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

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[b] M			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)

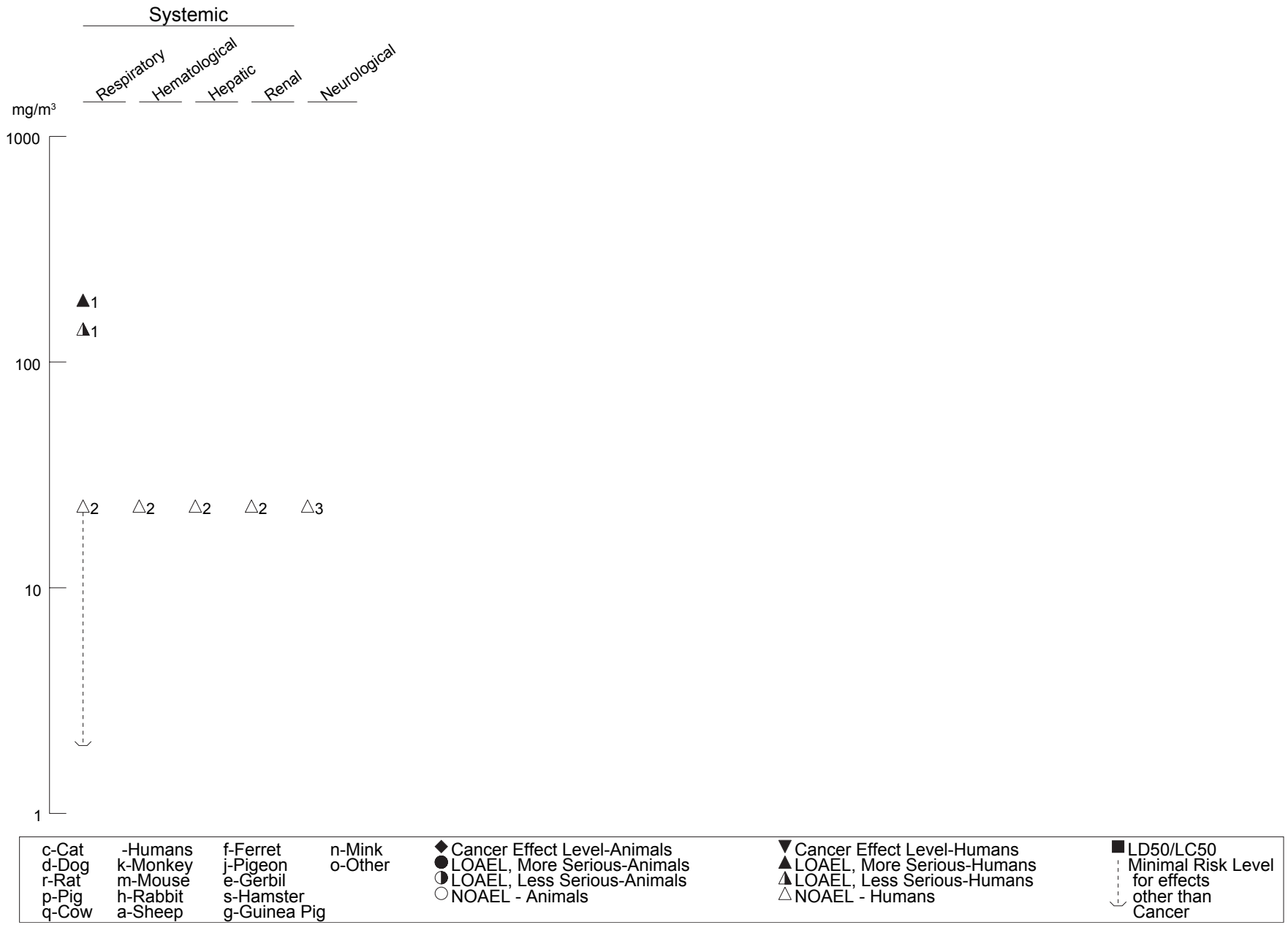
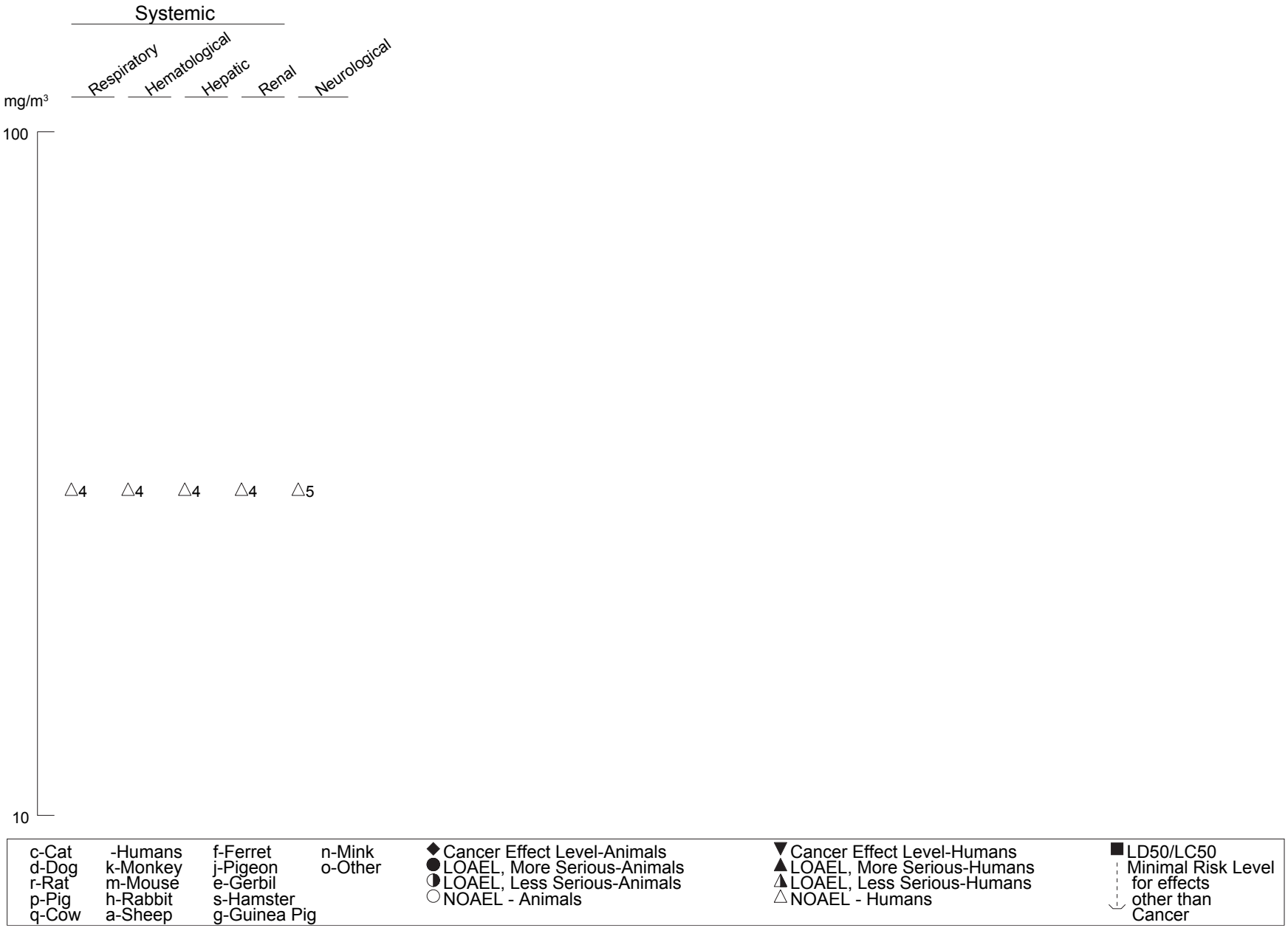


