National Toxicology Program
U.S. Department of Health and Human Services

Center For The Evaluation Of Risks To Human Reproduction

NTP-CERHR EXPERT PANEL REPORT on the REPRODUCTIVE and DEVELOPMENTAL TOXICITY of ETHYLENE GLYCOL

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PREFACE

The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences (NIEHS) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the Center is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction, including development, caused by agents to which humans may be exposed.

Ethylene glycol was selected for evaluation by the CERHR based on its high production, most of which is used in the production of polyester compounds. There is widespread public exposure to ethylene glycol due to its use in automotive antifreeze and as a de-icer for aircraft.

This evaluation results from the effort of a nine-member panel of government and non-government scientists that culminated in a public expert panel meeting held February 11-13, 2003. This report has been reviewed by CERHR staff scientists, and by members of the Ethylene Glycol / Propylene Glycol Expert Panel. Copies have been provided to the CERHR Core Committee, which is made up of representatives of NTP-participating agencies. This report is a product of the Expert Panel and is intended to (1) interpret the strength of scientific evidence that ethylene glycol is a reproductive or developmental toxicant based on data from in vitro, animal, or human studies, (2) assess the extent of human exposures to include the general public, occupational groups, and other sub-populations, (3) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/developmental health effects may be associated with such exposures, and (4) identify knowledge gaps to help establish research and testing priorities to reduce uncertainties and increase confidence in future assessments of risk.

The Expert Panel Report on Ethylene Glycol will be a central part of the subsequent NTP CERHR Monograph. The monograph will include the NTP CERHR Brief, the expert panel report, and all public comments on the expert panel report. The NTP CERHR Monograph will be made publicly available and transmitted to appropriate health and regulatory agencies.

The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC and is staffed and administered by scientists and support personnel at NIEHS and at Sciences International, Inc., Alexandria, Virginia.

Reports can be obtained from the website (http://cerhr.niehs.nih.gov**) or from:**

Michael D. Shelby, Ph.D.
NIEHS EC-32
PO Box 12233
Research Triangle Park, NC 27709
919-541-3455
shelby@niehs.nih.gov

A Report of the CERHR Ethylene Glycol and Propylene Glycol Expert Panel:

Name	Affiliation
John A. Thomas, Ph.D., Chair	Consultant; San Antonio, TX
John M. DeSesso, Ph.D.	Mitretek Systems; Falls Church, VA
Bruce A. Fowler, Ph.D.	ATSDR; Atlanta, GA
Gary L. Ginsberg, Ph.D.	Connecticut Department of Public Health; Hartford, CT
Deborah Hansen, Ph.D.	Division of Genetic and Reproductive Toxicology; FDA/NCTR; Jefferson, AR,
Cynthia J. Hines, M.S.	NIOSH; Cincinnati, OH
Ronald Hines, Ph.D.	Medical College of Wisconsin; Milwaukee, WI
Kenneth Portier, Ph.D.	Institute of Food and Agricultural Sciences; Gainesville, FL
Karl K. Rozman, Ph.D.	University of Kansas Medical Center; Kansas City, KS

With the Support of CERHR Staff:

NTP/NIEHS

Michael Shelby, Ph.D.	Director, CERHR
Christopher Portier, Ph.D.	Director, Environmental Toxicology Program

Sciences International, Inc.

John Moore, D.V.M., D.A.B.T.	Principal Scientist
Annette Iannucci, M.S.	Toxicologist
Gloria Jahnke, M.S., D.V.M.	Toxicologist

Note to Reader:

This report is prepared according to the Guidelines for CERHR Panel Members established by NTP/NIEHS. The guidelines are available from the CERHR web site (http://cerhr.niehs.nih.gov/). The format for Expert Panel Reports includes synopses of studies reviewed, and an evaluation of the Strengths/Weaknesses and Utility (Adequacy) of the study for a CERHR evaluation. Statements and conclusions made under Strengths/Weaknesses and Utility evaluations are those of the Expert Panel and are prepared according to the NTP/NIEHS guidelines. In addition, the Panel often makes comments or notes limitations of the study in the synopses. Bold, square brackets are used to enclose such statements. As discussed in the guidelines, square brackets are used to enclose key items of information not provided in a publication, limitations noted in the study, conclusions that differ from authors, and conversions or analyses of data conducted by the Panel.

Abbreviations

ACC	American Chemistry Council
ACGIH	American Conference of Governmental Industrial Hygienists
ADH	alcohol dehydrogenase
ALDH	aldehyde dehydrogenase
ANOVA	analysis of variance
atm	atmospheric pressure
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the concentration versus time curve
BUN	blood urea nitrogen
bw	body weight
C	Celsius
$^{13}C_2$	carbon-13
^{14}C	carbon-14
CAS RN	Chemical Abstract Service Registry Number
CERHR	Center for the Evaluation of Risks to Human Reproduction
Cl_{oral}	clearance after oral dosing
Cl_{total}	total clearance
cm	centimeter
cm^2	centimeter squared
cm^3	centimeter cubed
C_{max}	maximum concentration
CNS	central nervous system
CO_2	carbon dioxide
$^{13}CO_2$	carbon-13 labeled carbon dioxide
$^{14}CO_2$	carbon-14 labeled carbon dioxide
CYP2E1	cytochrome P450 2E1
dL	deciliter
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
EEF	extraembryonic fluids
EG	ethylene glycol
f	female
F_0	parental generation
F_1	first filial generation
F_2	second filial generation
FSH	follicle stimulating hormone
g	gram
GA	glycolic acid
Ga_2O_3	gallium oxide
GC	gas chromatography
gd	gestation day
GLP	Good Laboratory Practices
H_2O_2	hydrogen peroxide
HazDat	Hazardous Substance Release and Health Effects Database
HDT	highest dose tested
Hg	Mercury
HPLC	high pressure liquid chromatography
hr	hour
HSDB	Hazardous Substances Data Bank
IP	intraperitoneal

