2. RELEVANCE TO PUBLIC HEALTH

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

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

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

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

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

2.2 SUMMARY OF HEALTH EFFECTS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2.3 MINIMAL RISK LEVELS (MRLs)

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

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

Inhalation MRLs

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Oral MRLs

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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