Figure 3-1 Levels of Significant Exposure to Ethylene Glycol - Inhalation *(Continued)*

Intermediate (15-364 days)



that included a burning sensation in the trachea and a burning cough. The NOAEL of 23 mg/m^3 for days 1–14 was used to derive an acute-duration inhalation MRL for ethylene glycol 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).

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 $\leq$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 and the sweet taste make 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). Ingestion is the exposure route most commonly associated with adverse effects.

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 (Corley et al. 2008).

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). 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

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				Reference Chemical Form	Comments
					Less Serious (mg/kg/day)		Serious (mg/kg/day)			
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 (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 (CD-1)	2 gen (W)			897 F (reduced pup weight in F0 females)		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
CHRONIC EXPOSURE								
Death								
90	Rat (Sprague-Dawley)	2 yr (F)				750 M (100% mortality within 100 days)	Blood 1965	
						3000 F (100% mortality during second year)		
91	Rat (Wistar)	12 mo (F)				300 M (4/10 died or were moribund on days 111-221)	Corley et al. 2008	
92	Rat (Fischer 344)	16 mo (F)				1000 M (100% dead or moribund by day 475)	DePass et al. 1986a; Woodside 1982	

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								
93	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)			
94	Rat (Wistar)	12 mo (F)	Renal	150 M		300 M (oxalate nephrosis and bladder inflammation and hemorrhage)	Corley et al. 2008	
			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
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	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
97	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 12000 F	6000 M (oxalate-like crystals and calculi in tubules)			
			Endocr	12000 F				
			Dermal	12000 F				
			Bd Wt	12000 F				
Immuno/ Lymphoret								
98	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
99	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.
100	Mouse (CD-1)	24 mo (F)		1000			DePass et al. 1986a; Woodside 1982	NOAEL is for histopathology of spleen and lymph nodes.
101	Mouse (B6C3F1)	24 mo (F)		12000 F			NTP 1993	NOAEL is for histopathology of spleen, thymus and lymph nodes.
Neurological								
102	Rat (Sprague-Dawley)	2 yr (F)		3000 F			Blood 1965	NOAEL is for histopathology of brain.
103	Rat (Fischer 344)	24 mo (F)		1000			DePass et al. 1986a; Woodside 1982	NOAEL is for histopathology of brain and spinal cord.
104	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
105	Mouse (B6C3F1)	24 mo (F)		12000 F			NTP 1993	NOAEL is for histopathology of brain.
Reproductive								
106	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.
107	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.
108	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 MRL based on Cruzan et al. (2004) would be higher than the acute-duration MRL, as discussed in Section 2.3. 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)

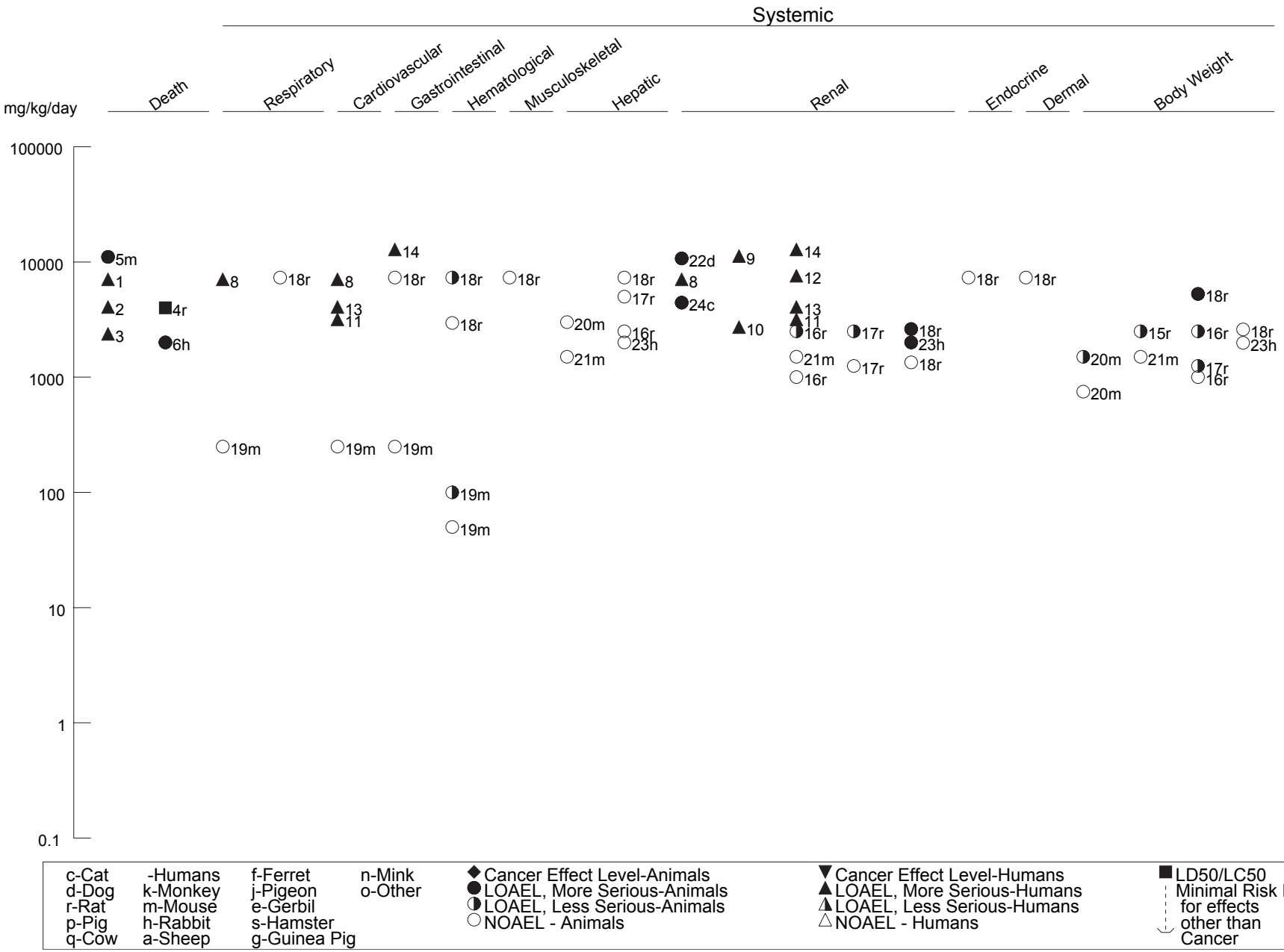


Figure 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral *(Continued)*

Acute (≤14 days)

