the trachea and a burning cough. Because the near-continuous exposures were tolerated with respiratory irritation that was infrequent and not serious, and not accompanied by neurological, hematology, clinical chemistry, or urinalysis findings indicative of renal or other systemic effects, the interim (12–14-day) findings in this study identified a

NOAEL of 23 mg/m^3 for acute-duration exposure in humans. The LOAEL was 140 mg/m^3 because brief exposures to this concentration commonly caused respiratory irritation.

Dose and end point used for MRL derivation:

[X] NOAEL [ ] LOAEL

Uncertainty factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[ ] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable (inhalation study).

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable (human study).

Exposure concentrations were not converted from mg/m^3 to ppm because ppm is unsuitable for aerosols.

Was a conversion used from intermittent to continuous exposure? The NOAEL of 23 mg/m^3 was not adjusted for discontinuous daily exposure (20 hours/24 hours) because the critical effect is concentration dependent and not duration dependent.

Other additional studies or pertinent information that lend support to this MRL: The only other information on effects of acute-duration inhalation exposure to ethylene glycol is available from three developmental toxicity studies in rats and mice (Tyl 1988a; Tyl et al. 1995a, 1995b).

In a developmental toxicity study in CD-1 mice using whole-body exposure to nominal concentrations of 0, 150, 1,000, or 2,500 mg/m^3 for 6 hours/day on Gd 6–15, maternal body weight gain was decreased, but corrected weight was unaffected, at concentrations ≥1,000 mg/m^3 (Tyl 1988a; Tyl et al. 1995a). Significant effects on implant viability, weight of live fetuses, and on the incidence of external, visceral, and skeletal malformations were observed at concentrations ≥1,000 mg/m^3. Maternal toxicity (e.g., increased liver weight in rats and reduced body weight gain in mice) was evident at 2,500 and 1,000 mg/m^3 in rats and mice, respectively (Tyl et al. 1995a). In CD rats exposed similarly in the same study, there were significant increases in absolute and relative liver weight among maternal animals exposed to 2,500 mg/m^3; kidney weights were unchanged, and liver and kidney histopathology was not evaluated. In addition, reduced ossification at some sites in the axial skeleton was observed with exposure to 1,000 and 2,500 mg/m^3; however, in an Expert Panel Review of this study, NTP-CERHR (2004) concluded that the relationship of this effect to treatment was uncertain due to the lack of a dose-response relationship. Both of the whole-body experiments were confounded by significant ingestion of ethylene glycol deposited on the fur and consumed during grooming; the authors estimated that the ingestion dose comprised the majority of exposure (Tyl 1985, 1988a; Tyl et al. 1995a).

In a follow-up developmental study aimed at reducing the confounding from ingestion exposure, pregnant CD-1 mice were exposed nose-only to target concentrations of 0, 500, 1,000, or 2,500 mg/m^3 aerosolized ethylene glycol for 6 hours/day on Gd 6–15 (Tyl 1988a; Tyl et al. 1995b). In maternal animals, there were no effects other than changes in kidney weights. Absolute kidney weight was significantly increased at 1,000 and 2,500 mg/mg^3, and relative kidney weight was increased at 2,500 mg/m^3; however, microscopic examination of kidneys did not indicate any histopathological changes. At 2,500 mg/m^3, live

fetal body weight was significantly reduced, and there was a significant increase in the one type of skeletal malformation (fused ribs). Increases in some skeletal variations were observed at 2,500 mg/m^3, and one type (extra ossification sites in the sagittal suture) was significantly increased at concentrations ≥500 mg/m^3. The authors designated the 1,000 mg/m^3 concentration a developmental NOAEL and the 500 mg/m^3 concentration a NOAEL for maternal effects. However, the authors noted that the animals in the nose-only experiment were also exposed by ingestion of ethylene glycol during preening of the face after exposure (Tyl 1988a; Tyl et al. 1995b). Furthermore, NTP-CERHR (2004) noted that stress from restraint in the single nose-only exposure study may have contributed to the developmental effects observed with ethylene glycol, which were similar in nature to effects observed in a study of restrained nose-only exposure to water vapor (Tyl et al. 1994).