IPCS	International Programme on Chemical Safety
IV	intravenous
kg	kilogram
km	kilometer
K_m	Michaelis constant
K_{ow}	octanol-water partition coefficient
K_p	permeability constant
L	liter
LC_{50}	lethal concentration, 50% mortality
LD_{50}	lethal dose, 50% mortality
LDH	lactate dehydrogenase
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
LOEL	lowest observed effect level
LQ	limit of quantification
m	male
m^3	meter cubed
MAC	maximum allowable concentration
mg	milligram
min	minute
mL	milliliter
MLD	minimum lethal dose
mm	millimeters
mM	millimolar
MMAD	mass median aerodynamic diameter
mmol	millimole
mol	mole
mRNA	messenger ribonucleic acid
MRT_∞	mean residence time
MS	mass spectrometry
mw	molecular weight
n or no.	number
NA	not analyzed
NAD	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
$NaHCO_3$	sodium bicarbonate
ng	nanogram
ND	not determined
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute of Occupational Safety and Health
nmol	nanomole
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
NOES	National Occupational Exposure Survey
NP	no peak detected
NS	not specified
NTP	National Toxicology Program
OA	oxalic acid
OSHA	Occupational Safety and Health Administration
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic

PCO_2	carbon dioxide partial pressure
PET	polyethylene terephthalate
pnd	postnatal day
ppb	parts per billion
ppm	parts per million
RCF	regenerated cellulose film
SAR	structure activity relationship
SC	subcutaneous
SCE	sister chromatid exchange
SD	standard deviation
$t_{1/2}^{\beta}$	half-life of elimination
TFT	trifluorothymidine
TLV	Threshold Limited Value
T_{max}	time to maximum blood levels
TRI	Toxics Release Inventory
TV	threshold value
TWA	time weighted average
U_{∞}	percent dose excreted
$U_{\infty}^{\text{ethylene glycol}}$	percent dose excreted as ethylene glycol in urine
USEPA	United States Environmental Protection Agency
V_{max}	maximal velocity of metabolism
w/v	weight per volume
µg	microgram
µL	microliter
µM	micromolar
µmol	micromole
µm	micrometer

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1.0 CHEMISTRY, USE, AND HUMAN EXPOSURE

1.1 Chemistry

1.1.1 Nomenclature

The Chemical Abstracts Service Registry Number (CAS RN) for ethylene glycol is 107-21-1. Synonyms or trade names for ethylene glycol include 1,2-Dihydroxyethane; 1,2-Ethanediol; Dowtherm 4000; Dowtherm SR 1; DuPont Zonyl FSE Fluorinated Surfactants; DuPont Zonyl FSO Fluorinated Surfactants; EG; Ethane-1,2-diol; ethylene dihydrate; ethylene alcohol; Ethylene glycol; Ethylene Glycol; Fridex; Glycol; Glycol Alcohol; lutrol-9; macrogol 400 bpc; M.E.G.; monoethylene glycol; norkool; tescol; ucar 17 *(1)*.

1.1.2 Formulas and Molecular Weight

Figure 1-1. Formulas and Molecular Weight (mw) of Ethylene Glycol

Chemical formula=$C_2H_6O_2$

$OH—CH_2—CH_2—OH$

mw= 62.07

1.1.3 Chemical and Physical Properties

Table 1-1 lists the physical and chemical properties of ethylene glycol.

Table 1-1. Physicochemical Properties of Ethylene Glycol

Property	Value
Vapor Pressure	0.092 mm Hg @ 25°C
Melting Point	-13°C
Boiling Point	197.6°C @ 760 mm Hg
Specific Gravity	1.1088 @ 20°C
Solubility in Water	Miscible
Log K_{ow}	-1.36
Stability	Stable[a]
Reactivity	Reactive with acids, bases, and oxidizing materials.[a]
Odor	0.23 mg/m^3[b]

HSDB *(2)*

[a]Hills Brothers *(3)*

[b]Absolute perception threshold (Verschueren *(4)*)

1.1.4 Technical Products and Impurities

Purity of ethylene glycol generally exceeds 99% *(5)*. Possible trace impurities of ethylene glycol include formaldehyde, ethylene oxide, and 1,4-dioxane *(3)*.

Some trade names for ethylene glycol are listed under Section 1.1.1. Past or current manufacturers of ethylene glycol include: BASF Corporation; Dow Chemical USA; Eastman Kodak Company; Formosa Plastics Corporation USA; Hoechst Celanese Corporation; Huntsman Corporation; Shell Oil Company; Sun Company, Inc.; Union Carbide Corporation; Occidental Petroleum Corporation; and PD Glycol *(2)*. Quantum Chemical Corp and Texaco Chemical Company have also been identified as manufacturers of ethylene glycol *(6)*.

1.2 Use and Human Exposure

1.2.1 Production

Ethylene glycol may be manufactured by one of the following methods *(7)*:

1) Oxidation of ethylene to ethylene oxide, followed by hydration;
2) Acetoxylation: Reaction of ethylene with acetic acid in the presence of a catalyst (e.g., tellurium bromide) to form mixed mono- and diacetates that are hydrolyzed to form ethylene glycol and acetic acid;
3) From carbon monoxide and hydrogen derived from coal gasification; or
4) Oxirane Process: Catalytic oxidation of ethylene to the diacetate followed by hydrolysis to ethylene glycol.

In 1999, U.S. production of ethylene glycol was 6,320 million pounds, while actual consumption was 5,497 million pounds *(8)*.