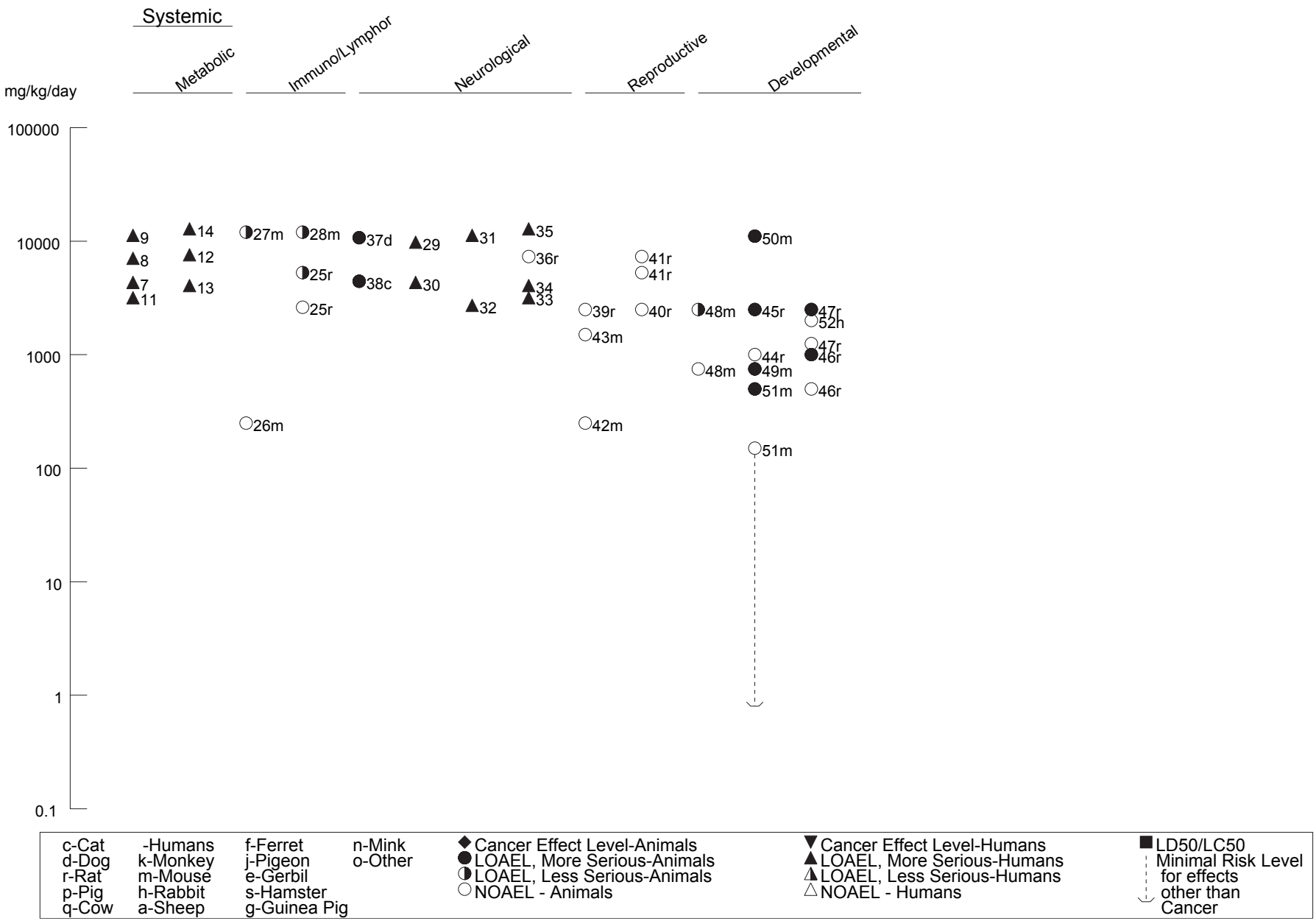
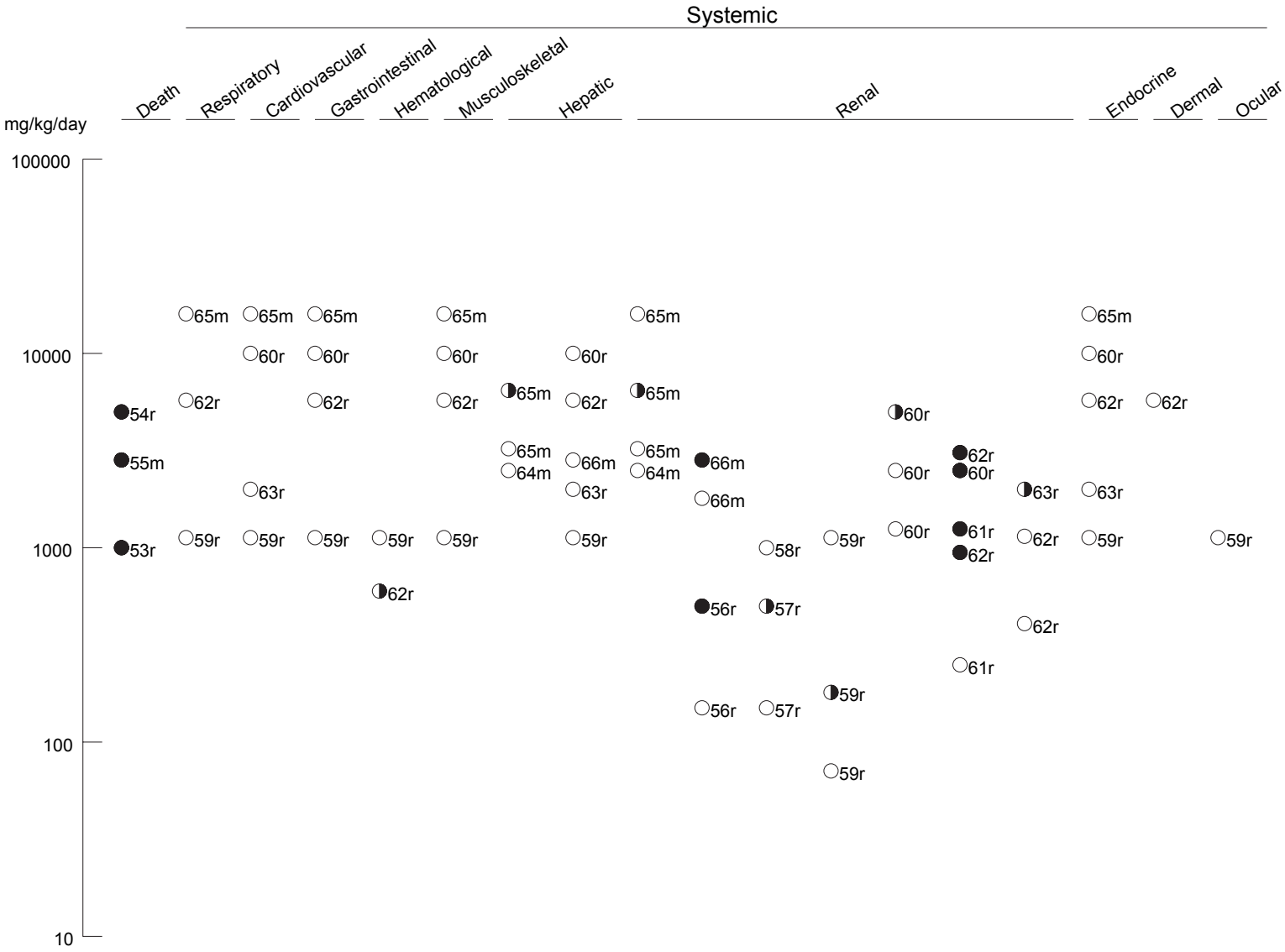