Because of the confounding oral exposures in both the whole-body and nose-only developmental toxicity studies, NTP-CERHR (2004) concluded that the data from these studies were not suitable for evaluation of effect levels from inhalation exposure to ethylene glycol. The available data do, however, provide a conservative estimate of the inhalation NOAEL, with the caveat that total exposure to ethylene glycol in these studies included intake via ingestion. Collectively, these studies suggest that inhalation exposure to ethylene glycol at a nominal concentration of about 150 mg/m^3 is not associated with developmental toxicity in mice or rats, or renal toxicity in mice (kidney histopathology not assessed in rats). The next highest concentration (500 mg/m^3 in the nose-only study) was associated with developmental effects (increased incidence of skeletal variations), but it is not possible to conclusively relate these effects to inhalation of ethylene glycol.

As indicated above, the developmental studies (Tyl 1988a; Tyl et al. 1995a, 1995b) collectively suggest that 150 mg/m^3 is a conservative NOAEL for developmental toxicity in rats and kidney toxicity in mice. This concentration is similar to the 140 mg/m^3 LOAEL for respiratory tract irritation in humans (Wills et al. 1974). The human NOAEL of 23 mg/m^3 is a suitable basis for MRL derivation because it is based on evaluations for renal and other systemic effects as well as for local irritation, and is well within the NOAEL range for developmental toxicity in animals.

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APPENDIX A

MINIMAL RISK LEVEL WORKSHEET

Chemical name(s): Ethylene glycol
CAS number(s): 107-21-1
Date: August 2007
Profile status: Final Draft Pre-Public Comment
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Key to figure: 51
Species: Mouse

MRL: 0.8 [X] mg/kg/day [ ] ppm [ ] mg/m^3

References: Neeper-Bradley TL, Tyl RW, Fisher LC, et al. 1995. Determination of a no-observed-effect level for developmental toxicity of ethylene glycol administered by gavage to CD rats and CD-1 mice. Fundam Appl Toxicol 27:121-130.

Tyl RW. 1989. Developmental toxicity evaluation of ethylene glycol administrated by gavage to CD-1 mice: Determination of a "no-observed-effect-level" (NOEL). Bushy Run Research Center, CMA Project Report 51-591.

Experimental design: Groups of 30 timed-pregnant CD-1 mice were given doses of 50, 150, 500, or 1,500 mg/kg ethylene glycol daily by gavage on Gd 6–15; vehicle controls were given water on the same schedule (Neeper-Bradley et al. 1995; Tyl 1989). Maternal animals were observed daily for clinical signs and weighed periodically; water intake was measured throughout gestation. At sacrifice on Gd 18, body weight, gravid uterine weight, liver weight, and kidney weight were measured in dams. Kidneys from control and high-dose dams were examined microscopically. Corpora lutea and uterine contents were evaluated, and live fetuses were weighed and sexed. External, visceral, and skeletal malformations and variations in the fetuses were evaluated.

Effects noted in study and corresponding doses: No effects on maternal body weight, water consumption, or liver or kidney weight were observed. There were no significant effects on the number of corpora lutea/dam, on the number of total, nonviable, or viable implants/litter, or on sex ratio. Average fetal body weight per litter was reduced (13% below controls) at 1,500 mg/kg/day. The incidence of individual external or visceral malformations was not significantly increased in any treatment group relative to the vehicle control; however, exencephaly (a malformation observed by Price et al. [1985] at higher doses) was observed in two fetuses in the 500 mg/kg/day group and in three fetuses of the 1,500 mg/kg/day dose group. There was a significant increase in the incidence of two skeletal malformations (fused ribs or thoracic arches) in the 1,500 mg/kg/day group (15/21 litters with fused ribs vs. 1/19 controls; 8/21 litters with fused thoracic arches vs. 0/19 controls). Further, the incidence of total malformations per litter (external, visceral, and skeletal) was significantly increased both at 500 and 1,500 mg/kg/day (3/19, 7/20, 5/24, 12/24, and 17/21 from control to high dose). The incidences of 23 skeletal variations were increased in the 1,500 mg/kg/day group. One of these variations (bilateral extra rib 14) was also significantly increased at ≥500 mg/kg/day (4/19, 4/20, 6/24, 17/24, and 21/21 in control through high dose groups, respectively). This study identified a developmental NOAEL of 150 mg/kg/day and LOAEL of 500 mg/kg/day for increased incidence of total malformations and bilateral extra rib 14. The high dose (1,500 mg/kg/day) was a NOAEL for maternal effects.