1.2.2 Use

Of the 5,497 million pounds of ethylene glycol consumed in the U.S., 1,703 million pounds (31%) was used in polyethylene terephthalate (PET) resins, 1,670 million pounds (30.4%) in polyester fibers and films, 1,610 (29.3%) in antifreeze, and the remaining 514 million pounds (9.4%) was used in other applications (in million pounds: deicing fluid, 130; surface coatings, 83; heat transfer fluids and industrial coolants, 55; unsaturated polyester resins, 51; hydraulic fluids, 19; surfactants and emulsifiers, 19; and miscellaneous, 157) *(8)*.

Automotive antifreezes generally contain 50% ethylene glycol *(9)*. Ethylene glycol levels of 45–50% were reported for deicing solutions *(10, 11)*. Ethylene glycol levels were reported at 2.3–2.6% in 4 U.S. latex paints; 11 Canadian paint and coating companies reported that their paints may contain up to 5% ethylene glycol *(9)*. ATSDR *(6)* reported that brake fluids currently have less than 0.1% ethylene glycol. Other uses of ethylene glycol are less clear. Based on information published in 1986 or 1989, IPCS *(9)* reported that tub and tile cleaners, windshield washer fluids, automotive wax and polish, household floor wax, and cement sealers may contain ethylene glycol. However, the American Chemistry Council (ACC) *(12)* noted that the most recent information available did not report the presence of ethylene glycol in any of those products *(13)*. The preface to that report states that the current volume contains different formulations than previous volumes but notes that cleaning product formulations have changed significantly in the past few years *(13)*. One cosmetic registered in Canada contains ethylene glycol *(9)*, but it is not known if ethylene glycol is present in any cosmetics sold in the U.S. Eyedrops sterilized with ethylene oxide may contain ethylene glycol and/or ethylene chlorohydrin residues, and in 1979 ethylene glycol was detected in 4 of 15 eyedrop samples at 10–28 mg/L. Ethylene glycol is not

approved as an active ingredient in eyedrops in the U.S. *(14)*, but it is not known if ethylene oxide is currently used as a sterilant *(15)*.

1.2.3 Occurrence

In 2000, the Toxics Release Inventory (TRI) reported an estimated 7.1 million pounds of ethylene glycol released to the atmosphere from U.S. manufacturing and processing facilities *(16)*. Airport deicing operations also release ethylene glycol to the environment. In 1994, it was estimated that 58 million pounds of ethylene glycol per year were released at the 17 busiest airports in the U.S. *(17)*. In a more recent preliminary summary report *(18)*, it was estimated that approximately 28 million gallons of aircraft deicing fluid/year (12.6 million gallons of pure glycols/year) were released to surface waters by airports prior to the implementation of Phase I Storm Water Discharge Permit regulations around 1990. As a result of these regulations, current discharges of aircraft deicing fluid are estimated at 21 million gallons/year with an additional 2 million gallons/year discharged to publicly owned treatment works. Because the regulations are generally enforced by state governments, there are large disparities in permit requirements for airports. The EPA found 70% collection efficiency at airports that were most effective at wastewater collection, containment, and recycling or treatment. Overall trends noted by the EPA include increased use of aircraft or pavement deicers solutions containing propylene glycol or potassium acetate in place of ethylene glycol, increased use of anti-icing fluids to reduce needed quantities of deicing fluid, increased efforts to use fluids that are less toxic to aquatic life, increased use of source reduction methods (e.g., forced air and infrared deicing), increased recycling/recovery of spent deicing fluids, and increased collection, containment, and treatment of fluids.

Health Canada *(19)* cited studies reporting that, following the life cycle of antifreeze from manufacture to disposal, approximately 0.87 g of ethylene glycol is released into the environment for every liter of antifreeze solution used in automobiles, and that approximately 39% of all consumed antifreeze is lost to storm sewers. Although the information is from a single study published in 1994, the Panel believes it is reasonable to assume that some ethylene glycol used in cars is lost to the environment. In 1995, the Hazardous Substance Release and Health Effects Database (HazDat), maintained by the Agency for Toxic Substances and Disease Registry (ATSDR), reported that at least 34 National Priority List sites in the U.S. contain measurable amounts of ethylene glycol in some environmental media *(6)*.

Ethylene glycol has a low vapor pressure (0.092 mmHg at 25°C) and Henry's law constant (6.0×10^{-8} atm-m^3/mole at 25°C) *(2)*, and is therefore expected to partition to the air only slightly from soil and not at all from water. Vapor phase ethylene glycol is oxidized rapidly by photochemically produced hydroxyl radicals and has an estimated half-life of about 50 hours *(2)*. The California Air Resources Board stated that no data are available for ambient levels of ethylene glycol in outdoor or non-workplace indoor air *(19)*. Health Canada also identified no sources of data on non-workplace indoor air levels of ethylene glycol, but did present some levels in outdoor air that were associated with point source emissions *(19)* (see Table 1-2). Thus, general population exposure through inhalation is not expected to be great.

Because it is miscible in water and highly mobile in soils, ethylene glycol spilled on the ground will leach through soil into ground water or surface water, thereby producing an exposure pathway of concern *(6)*. Data on levels measured in drinking water, however, are not available, and reported levels in surface and ground water are limited mostly to areas of known contamination, particularly airports. The limited data available indicate that surface water levels of ethylene glycol are generally low (a few micrograms per liter), while wastewater from glycol production plants have averaged up to 1,300 mg/L, and runoff water samples from airports have

shown the highest levels *(20)*. Ethylene glycol concentrations were measured at 19,000 mg/L in stormwater runoff at Salt Lake City International Airport and 0–100,000 mg/L at Denver's Stapleton Airport *(17)*. Table 1-2 provides examples of measured levels of ethylene glycol in surface and ground waters associated with deicing operations at Canadian airports *(19)*. These levels are not representative of background levels likely to be found in areas unassociated with a known ethylene glycol source.