Figure 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral *(Continued)*

Intermediate (15-364 days)



c-Cat -Humans f-Ferret n-Mink
d-Dog k-Monkey j-Pigeon o-Other
r-Rat m-Mouse e-Gerbil
p-Pig h-Rabbit s-Hamster
q-Cow a-Sheep g-Guinea Pig

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◮ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

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

Intermediate (15-364 days)

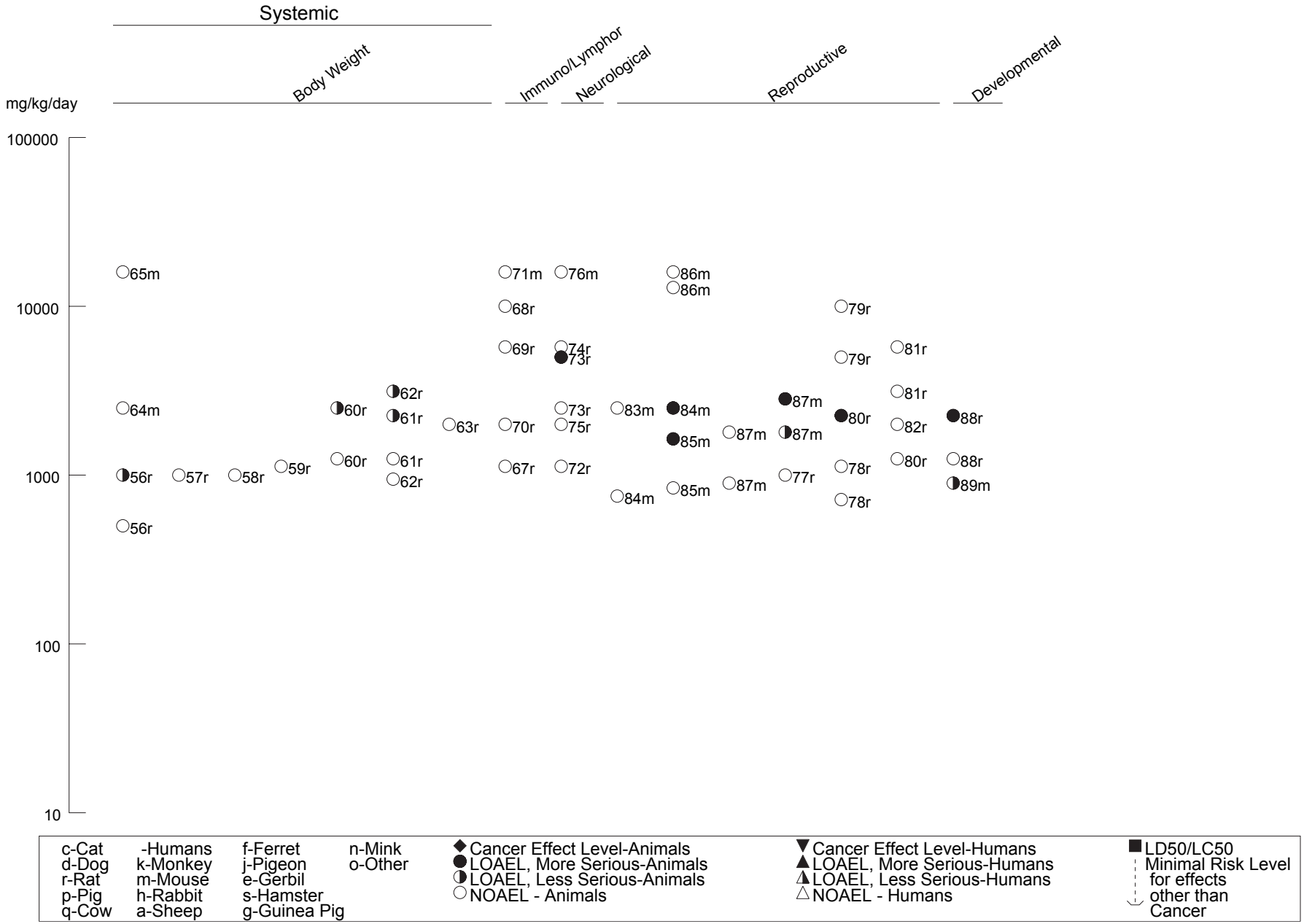


Figure 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral *(Continued)*

Chronic (≥365 days)