Dose and end point used for MRL derivation:

[ ] NOAEL [ ] LOAEL [X] BMDL

To derive a point of departure for MRL derivation, BMD dose modeling was conducted using the mouse data on the incidence of litters with malformations (of any kind) and on the incidence of one skeletal variation (bilateral extra rib 14). The incidences for both end points are presented in Table A-1. These two end points were observed at lower doses than other observed effects (skeletal malformations, pup body weight reductions).

Table A-1. Incidences of Developmental Effects in Offspring of Mice Exposed to Ethylene Glycol by Gavage on Gestation Days 6–15

	Dose (mg/kg/day)				
Effect[a]	0	50	150	500	1,500
Extra lumbar rib[b]	4/19	4/20	6/24	17/24[c]	21/21[c]
Total malformations	3/19	7/20	5/24	12/24[d]	17/21[c]

[a]Number of litters with effects/number of litters examined.
[b]Extra rib 14, first lumbar arch, bilateral.
[c]$p<0.01$.
[d]$p<0.05$.

Sources: Neeper-Bradley et al. 1995; Tyl 1989

All dichotomous variable models in the EPA Benchmark Dose Software (Version 1.4.1) were fit to the malformation and skeletal variation data. Although one of the end points modeled (total malformations) represents a more serious effect, the group sizes in this study (19–24 litters/dose examined) did not support a BMR lower than 10%; thus, an extra risk incidence of 10% above controls was selected as the BMR. Model results for the data on total malformations are shown in Table A-2. All available dichotomous models provided adequate fit to the data ($p>0.1$). Comparing across models, a better fit is generally indicated by a lower Akaike's Information Criteria (AIC). The multistage and quantal linear models converged on the same model providing the best fit (as assessed by AIC) to the data on total malformations; these models both predicted a BMD_{10} of 113.84 mg/kg/day and a $BMDL_{10}$ of 75.59 mg/kg/day. Figure A-1 shows the fit of the multistage (1-degree polynomial) model to the malformation data.

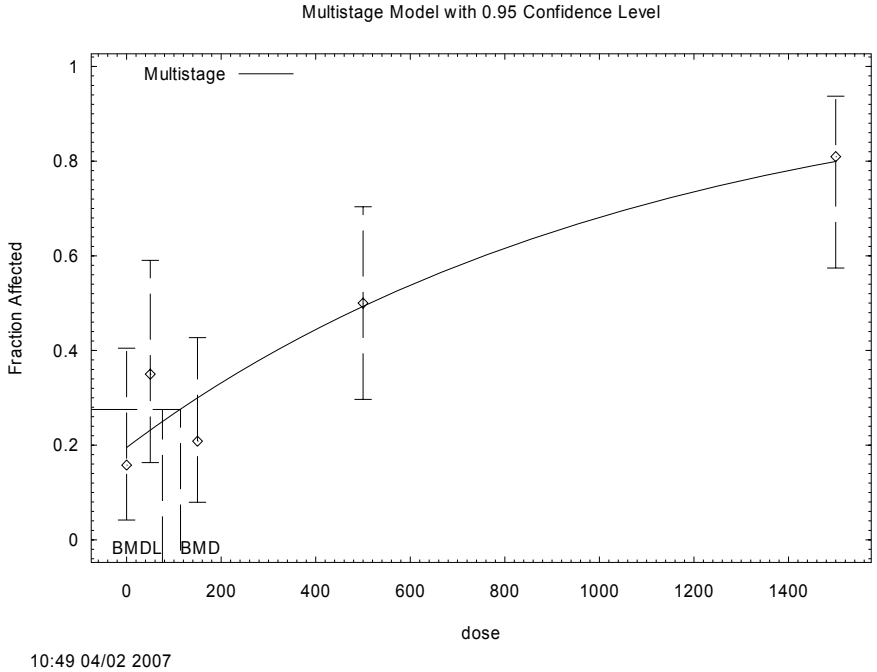
Table A-2. Model Predictions for the Incidence of Total Malformations in Offspring of Mice Exposed to Ethylene Glycol by Gavage on Gestation Days 6–15