Migration rates in various soils of 4–27 cm per 12-hour period for ethylene glycol have been reported *(20)*. In soil and water, biodegradation is the primary means of ethylene glycol removal, with aerobic conditions effecting complete biodegradation within several days, and anaerobic conditions requiring slightly more time *(2)*. ATSDR *(6)* reported estimated half-lives of ethylene glycol in various environmental media as follows: 2–12 days in water under aerobic conditions, 8–48 days in water under anaerobic conditions, 0.3–3.5 days in the atmosphere, and 0.2–0.9 days in soil. The low octanol/water partition coefficient and measured bioconcentration factors in a few organisms indicate low capacity for bioaccumulation *(20)*. Freitag et al. *(21)* reported a bioconcentration factor of 10 in golden ide fish using ^{14}C-labelled ethylene glycol. **[The Expert Panel reviewed the Freitag et al. *(21)* data and concluded that a bioconcentration factor of 10 was not plausible, i.e. too high.]** Therefore, exposure to ethylene glycol through ingestion of fish or other animal products is not expected to be significant.

Ethylene glycol can be found in food due to its approved uses as an indirect food additive. Polyethylene glycol, an ingredient of regenerated cellulose films (RCF) used as food wraps, can contain ethylene glycol at ≤0.2% by weight [2,000 ppm] if its mean molecular weight is ≥350 or at ≤0.5% if its mean molecular weight is <350 *(22)*. However, it appears that RCF food wraps containing ethylene glycol are not manufactured in the U.S. Ethylene glycol is also approved as an ingredient starting material in the manufacture of PET, the material used to produce soft drink bottles *(23)*. Results of migration studies conducted with experimental RCFs and a PET bottle are described in Section 1.2.4.1.

Table 1-2. Levels of Ethylene Glycol in Selected Environmental Samples[a].

Source[b]	Vicinity of measurement to source	Levels measured or modeled	Reference
Outdoor Air:			
Airport (Ontario)	Unknown	3,200 and 4,100 μg/m^3	Health Canada *(19)*
Bridge deicing operation (Louisiana)	Unknown	<50 to 10,570 μg/m^3 (total airborne); <50 to 330 μg/m^3 (aerosol)	Health Canada *(19)*; Abdelghani et al. *(10)*
Ethylene glycol manufacturing plant (Alberta)-Predicted maximum 24-hour average ground-level concentration at specified distance	1.8 km 4.0 km 6.8 km Surrounding prairie	100 μg/m^3 (modeled) 50 μg/m^3 (modeled) 25 μg/m^3 (modeled) 0.0012 μg/m^3 (modeled)	Health Canada *(19)*
Ethylene glycol manufacturing plant (Alberta) from 1995-1999.			
Estimated maximum 24-hour average ambient air concentration.	Nearby residence Company outer property boundary	154 μg/m^3 (modeled) 240 μg/m^3 (modeled)	Sciences International *(24)*
Estimated maximum annual average ambient air concentration.	Nearby residence Company outer property boundary	9.49 μg/m^3 (modeled) 13.4 μg/m^3 (modeled)	
Surface Water:			
Airport (Winnipeg)	Tributary, < 2 km downstream	1996: 2 to 660 mg/L 1997: <10 mg/L 1998: undetected (limit not specified) to 83 mg/L	Health Canada *(19)*
Airport (Toronto)	Tributary, <1 km downstream	<25 mg/L (detection limit)	Health Canada *(19)*
Airport (Newfoundland)	Tributary	1997/1998: 5 mg/L (detection limit) to 80 mg/L (median=5 mg/L) 1998/1999: 5 mg/L (detection limit) to 170 mg/L (median=12 mg/L)	Health Canada *(19)*
Ground Water:			
Airport (Calgary)	Unknown	4 mg/L to 38 mg/L	Health Canada *(19)*
Airport (Montreal)	Unknown	8 mg/ L to 42 mg/L	Health Canada *(19)*

[a]This table demonstrates that ethylene glycol has been detected or is likely to be detected in environmental samples. The values represent those reported in the literature for particular points in time. No assumptions should be made that the values represent ethylene glycol levels during other time periods.
[b]No data were available for indoor air and drinking water.

1.2.4 Human Exposure

1.2.4.1 General Population Exposure

The general population can be exposed to ethylene glycol through dermal contact with consumer products such as antifreeze, coolant, or brake fluids. Accidental or intentional ingestion of ethylene glycol-containing products has been reported, and levels resulting in toxicity are discussed in Chapter 2. In the year 2000, more than 5,000 cases of ethylene glycol poisonings were reported to U.S. poison control centers *(25*). Because ethylene glycol is readily soluble in water, drinking, bathing in, or showering with contaminated water are potential exposure routes. However, there are no known reports on levels of ethylene glycol in drinking water *(9*). The EPA has established 70 mg/L as the drinking water equivalent level (DWEL) for ethylene glycol. A DWEL is the maximum exposure over a lifetime that is considered to be protective of health *(26*). It further assumes that the only route of exposure is through water.

Consumer exposure to ethylene glycol through food ingestion is possible if the food is packaged in PET bottles, which may contain trace amounts of unreacted ethylene glycol, or in RCFs, which may contain polyethylene glycol as a softening agent. Ethylene glycol was found to migrate in small amounts from 32-ounce PET bottles to a 3% acetic acid solution (a simulant for foods of pH 5.0 and below) after a 6-month storage period at 32°C, resulting in a concentration of about 100 ppb, or 94 μg ethylene glycol per bottle *(27*). In a U.K. laboratory study to determine migration of ethylene glycol from experimental RCFs coated with polyethylene glycol, ethylene glycol was detected at levels of <10–34 ppm in various cakes, pies, and sweets that had been wrapped in those RCFs for various lengths of time *(28*). There are no known studies that examine ethylene glycol levels in food packaged in RCF wraps currently approved for use in the U.S. It appears that such wraps are not currently manufactured in the U.S.