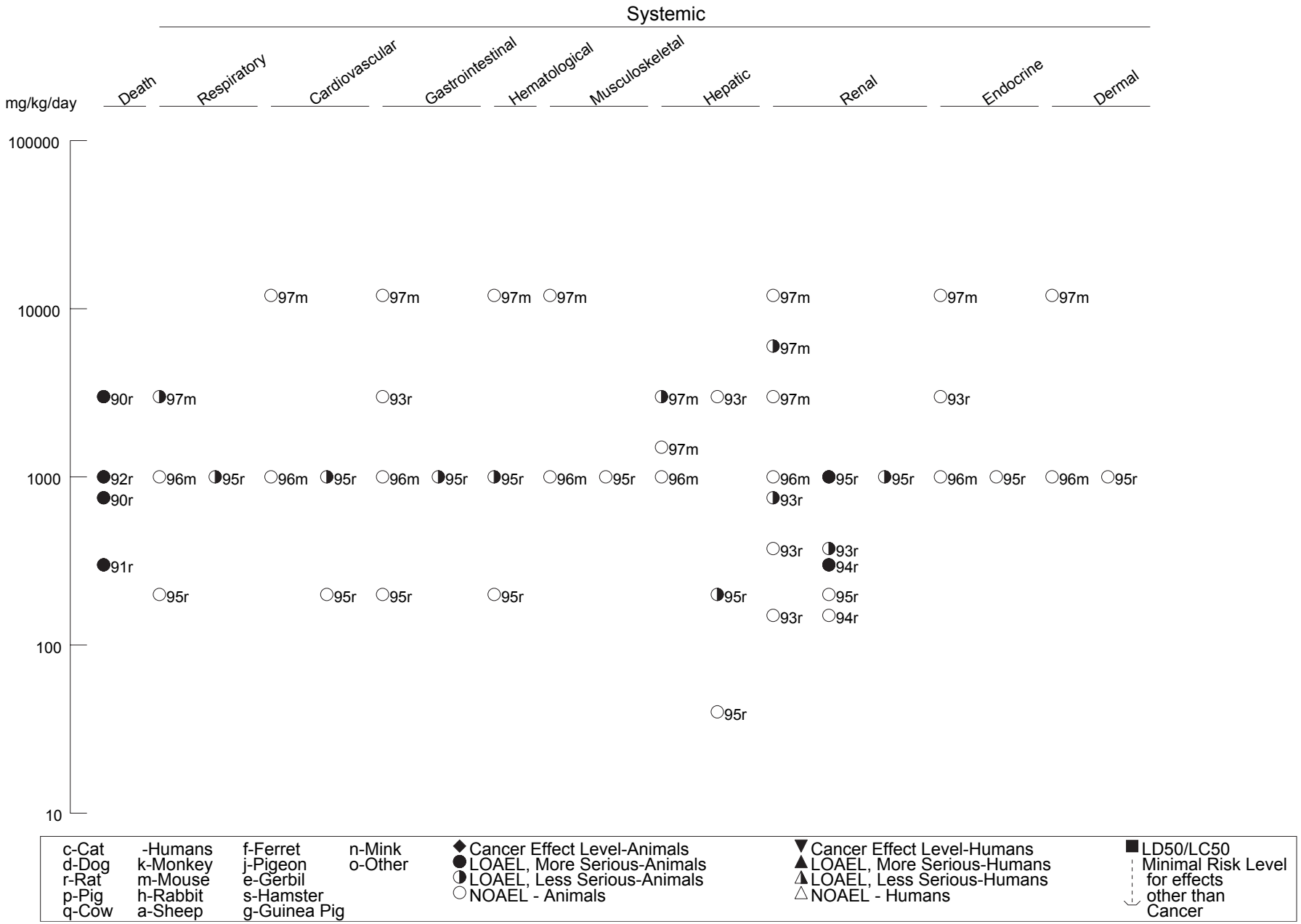
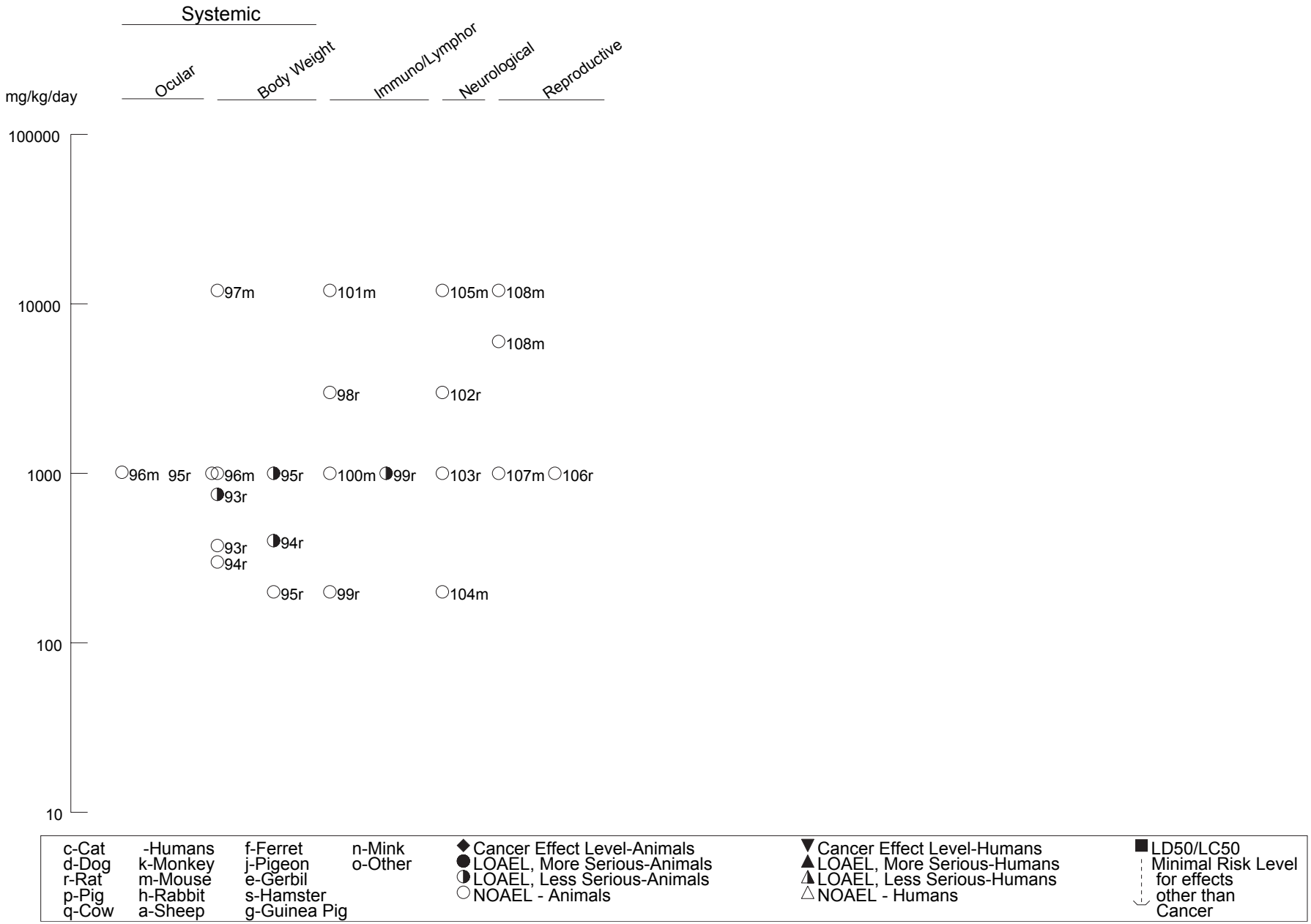


Figure 3-2 Levels of Significant Exposure to Ethylene Glycol - Oral *(Continued)*

Chronic (≥365 days)



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 aminotransferase (ALT), aspartate aminotransferase (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; Lovrić et al. 2007; 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 (Corley et al. 2008). 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 (Corley et al. 2008) 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 (Corley et al. 2008). 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 (Corley et al. 2008).