Model	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	χ^2 p-value	AIC
Gamma[a]	162.51	75.95	0.27	129.33
Logistic	211.23	156.53	0.41	127.64
Log-logistic[b]	213.64	48.14	0.28	129.24
Multi-stage[c]	**113.84**	**75.59**	**0.44**	**127.40**
Probit	208.48	159.25	0.41	127.66
Log-probit[b]	242.07	140.87	0.28	129.21
Quantal linear	**113.84**	**75.59**	**0.44**	**127.40**
Quantal quadratic	392.59	307.59	0.26	128.84
Weibull[a]	152.27	75.98	0.27	129.33

[a]Power restricted to ≥1.
[b]Slope restricted to ≥1.
[c]Betas restricted to ≥0; lowest degree polynomial with adequate fit is reported; degree of polynomial = 1.

Sources: Neeper-Bradley et al. 1995; Tyl 1989

Figure A-1. Predicted and Observed Incidence of Total Malformations in Offspring of Mice Exposed to Ethylene Glycol by Gavage on Gestation Days 6–15*



*BMD and BMDL associated with a 10% extra risk increase over control are shown; doses given in units of mg/kg/day.

Sources: Neeper-Bradley et al. 1995; Tyl 1989

Model results for the data on bilateral extra rib 14 are shown in Table A-3. For these data, the probit model provided the best fit (as assessed by AIC); a BMD_{10} of 99.35 mg/kg/day and $BMDL_{10}$ of 75.56 mg/kg/day were predicted. Figure A-2 shows the fit of the probit model to the skeletal variation data.

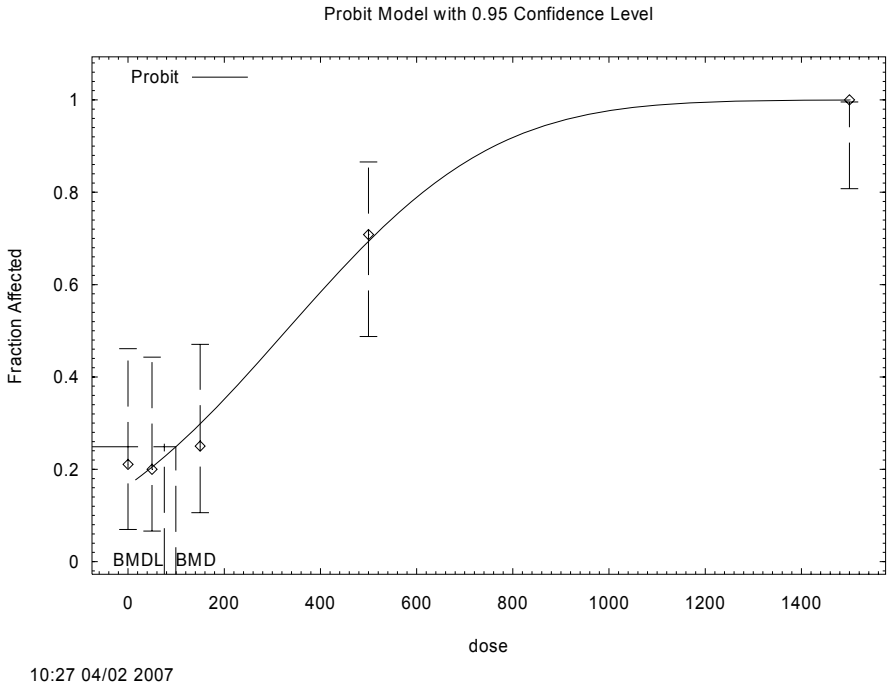
Table A-3. Model Predictions for the Incidence of Bilateral Extra Lumbar Ribs in Offspring of Mice Exposed to Ethylene Glycol by Gavage on Gestation Days 6–15

Model	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	χ^2 p-value	AIC
Gamma[a]	200.86	64.02	0.98	101.59
Logistic	103.80	77.82	0.90	100.16
Log-logistic[b]	419.85	101.67	0.91	101.72
Multi-stage[c]	49.956	35.31	0.36	103.53
Probit	**99.35**	**75.56**	**0.90**	**100.12**
Log-probit[b]	353.71	97.62	0.91	101.72
Quantal linear	49.96	35.31	0.36	103.53
Weibull[a]	192.89	60.78	0.99	101.55

[a]Power restricted to ≥1.
[b]Slope restricted to ≥1.
[c]Betas restricted to ≥0; lowest degree polynomial with adequate fit is reported; degree of polynomial = 1.