Food surveys *(9*) have found ethylene glycol in Italian wines at levels up to 6.25 mg/L (origin of contamination unknown) and in French breads preserved with ethylene oxide in airtight bags at levels up to 92.2 mg/kg. The documents reporting this information were published in 1987 and 1970, respectively, and more recent data in these types of food are not available. A French study published in 1993 did not report ethylene glycol levels, but authors noted that residual ethylene glycol was rapidly lost *(9*). It is not known if ethylene oxide is used to sterilize foods sold in the U.S. IPCS *(9*) assumes that "…the vast majority of foods consumed in Canada contain no ethylene glycol." Ethylene glycol has been reported to be produced naturally in small, and presumably negligible, amounts in certain plants and edible fungi *(19*).

Health Canada *(19*) [also IPCS *(9*)] estimated human exposures to ethylene glycol occurring through dermal contact with consumer products, dietary intake, and inhalation of air and ingestion of soil near point sources. Available exposure data were very limited *(9*). CERHR agrees that the exposure data are limited. For example, food estimates were based on levels of ethylene glycol measured in foods packaged in an experimental RCF manufactured in the U.K. and not a market-basket survey. Due to the limitations in data, Health Canada used conservative assumptions in their estimates, as is typical for a regulatory agency. Health Canada estimated human exposure to ethylene glycol as a worst-case-scenario for persons living next to an industrial point source in the range of 22–88 μg/kg bw/day *(19*). **[The Expert Panel acknowledges the limitations in these estimates as stated in ICPS *(9*).]**

In a small study of 16 persons with no known occupational exposure to ethylene glycol, ethylene glycol levels in urine ranged from 0.07 to 2.93 mg/L (median=0.23 mg/L) (0.12–2.64 mg/g creatinine, median 0.31 mg/g creatinine) *(29)*.

1.2.4.2 Occupational Exposure

Occupational exposure to ethylene glycol may occur through dermal contact while handling products containing this compound or through inhalation of airborne ethylene glycol that results from heating or spraying processes *(6)*. Ethylene glycol releases can occur during the manufacturing of PET and other synthetic organic chemicals *(2)*. Ethylene glycol is also present in industrial adhesives, as well as paint, primer, and varnish formulations *(2)*; thus, workers who manufacture or use such formulations may be potentially exposed either dermally or through inhalation of the volatilized compound.

A 1981−1983 National Occupational Exposure Survey (NOES) of U.S. workers led NIOSH to estimate that 1,133,792 people (352,752 of whom were female) were potentially exposed to ethylene glycol at the workplace *(2)*. Deicing operations on airport runways are another important exposure scenario for workers. Health Canada *(19)* cited studies reporting that 50% of the glycols used in aircraft deicing falls to the ground in the vicinity of the airplane, while 16% remains on the airplane and 35% is blown off by wind.

Gerin et al. *(11)* conducted a study to measure ethylene glycol exposure and kidney function in 33 male Canadian aviation workers exposed to ethylene glycol deicing fluid. The discussion of kidney function is included in Section 2.2.1.2. The deicing fluid (Union Carbide's UCAR D) contained about 45% ethylene glycol, 5% diethylene glycol, and 50% water and other additives. Before spraying, the fluid was diluted with 70% water and heated to 70−80 °C. The study was conducted in Quebec from January to March of 1992. Table 1-3 outlines concentrations of ethylene glycol measured in the breathing zones and urine of workers. A total of 154 ethylene glycol vapor and mist samples were taken. Because the values were not weighted for exposure time, exposure durations for detected concentrations are listed in Table 1-3. Eighteen vapor samples exceeded the quantification limit (2.5 mg/m^3) of the analytical method, but only 3 samples had quantifiable levels of ethylene glycol mists (≥17 mg/m^3). Urine samples were obtained prior to, immediately after, and the morning after the shift. Thirty-three to forty-two urine samples were collected for each period. A threshold value of 5 mmol/mol creatinine was selected because levels below that limit were thought to be unrelated to occupational exposure. A total of 16 urine samples had ethylene glycol concentrations (normalized for creatinine) that exceeded the threshold value. Diethylene glycol was found in some air and urine samples at about one-tenth the level of ethylene glycol. The authors concluded that the highest exposures to ethylene glycol occurred in basket operators and coordinators who were most likely to have contact with the greatest concentrations or to be accidentally sprayed. Most basket operators wore masks that offered some protection against mists but not vapors. Because ethylene glycol was not detected in air samples of some workers with the highest urine values, the authors suggested that the workers could also be exposed through oral intake and dermal contact. **[The Panel noted that appropriate air sampling methods were used in this study. Personal breathing samples were obtained to measure both vapors and mists. The ability to examine the statistical correlation between air and urinary ethylene glycol levels was limited by the small number of air and urine samples with values exceeding their respective limit of quantification and threshold values. The limited amount of data do not allow firm conclusions to be made regarding the most significant exposure route(s). The study does demonstrate that deicing operations can result in ethylene glycol mist exposures higher than the ACGIH limit of 100 mg/m^3 in**

workers, with exposures possibly occurring through multiple routes. However, exposures exceeding the Threshold Limit Value (TLV) were rare (1 out of 154 worker measurements) and limited to a single basket operator.]

Table 1-3. Ethylene Glycol in Air Samples and Urine of Aviation Workers *(11)*.