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 (Amathieu et al. 2006; 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 glyoxylate 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) (Amathieu et al. 2006; 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 agnosia developed. Unusual brain findings in other cases of ethylene glycol poisoning included bilateral pallidal hemorrhage

(Caparros-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 decreased live implants and significantly increased 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 spermatocytes, vacuolization of epithelial cells, and interstitial cell hyperplasia) and F_1 males (seminiferous tubule degeneration and interstitial 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 embryotoxicity in laboratory animals exposed to ethylene glycol exposure includes reduction in fetal body weight.

Studies in laboratory animals indicate that acute-duration oral 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 ethylene glycol doses of 0, 750, 1,500, or 3,000 mg/kg/day 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 ≥1,500 mg/kg/day. In a later study aimed at identifying a NOAEL for developmental effects in CD-1 mice, an increased incidence of malformations was observed at gavage doses ≥500 mg/kg/day 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 gavage doses of at least 1,000 mg/kg/day 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 ≥2,500 mg/kg/day in CD rats treated 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 administered gavage doses ≥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 gavaged with ethylene glycol at 2,500 mg/kg/day 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 embryotoxicity 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 ≥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 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 ≥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 administered 2,250 mg/kg/day ethylene glycol by gavage 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 administered 2,250 mg/kg/day ethylene glycol by gavage on Gd 6–20 (NTP 1988).

In summary, there is a substantial database demonstrating developmental toxicity in rats and mice 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 ≥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).

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.

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

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
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

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 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).

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

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), ^{13}C-ethylene glycol and $^{13}C_2$-glycolic acid were detected in the plasma and urine, providing evidence of absorption (Carstens et al. 2003). Similar results were obtained from two other volunteers exposed to ^{13}C-ethylene glycol vapors (Upadhyay et al. 2008). 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-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, dogs, and pregnant rabbits (Carney et al. 2008; 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

Information regarding the dermal absorption of ethylene glycol in humans is limited. Upadhyay et al. (2008) applied 0.8 mL of ^{13}C-ethylene glycol to the forearm (occluded) of each of three volunteers for 4 or 6 hours and assessed the appearance of ^{13}C-ethylene glycol and ^{13}C-glycolic acid in serum and urine. Their results indicated that only 1.0–1.3% of the epidermally-applied dose was absorbed. A skin permeability constant of 0.000027 cm/hour was calculated.

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. A similar result was obtained from another volunteer who inhaled ethylene glycol vapors (Upadhyay et al. 2008).

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 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).

Ethylene glycol crosses the placenta and enters the developing fetus. In a toxicokinetic study of orally exposed pregnant rabbits, ethylene glycol levels were measured in maternal blood and kidney tissue as well as yolk sac cavity fluid, the yolk sac itself, and fetal tissues (Carney et al. 2008). Measured ethylene glycol concentrations were initially somewhat lower in the yolk sac cavity fluid than those of maternal blood, but were slightly higher during the elimination phase (Carney et al. 2008). Levels of unchanged ethylene glycol in yolk sac and embryos were approximately 14–20% of the level in maternal blood. In a study designed to compare levels of ethylene glycol, glycolic acid, and oxalic acid in maternal blood and extraembryonic fluids of rats and rabbits (Carney et al. 1998), gavage doses of 500 or 2,500 mg/kg ethylene glycol were administered to pregnant rats and rabbits on Gd 10 or 9, respectively. Maternal blood levels of ethylene glycol were similar in the rats and rabbits, indicating no significant species differences in absorption. Ethylene glycol levels were determined in the extraembryonic fluid of both species and were found to be approximately 2-fold higher in the rat compared to the rabbit.

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

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

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

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

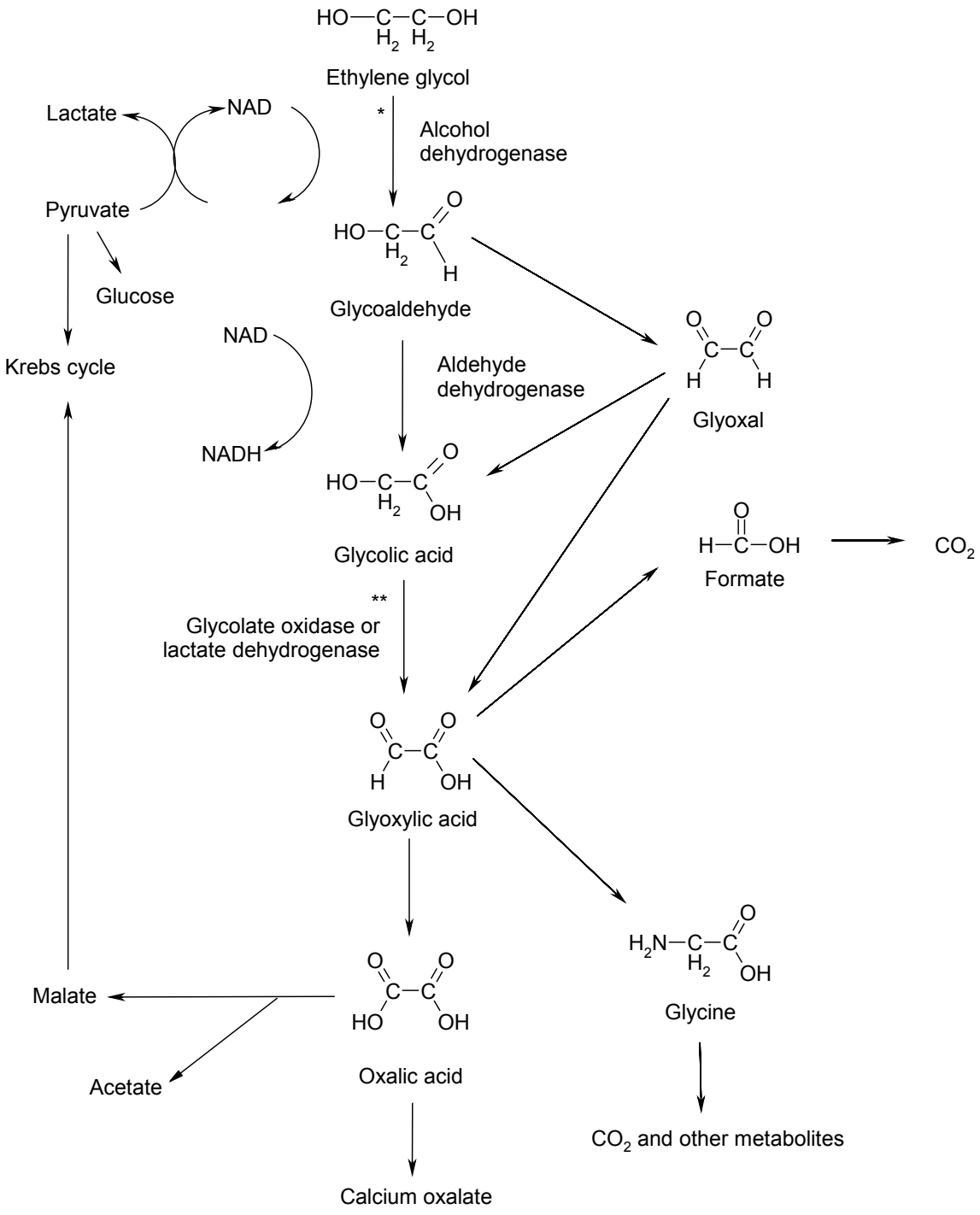
The metabolic pathway for ethylene glycol is shown in Figure 3-3. Asterisks indicate rate-limiting steps in the pathway. The metabolism of ethylene glycol was reviewed by NTP-CERHR (2004) and Slikker et al. (2004). Ethylene glycol is converted to glycolaldehyde by nicotinamide adenine dinucleotide (NAD)-dependent 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. The conversion of glycolic acid to glyoxylic acid is the most rate-limiting step in ethylene glycol metabolism (Slikker et al. 2004).