Sources: Neeper-Bradley et al. 1995; Tyl 1989

Figure A-2. Predicted and Observed Incidence of Extra Lumbar Ribs in Offspring of Mice Exposed to Ethylene Glycol by Gavage on Gestation Days 6–15*



*BMD and BMDL associated with a 10% extra risk increase over control are shown; doses given in units of mg/kg/day.

Sources: Neeper-Bradley et al. 1995; Tyl 1989

Modeling of both the malformation and skeletal variation end points resulted in the same $BMDL_{10}$, indicating that an acute oral MRL based on this point of departure should provide protection against both effects.

Uncertainty factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Although some mechanistic information suggests that humans may be less sensitive than rodents to the developmental effects of ethylene glycol, the available data are not adequate to support a lower interspecies uncertainty factor; thus, a full 10-fold uncertainty factor was used for interspecies extrapolation. While *in vitro* data suggest that humans metabolize glycolic acid (the proximate developmental toxicant) more efficiently than rats (Booth et al. 2004; Corley et al. 2005a), NTP-CERHR (2004) observed that the data supporting the glycolic acid metabolic rate in humans are limited. In addition, NTP-CERHR (2004) reviewed preliminary data indicating that the inverted yolk sac placenta, a stage in placental development that does not exist in humans, tends to concentrate weak acids such as glycolic acid in the embryonic fluids. These data suggest enhanced sensitivity to ethylene glycol developmental effects in rodents compared with humans; however, NTP-CERHR (2004) characterized the available data as inconclusive. A 10-fold uncertainty factor for interindividual variability was also used. Ethylene glycol metabolism is known to involve alcohol dehydrogenase and aldehyde

dehydrogenase, and may also involve cytochrome p450 isozymes (NTP-CERHR 2004). Polymorphisms in the genes encoding these enzymes may lead to wide variability in the production and elimination of glycolic acid and other metabolites in humans exposed to ethylene glycol, but data quantifying the range of variability are not currently available (NTP-CERHR 2004). In addition, fetal and/or placental differences in expression of these enzymes over the course of gestation will affect local concentrations of glycolic acid and other metabolites to which the developing conceptus is exposed, yet little is known about these differences (NTP-CERHR 2004).

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable (gavage study).

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? Not applicable.

Other additional studies or pertinent information that lend support to this MRL: In acute-duration oral developmental toxicity studies in rodents, fetal effects have consistently been observed at doses that are not maternally toxic. Furthermore, the teratogenic effects observed after ethylene glycol exposure appear to be generally consistent across studies and across species, with the primary end point consisting of skeletal malformations. The incidence of malformations was increased in CD-1 mice at doses of ≥500 mg/kg/day when administered by gavage during gestation (Gd 6–15) (Neeper-Bradley et al. 1995; Tyl 1989). Embyrotoxicity was also manifested as a reduction in fetal body weight in CD-1 mice given doses of ≥750 mg/kg/day on Gd 6–15 (Neeper-Bradley 1990; Price et al. 1985; Tyl 1989). In rats, doses of ≥1,000 mg/kg/day by gavage on Gd 6–15 resulted in an increased incidence of skeletal malformations in offspring (Neeper-Bradley 1990; Neeper-Bradley et al. 1995; Price et al. 1985). Decreases in pup body weight and increases in both the number of litters with malformations and the number of malformed fetuses per litter were observed in rats treated during Gd 6–15 with doses ≥2,500 mg/kg/day (Price et al. 1985; Neeper-Bradley 1990; Neeper-Bradley et al. 1995). In mice given doses of 3,000 mg/kg/day during Gd 6–15, neural tube and craniofacial defects were increased, and the number of live fetuses per litter was decreased (Price et al. 1985). In contrast to the results in rodents, no teratogenic effects were observed in rabbits exposed to maternally lethal doses of 2,000 mg/kg/day during gestation (Tyl et al. 1993).