Job (Number of air samples)	Vapor Level; n=number samples≥LQ (Duration of exposure)	Mist Level, n=number samples≥LQ (Duration of exposure)[a]	Urine Concentrations (mmol/mol creatinine), n=number samples≥TV[a,b]		
			Pre-Shift	Post-Shift	Next Morning
Lead (n=25)	5 mg/m^3; n=1 (25 minutes) [a]	<17 mg/m^3; n=0	<5; n=0	<5; n=0	<5; n=0
Truck Driver (n=27)	2.5, 3.4 mg/m^3; n=2 (43, 62 minutes) [a]	<17 mg/m^3; n=0	<5; n=0	6.8; n=1	6.4; n=1
Coordinator (n=18)	7.3 mg/m^3; n=1 (15 minutes) [a]	91 mg/m^3; n=1 (118 minutes)	<5; n=0	129; n=1	11.6, 13.6, 14.8; n=3
Basket Operator (n=84)	0.9–22.0 mg/m^3; n=14 (117–118 minutes)	76, 190 mg/m^3; n=2 (45, 75 minutes)	<5; n=0	5.2, 5.6, 6.2, 7.6, 10.4, 10.6, 11.8, 15.9; n=8	7.7, 14.5; n=2

LQ=Limit of Quantification=2.5 mg/m^3 for vapor and 17 mg/m^3 for mist
TV=threshold value for urine= 5 mmol/mol creatinine
[a]Values for individual workers.
[b]Total number of urine samples taken/time period were 16–22 for basket operators, 5–6 for leads, 7–9 for truck drivers, and 5 for coordinators.

Many U.S. airlines now use trucks with enclosed baskets that protect workers from exposures during deicing spraying operations *(18)*.

Laitinen et al. *(30)* examined exposure to ethylene and propylene glycol in Finnish motor servicing workers using the method of Tucker and Deye *(31)*. Ten male mechanics from five different garages participated in the study. The only protective equipment used by some workers was leather gloves. Ten age-matched male office workers served as controls. Differences between groups were evaluated by Student's t-test. Air concentrations of ethylene glycol and propylene glycol were measured during the entire shift. Neither ethylene glycol nor propylene glycol vapors were detected in the breathing zones of workers; detection limits for each compound were 1.9 cm^3/m^3 and 3.2 cm^3/m^3, respectively **[cm^3/m^3 equivalent to ppm]**. Urine samples were collected after the work shift and analyzed for ethylene glycol, oxalic acid, and propylene glycol. Possible biochemical indicators of toxicity were also analyzed in urine and are discussed in Chapter 2. Urinary concentrations of ethylene glycol were significantly higher in mechanics versus controls (7.3±4.7 vs 1.7±0.7 mmol/mol creatinine, respectively). Levels of oxalic acid were also greater in mechanics, but statistical significance was not achieved (47±11 vs 36±14 mmol/mol creatinine). Propylene glycol concentrations were not increased in urine from mechanics. The study authors noted that ethylene glycol excretion was higher in workers who conducted major engine repairs and were exposed to ethylene glycol for longer time periods. Because ethylene glycol was not detected in air, but was detected in the urine of workers, the study authors concluded that

exposure occurred through dermal contact. **[The Expert Panel noted that only exposures to vapors, and not mists, were measured. Because only vapors were measured, it is not known if mists were present due to aerosol generation while handling the fluid or due to temperature-related condensation. Ethylene glycol exposure would be underestimated if mists were present. However, mist exposure during automotive maintenance seems less likely than during aircraft deicing operations. The Panel also notes that urine sample storage conditions were not specified and limited information was provided regarding the quantification method for ethylene glycol in urine. The study suggested a difference in exposure between exposed workers and controls. However, due to the small sample size and lack of methodology and characteristics of the data set (e.g., how many values were below the limit of detection; actual range of values) this study should be considered preliminary.]**

Abdelghani et al. *(10)* measured short-term (15-minute) exposures to ethylene glycol vapors and mists in 8 workers who were deicing bridges with a 50% ethylene glycol solution sprayed from a truck. Sampling was conducted on two separate dates to obtain a total of 16 samples. During sampling, the window on the driver side of the truck was closed while the passenger side window was open. During normal operations, both windows are usually closed. Fifteen-minute ceiling values were measured at <0.05–2.33 mg/m^3 for aerosol and <0.05–3.37 mg/m^3 for vapor.

A ceiling limit of 50 ppm (125 mg/m^3), established for ethylene glycol by the Occupational Safety and Health Administration (OSHA), was vacated in 1989, although it is still enforced in some states *(2)*. The National Institute for Occupational Safety and Health (NIOSH) has questioned whether this level will protect workers from recognized health hazards *(2)*. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a ceiling exposure limit of 100 mg/m^3 for ethylene glycol aerosol, to minimize potential respiratory and ocular irritation *(32)*. Irritative properties of ethylene glycol may preclude prolonged voluntary high exposures, as noted in the study by Wills et al. *(33)*, where an ethylene glycol air concentration of 188 mg/m^3 (mist) could be tolerated for 15 minutes; however an air concentration of 244 mg/m^3 could only be tolerated for, at most, 2 minutes.

1.3 Utility of Data

Limited exposure data for ethylene glycol were available for review by the Panel. The utility of occupational exposure data is limited by either small sample size or a high proportion of non-detected values. Estimates of ethylene glycol-exposed workers are based on a 1981–1983 NOES that is approximately 20 years old and may not accurately reflect the number of workers currently exposed. The applicability of reported ethylene glycol levels in food for assessing consumer exposure is also unclear due to testing of an experimental food wrap and the limited number of foods tested.