Both glycolic acid and oxalic acid 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 volunteers who inhaled ^{13}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 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. Similar results were obtained for two other similarly exposed volunteers (Upadhyay et al. 2008).

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

Figure 3-3. Metabolic Pathway for Ethylene Glycol



*Rate-limiting step
**Most rate-limiting step

Sources: NTP-CERHR 2004; Slikker et al. 2004

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 levels ≥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 do rats. *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. 2005). 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 ^{13}C-ethylene glycol at doses estimated by the authors to be 0.96 and 1.51 mg/kg. Urinary excretion of ^{13}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 ^{13}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 ^{13}C-ethylene glycol was not detectable in exhaled air, but did not assess expiration of $^{13}CO_2$. Similar results were obtained for two other similarly exposed volunteers (Upadhyay et al. 2008).

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 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.

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

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

^{13}C-Glycolytic acid was detected in the urine of three volunteers following 4- or 6-hour dermal exposure to ^{13}C-ethylene glycol (Upadhyay et al. 2008).

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.

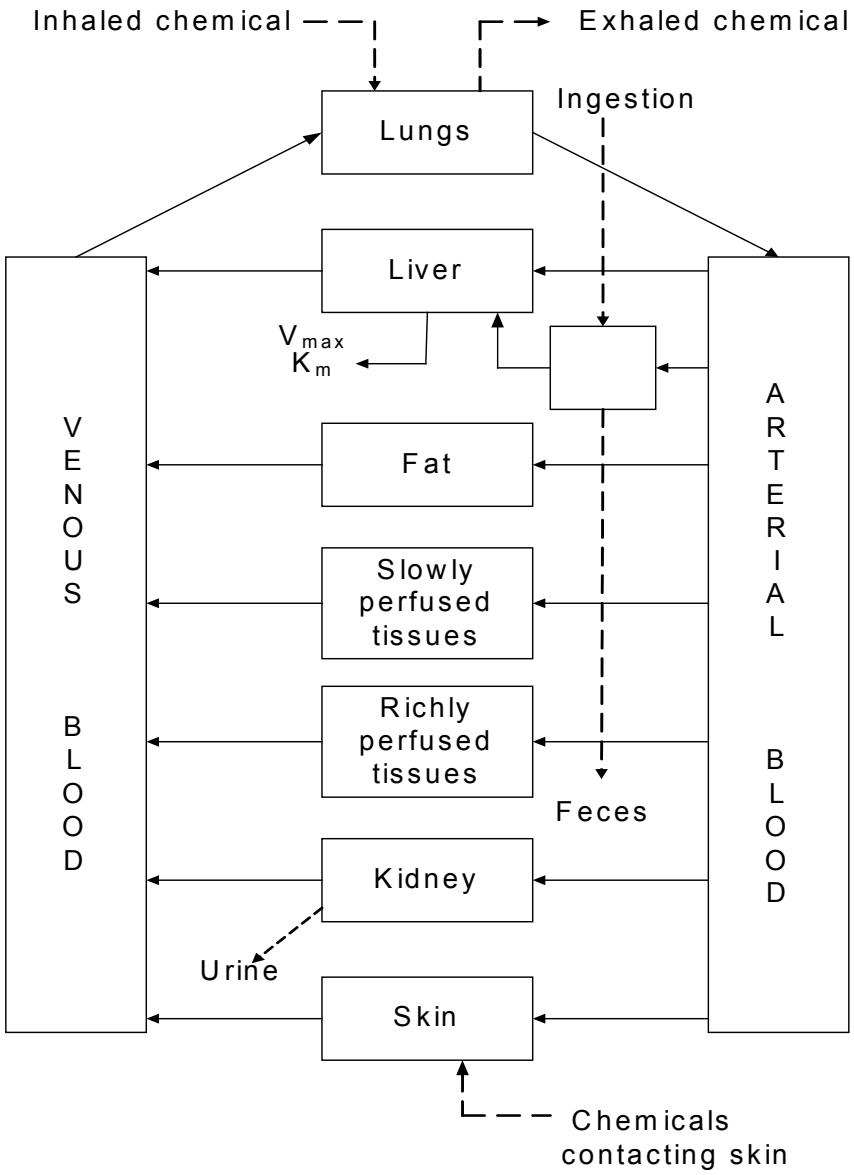
PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 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.

A PBPK model is available to describe the disposition of ethylene glycol and its intermediate metabolite, glycolic acid, in rats and humans after inhalation, oral consumption, intraperitoneal injection, intravenous injection, or subcutaneous infusion of ethylene glycol or glycolic acid (Corley et al. 2005). The model is composed of submodels for ethylene glycol and glycolic acid, which are linked via saturable metabolism in the liver. The submodel for ethylene glycol consists of eight compartments connected by blood flow (lungs, richly perfused tissues, poorly perfused tissues, fat, skin, gastrointestinal tract, liver, and kidney); the submodel for glycolic acid has 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. The model assumes instantaneous dispersion of ethylene glycol and glycolic acid through each compartment based on blood perfusion rates and relative tissue:blood partition coefficients. Urinary elimination of ethylene glycol and glycolic acid were 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. The elimination of ethylene glycol into urine was described as a first-order clearance of arterial blood scalable by $(\text{body weight})^{0.70}$. Urinary elimination of glycolic acid 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.

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. 2005

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. 2005

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

As identified in Section 3.12.3 (Ongoing Studies), Corley and coworkers are working to extend the PBPK model for ethylene glycol and glycolic acid in rats and humans (Corley et al. 2005) to include glyoxylic acid and oxalic acid. However, at the time of publication of this ATSDR update Toxicological Profile for Ethylene Glycol, the model revisions had not been completed.