No effects were observed on hematology parameters, but dose-related effects on bone marrow and erythropoiesis were observed when doses of 0, 50, 100, or 250 mg/kg/day ethylene glycol were given for 4 consecutive days by gavage to B6C3F1 mice. Seven mice per sex were sacrificed on 1 day postexposure for measurement of body, liver, thymus, spleen, kidney, and testis weights, and histopathology of these organs as well as the lung, heart, adrenals, stomach, bone marrow, urinary bladder, intestines, and uterus. Hematology, bone marrow parameters, and erythropoiesis were evaluated in other groups of mice evaluated between 1 and 14 days after exposure. Microscopic examination of the spleen and bone marrow did not reveal any histopathological changes, and there were no significant changes to hematological parameters (hemoglobin, hematocrit, mean corpuscular volume, erythrocyte and leukocyte counts) evaluated 5 days after exposure termination. Exposure to ethylene glycol resulted in statistically significant decreases in bone marrow cellularity (up to about 25% below control values) at doses of ≥100 mg/kg/day; this effect persisted up to 14 days after exposure in males. Granulocyte-macrophage progenitor formation was suppressed (~15% below controls) at 50 mg/kg/day in males evaluated 14 days postexposure and at higher doses in both males and females evaluated at earlier time points. The magnitude of reduction in granulocyte-macrophage progenitor formation ranged up to 40% below controls at 250 mg/kg/day. Iron uptake in the bone marrow was suppressed (38% below controls) in males exposed to 250 mg/kg/day.

While bone marrow effects were observed in male mice exposed to doses of 50–250 mg/kg/day in this study, the biological significance of these effects is uncertain. No effects were observed on any hematological parameters, or on bone marrow or spleen histology (Hong et al. 1988). Histology was evaluated only 1 day after exposure, and hematological parameters were evaluated 5 days postexposure; thus, these evaluations would not have captured delayed effects on these parameters. However, studies using much higher doses and longer durations have failed to indicate effects on bone marrow, spleen, or hematology in mice, and provide inconsistent findings in rats. No histological changes in the bone marrow were observed in mice or rats exposed to higher doses of ethylene glycol for longer durations; these included B6C3F1 mice exposed to ≤16,000 mg/kg/day in diet for 13 weeks or ≤12,000 mg/kg/day in diet for 2 years (Melnick 1984; NTP 1993), F344 rats exposed to ≤10,000 mg/kg/day in diet for 13 weeks (Melnick 1984), and Sprague-Dawley rats exposed to ≤7,327 mg/kg/day in drinking water for 10 or 90 days (Robinson et al. 1990). Results of routine hematology evaluations in these studies were unremarkable except for some alterations in the 10- and 90-day studies in rats. In the 10-day study, statistically significant decreases in hemoglobin, hematocrit, erythrocytes, and total leukocytes (7.3, 8.9, 8.5, and 34.8% less than controls, respectively) occurred in female rats at 7,327 mg/kg/day (Robinson et al. 1990). In the 90-day study, total leukocyte counts were significantly reduced in female rats at 597, 3,087, and 5,744 mg/kg/day (32, 30, and 50% less than controls, respectively) (Robinson et al. 1990). Results of differential counts were not reported, and no clear hematological changes occurred in male rats in either study. Hematology evaluations were also negative in other studies that did not examine bone marrow histology; these included studies of B6C3F1 mice exposed to ≤12,000 mg/kg/day in diet for 2 years (DePass et al. 1986a), Wistar rats exposed to ≤2,000 mg/kg/day by gavage for 4 weeks (Schladt et al. 1998), and Wistar rats exposed to ≤1,128 mg/kg/day in diet for 16 weeks (Gaunt et al. 1974). In male F344 rats exposed to 1,000 mg/kg/day in the diet for 2 years, significant hematological changes were observed, but this dose also caused mortality due to renal toxicity (DePass et al. 1986a).