1.4 Summary of Human Exposure Data

Ethylene glycol is used as an engine coolant, in the manufacture of polyester and PET resins, and is found in deicing solutions, industrial coolants, hydraulic fluids, and surface coatings *(9, 19, 20)*. In 1999, U.S. production of ethylene glycol was 6,320 million pounds; U.S. consumption of ethylene glycol was 5,497 million pounds *(8)*. Older publications have reported additional uses of ethylene glycol in consumer products but this information could not be verified. Ethylene glycol is approved as an indirect food additive. It may be used to manufacture polyethylene glycol, an ingredient of the RCF used in food wraps *(22)*. Ethylene glycol is also an approved material for the manufacture of PET, the material used to produce soft drink bottles *(23)*. A single time-trend

study *(27*) reported 100 ppb ethylene glycol per bottle in 32-ounce PET bottles (n=2) containing 3% acetic acid solution at 6 months at 32°C.

Significant amounts of ethylene glycol are released to the atmosphere. In 2000, 7.1 million pounds of ethylene glycol were released into the environment by U.S. manufacturing and processing facilities *(16*). It is estimated that airplane deicing operations result in the release of millions of gallons of ethylene glycol per year from airports in the U.S. *(17, 18*). Implementation of Phase I Storm Water Discharge Permit requirements are reducing ethylene glycol discharges from some airports, with 70% collection efficiency at airports with the most effective wastewater collection, containment, and recycling/treatment programs *(18*). In addition to those practices, overall trends at airports include substitution of ethylene glycol with other chemicals, increased use of anti-icing fluid to reduce needed quantities of deicers, and use of source reduction methods such as forced air and infrared. For every liter of automobile antifreeze solution used, it is estimated that 0.87 grams of ethylene glycol is released to the environment *(19*).

The general public can be exposed to ethylene glycol from dermal contact with products such as antifreeze solutions, ingestion of food or beverages containing trace amounts of ethylene glycol leaching from packaging materials, and inhalation of air and ingestion of soil near point source emissions. Very little ethylene glycol is expected to be present in outdoor air, with the possible exception of point source emissions. Therefore, significant exposure through outdoor air is not expected for the majority of the general population. No information is available on ethylene glycol levels in drinking or bathing water. Health Canada estimated a worst-case-scenario human ethylene glycol exposure in the range of 22–88 μg/kg bw/day for persons living next to an industrial point source *(19*). Because the human exposure data are limited, Health Canada used very conservative assumptions in their estimates. These values likely overestimated actual human exposure levels. [The Expert Panel acknowledges the limitations in these estimates as stated by Health Canada]**.**

Occupational exposure to ethylene glycol can occur during its use as a chemical intermediate and as an ingredient of automotive antifreeze, deicing solutions, and paints. Exposure of workers is most likely to occur from dermal contact with ethylene glycol-containing solutions and inhalation of airborne vapors and mists generated through heating and spraying processes. However, exposures in workers are not well characterized. In a study of bridge deicing workers, Abdelghani et al. *(10*) obtained 16 short-term (15-minute) ethylene glycol exposure measurements of <0.05–2.33 mg/m^3 aerosol and <0.05–3.37 mg/m^3 vapor. A study of airport personnel measured personal air exposures ranging from <17 to 190 mg/m^3 mists and <2.5–22 mg/m^3 vapors *(11*); urinary concentrations of ethylene glycol were increased in some workers as compared to levels in a non-occupational comparison group, but correlations between personal breathing samples and urinary levels of ethylene glycol were not possible due to limited data. A study of automotive mechanics found increased urinary ethylene glycol levels compared to unexposed workers *(30*). Though ethylene glycol vapor levels were below the detection limit in personal air samples, the Panel noted that mist levels were not measured. Limitations in the occupational exposure studies *(11, 30*) do not allow a determination to be made as to whether the dermal or inhalation route predominates in occupational settings. ACGIH *(32*) recommends a workplace ceiling exposure limit of 100 mg/m^3 for ethylene glycol aerosols to minimize potential respiratory and ocular irritation.

2.0 GENERAL TOXICOLOGY AND BIOLOGICAL EFFECTS

2.1 Toxicokinetics and Metabolism

The first step in the evaluation of toxicokinetics and metabolism data for ethylene glycol was an examination of authoritative reviews (i.e., reviews by agencies such as ATSDR *(6)* and NTP *(34)*) to determine what is known as a function of route, dose, and species. Those reviews were summarized, compared, and contrasted to communicate facts about toxicokinetics and metabolism. Reviews written by independent authors *(35, 36)* were also examined and it was noted if conclusions were consistent or in contrast to those reached by authoritative sources. There were cases where it was found necessary or beneficial to summarize thoroughly and evaluate original studies. Such cases included important studies in interpreting developmental or reproductive toxicity issues, significant studies not included in reviews, and studies that clarified previous uncertainties. A limited number of studies in non-rodent species were also reviewed in detail to allow for comparison of interspecies variability. In reviewing key original studies, the Panel prepared statements on the strengths, weaknesses, and utility of the studies.

As described in the developmental toxicity section of this document (See Section 3) mice are more sensitive than rats to ethylene glycol-induced developmental toxicity. Compared to rodents, rabbits are more susceptible to maternal toxicity but less sensitive to adverse developmental outcomes. Mice are less sensitive to ethylene glycol-induced developmental toxicity when exposed dermally versus orally. These variations in toxicity among different species and through different exposure routes may be due to variations in toxicokinetics. Therefore, the focus in this section will be on studies that may provide insight on these issues.

2.1.1 Absorption

2.1.1.1 Human

2.1.1.1.1 Oral

Ethylene glycol is readily absorbed in humans following oral intake as evident by high levels in serum and rapid onset of clinical symptoms *(6, 35)*. A limited number of studies reported ethylene glycol blood levels in humans, mostly after acute poisonings. These levels were reported to range from 14.5 to 650 mg/dL **[2.3–105 mM]** *(6)*. Since most of these values were measured a number of hours following exposure, they may represent less-than-peak concentrations.