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). Recent *in vitro* studies confirm that intracellular calcium oxalate, not the oxalate ion, is responsible for cytotoxicity observed in cultured human proximal tubule cells (Guo and McMartin 2005; Guo et al. 2007). Results of studies using isolated rat kidney mitochondria indicate that calcium oxalate, not oxalate ion, induces changes in mitochondrial permeability, which may lead to renal cell death (McMartin and Wallace 2005).

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 ethylene glycol concentrations up to 50 mM did not cause morphological changes, while glycolic acid caused changes in the skeletal and craniofacial regions at concentrations ≥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. (2005) 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. 2005). 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. 2005), 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).

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.

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

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.

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 (Amathieu et al. 2006; 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.

Some recent investigations have focused on methods to prevent or reduce calcium oxalate formation. Calcium oxalate, an end metabolite of ethylene glycol and a major component of kidney stones, has been demonstrated to cause cytotoxicity in cultured human proximal tubule cells (Guo and McMartin 2005; Guo et al. 2007). Treatment with aluminum citrate was shown to significantly decrease calcium oxalate-

induced cytotoxicity (Guo and McMartin 2007). These results indicate that aluminum citrate may protect against tissue damage caused by high levels of calcium oxalate accumulation. In rats administered ethylene glycol in the drinking water at sufficient concentrations to induce the precipitation of calcium oxalate in proximal renal tubules, intraperitoneal injection of thymoquinone (a major component of *Nigella sativa* seeds) significantly decreased the number and size of the calcium oxalate deposits (Hadjzadeh et al. 2008). However, the usefulness of thymoquinone as a therapeutic drug for kidney calculi requires further investigation.

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

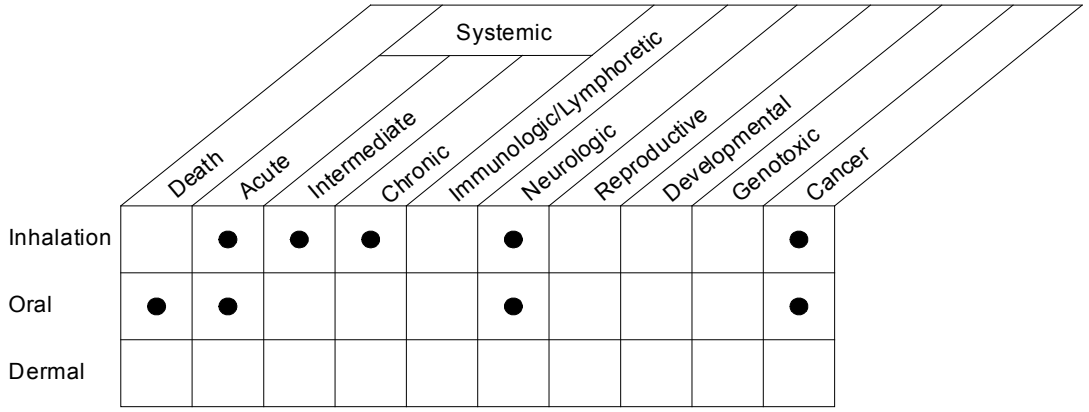
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of ethylene glycol is available. Where adequate information is 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.

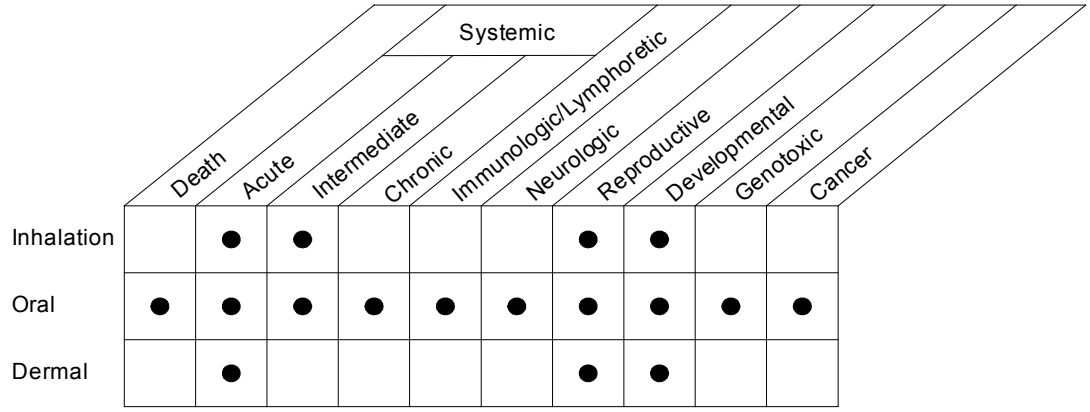
3.12.1 Existing Information on Health Effects of ethylene glycol

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

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



Human



Animal

● Existing Studies

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 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 ethylene glycol 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. The study authors stated that irritation became common at approximately 140 mg/m^3 (incidences and exposure duration not specified) and concluded that concentrations ≥200mg/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. 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 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). The near-continuous exposure was tolerated with effects that included occasional complaints of upper respiratory tract irritation, slight headache, and low backache. These occasional complaints were not associated with any particular exposure concentration and incidence and frequency data were not included in the study report; there was no mention of self-reported results from a control group. Therefore, neither a reliable LOAEL nor a definitive NOAEL were established in this study. The study found no indications of renal or other systemic effects as shown by urinalysis and hematology, clinical chemistry, and neurobehavioral evaluations. 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). 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 is considered adequate for deriving an intermediate-duration oral MRL for ethylene glycol because it does not have the limitations of the Gaunt et al. (1974) study. The data set for the critical effect (crystal nephropathy) is not appropriate for BMD analysis because the incidences increased from 0% at doses ≤150 mg/kg/day to 100% at the higher dose levels (i.e., 500 and 1,000 mg/kg/day). 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; Corley et al. 2008; DePass et al. 1986a) 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 doses ≥300 mg/kg/day in Wistar males (Corley et al. 2008), kidney lesions at doses ≥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 (Corley et al. 2008) 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 at doses ≤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 increase confidence in the chronic-duration oral MRL. 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 two studies evaluating toxicokinetic data in humans exposed via inhalation was identified (Carstens et al. 2003; Upadhyay et al. 2008). However, these studies are 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).

Information regarding the dermal absorption of ethylene glycol in humans is limited. Results of Upadhyay et al. (2008) indicate that only approximately 1% of an epidermally applied dose may be absorbed. No data were found regarding the kinetics of dermally absorbed ethylene glycol in humans. 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 percutaneous 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. (2005) 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. 2005; 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. 2005) 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). However, Corley and coworkers are presently extending the PBPK model for ethylene glycol and glycolic acid in rats and humans (Corley et al. 2005) to include glyoxylic acid and oxalic acid.