The hematopoietic system is an established target system for several ethylene glycol ethers (e.g., ethylene glycol monomethyl ether, ethylene glycol monobutyl ether (IRIS 2007). For these compounds, hematological effects are consistently observed across different species, doses, and exposure durations. In contrast, few studies have suggested hematological effects from ethylene glycol exposure, and those with positive findings were at higher doses than Hong et al. (1988) and gave inconsistent results. Given the lack of supporting evidence for hematological, bone marrow, or splenic effects in mice and rats exposed to much higher doses of ethylene glycol and for longer durations, the biological significance of the effects observed by Hong et al. (1988) is considered uncertain, and this study was not used to derive the acute oral MRL.

In a 10-day drinking water study, groups of 10 male and 10 female Sprague-Dawley rats were administered 0, 0.5, 1.0, 2.0, or 4.0% ethylene glycol; reported mean compound consumption was 649, 1,343, 2,615, and 5,279 mg/kg/day in males, and 794, 1,506, 2,953, and 7,327 mg/kg/day in females (Robinson et al. 1990). The incidence and severity of renal lesions were significantly increased and dose-related in males at ≥2,615 mg/kg/day; effects included tubular dilation, degeneration, necrosis, and intratubular calcium oxalate crystals. Effects on body weight, organ weights, and hematological parameters were observed at the high dose only. Changes in serum chemistry parameters were observed at lower doses in both males and females; however, these were not accompanied by histopathological changes in the liver. This study identified a LOAEL of 2,615 mg/kg/day for renal toxicity in male rats. As discussed above, other studies identified bone marrow effects and developmental toxicity at lower doses; thus, this study was not considered for use in acute oral MRL derivation.

Corley et al. (2005a) published a PBPK model for rats, but no model has yet been developed for mice, the species used in the study selected for MRL derivation. As a result, available data do not support the use of PBPK modeling to derive an acute oral MRL for ethylene glycol based on developmental toxicity in mice.

A key uncertainty in the acute-duration oral MRL stems from the use of gavage administration in the critical study. Bolus doses from gavage administration lead to higher peak concentrations of glycolic acid in the blood than occur with equivalent doses at slower dose-rates associated with environmentally-relevant exposures (Carney et al. 2001; NTP-CERHR 2004). Because the key study used gavage administration, the dose at which effects were observed may be lower than would be observed with non-bolus dosing. In support of this, Maronpot et al. (1983) observed neither fetal nor maternal toxicity at dietary doses up to 1,000 mg/kg in F344 rats, while Neeper-Bradley et al. (1995) reported skeletal malformations and effects on fetal body weight in CD rats given 1,000 mg/kg via gavage. While strain differences in susceptibility to ethylene glycol cannot be ruled out as the source of the differing results, the data supporting glycolic acid as the proximate toxicant, and the evidence for much lower serum levels of glycolic acid with continuous dosing than with bolus dosing, suggest that the lack of developmental toxicity observed by Maronpot et al. (1983) likely resulted from the difference in dose-rate.

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APPENDIX B. USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public, especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order that they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, and dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal Risk Levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See Sample LSE Table 3-1 (page B-6)

(1) Route of Exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.

(2) Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect. The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).

(5) Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to “Chemical x” via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).

(7) System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system, which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference. The complete reference citation is given in Chapter 9 of the profile.

(11) CEL. A CEL is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates that the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Sample Figure 3-1 (page B-7)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the acute and intermediate exposure periods are illustrated.

(14) Health Effect. These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL. Key number 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels. This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure. The Key explains the abbreviations and symbols used in the figure.

SAMPLE

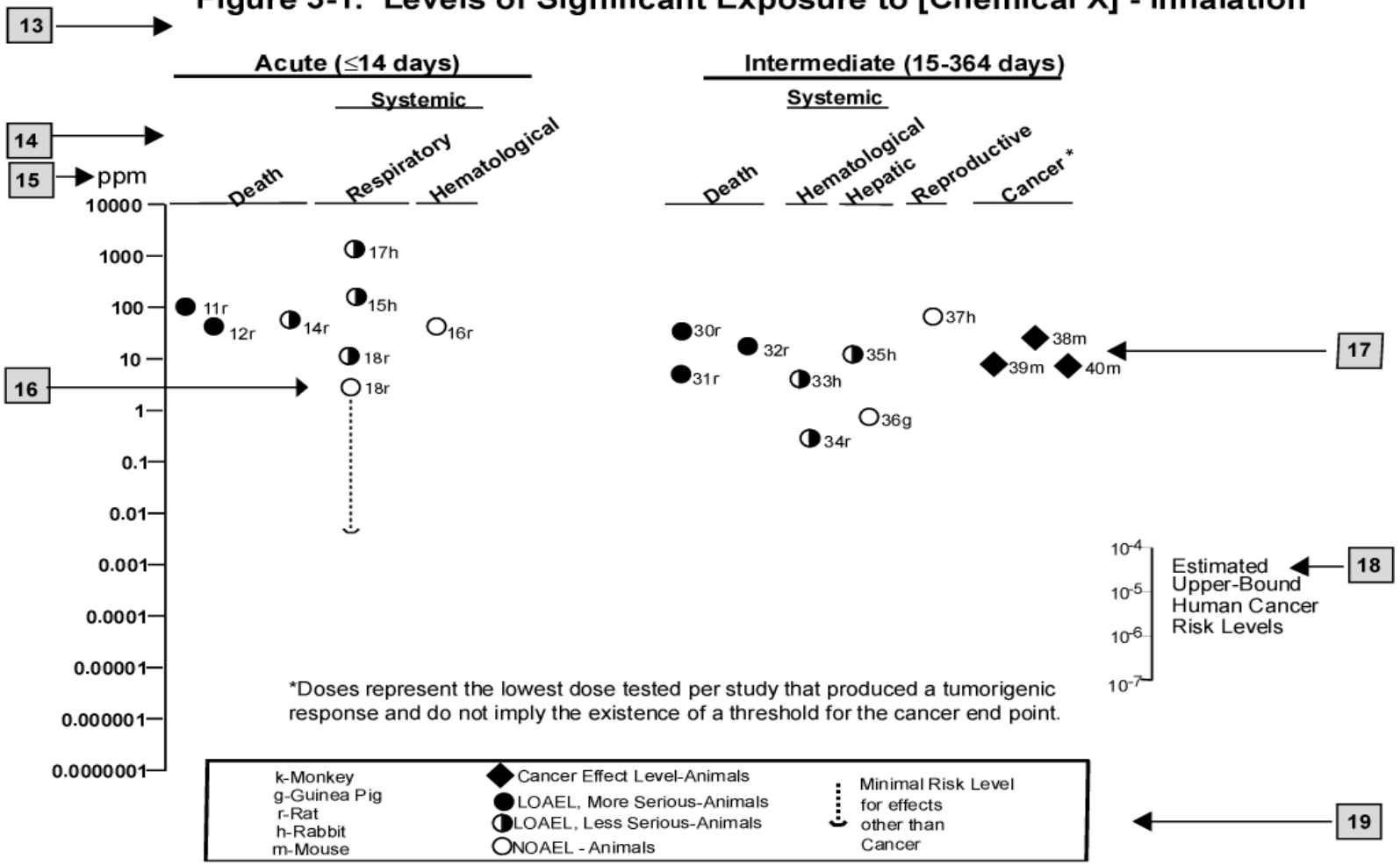
1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

	Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	Serious (ppm)	Reference
2 →	INTERMEDIATE EXPOSURE							
		5	6	7	8	9		10
3 →	Systemic	↓	↓	↓	↓	↓		↓
4 →	18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
	CHRONIC EXPOSURE							
	Cancer					11		
						↓		
	38	Rat	18 mo 5 d/wk 7 hr/d			20	(CEL, multiple organs)	Wong et al. 1982
	39	Rat	89–104 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
	40	Mouse	79–103 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

12 → [a] The number corresponds to entries in Figure 3-1.
[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of $5x10^{-3}$ ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



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APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	best available technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BMD	benchmark dose
BMR	benchmark response
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation
DOT/UN/ NA/IMCO	Department of Transportation/United Nations/ North America/Intergovernmental Maritime Dangerous Goods Code

DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
MA	*trans,trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor

MFO	mixed function oxidase
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
ND	not detected
NFPA	National Fire Protection Association
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OTS	Office of Toxic Substances
OW	Office of Water
OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon

PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
pg	picogram
PHS	Public Health Service
PID	photo ionization detector
pmol	picomole
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RQ	reportable quantity
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

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APPENDIX D. INDEX