2.1.1.1.2 Inhalation

A study where two individuals inhaled approximately 0.96 and 1.51 mg/kg bw ethylene glycol over 4 hours demonstrated an increase in urinary output of ethylene glycol over background levels *(37)*. **[The study suggests that ethylene glycol was absorbed into the lungs as would be expected due to its high water solubility and relatively low volatility.]** See section 2.1.3.1 for more details about this study.

Indirect evidence of absorption through inhalation exposure was previously assessed through a controlled study *(33)* and two occupational exposure studies *(11, 30)*. In a study conducted by Wills et al. *(33)*, men exposed to 17–49 mg/m^3 ethylene glycol aerosols for 30 days experienced no increase in serum or urine levels of ethylene glycol compared to controls. Complete details of this study are included in Section 2.2.1.2. The study authors stated that the study suggests poor

absorption of ethylene glycol through the respiratory tract of humans. **[The Expert Panel questioned the conclusion of poor absorption through the respiratory tract. They noted that the lowest amount of ethylene glycol detectable in urine via the study authors' analytical method was reported to be 7 mg/100 mL (detectable peak) or 10 mg/100 mL (bottom of standard curve). However, 7.7 mg/100 mL was reported as the maximum urine concentration in the exposed group. It would appear that the method detection limit was in the concentration range that these individuals had in urine and thus was not sensitive enough to reliably detect differences between exposed and control workers. Further, at the relatively low doses of ethylene glycol administered, it is possible that the majority of the dose was excreted in exhaled breath as CO_2 with some as metabolites in urine. Thus, the lack of detection of elevated ethylene glycol in urine cannot be taken as evidence of low percent absorption. The lack of ethylene glycol elevation in serum relative to unexposed controls might also be due to analytical difficulties. While the method detection limit was not reported, the authors state that a step for the removal of carbohydrates from serum had not been incorporated into the method; they state that this led to higher and more variable ethylene glycol results than would otherwise be obtained. This may have obscured any exposure/control differences in ethylene glycol serum levels.]**

In a Finnish study *(30)*, ethylene glycol levels in urine were higher in mechanics compared to unexposed controls even though levels of ethylene glycol vapors in the breathing zones of mechanics were below the detection limit. This finding led the study authors to conclude that exposure occurred through dermal contact. **[As discussed in Section 1.2.4.2, the Expert Panel noted limitations of the study that preclude making definitive conclusions about specific routes of exposure.]**

A study of Canadian aviation workers found that some workers who were not exposed to detectable levels of ethylene glycol in air had the highest levels of ethylene glycol in urine; study authors speculated that exposure could have occurred through oral intake and dermal contact *(11)*. **[As discussed in Section 1.2.4.2, the data in this study are too limited to draw conclusions regarding the importance of any single dose route in this industry.]**

2.1.1.1.3 Dermal

ATSDR *(6)* describes two *in vitro* skin absorption studies conducted with cadaver skin by Loden *(38)* and Driver et al. *(39)*. Average skin absorption rates were found to be quite variable between the two studies and ranged between 0.09 and 118 $\mu g/cm^2$/hour.

An additional study by Sun et al. *(40)* was identified and found to be the most comprehensive study, since *in vitro* dermal absorption rates of undiluted and 50% ^{14}C-ethylene glycol (97% purity) were compared in human and mouse skin. Fresh, full-thickness skin samples were obtained from the abdomens of 5–6 female volunteers (age 20–60 years old) and the dorsal trunks of 3 female Crl: CD-1 mice (8 weeks old). The entire skin surfaces were covered with an "infinite dose" of ethylene glycol (22–28 mg/cm^2) and incubated for 6 hours in covered cells containing minimum essential medium as the receptor fluid. At the end of the incubation period, radioactivity in receptor fluid, skin, and skin wash was measured by liquid scintillation spectrometry. ^{14}C-ethanol was used as a reference chemical to assess integrity of skin samples and it was verified that skin samples were normal since permeability rates of ^{14}C-ethanol were within historical ranges. Results of the study are listed in Table 2-1. As noted in Table 2-1, the lag times to steady state were three times longer in human compared to mouse skin. Steady state penetration rates and permeability constants (K_p) were 30–40 times lower in human compared to mouse skin for both undiluted and 50% ethylene glycol. Within both species, the permeability

constant for undiluted versus 50% ethylene glycol were approximately the same, while steady state penetration rates were twice as high for undiluted versus 50% ethylene glycol. In a comparison of results to those of other laboratories, the authors noted their penetration rate was much lower than that obtained by Loden *(38*), who used frozen skin. The authors speculated that deterioration may occur during storage and thawing of skin samples, thus reducing barrier properties. Authors also noted that their penetration rates were much higher than those obtained by Driver et al. *(39*) and they speculated that the low dose (8 μg/cm^2) used by Driver et al. does not represent an "infinite dose." In closing, the authors concluded that human skin is significantly less permeable to ethylene glycol than mouse skin.

Strengths/Weaknesses: Details of the Sun et al. *(40*) study are generally well reported. However, there is a key inconsistency in the data that may question the weight placed on the results. The mouse and human skin preparations were evaluated for integrity by first testing them against radiolabeled ethanol, an agent with known *in vitro* penetrant rates in both species. These results found K_p values to be essentially the same across the mouse and human skin specimens, with the ethanol K_p results being 2–3 times faster than the ethylene glycol results in mouse skin. This makes sense, as adding an extra polar group onto ethanol might be expected to retard ethylene glycol passage across the various dermal layers. However, the human skin specimens (n=5–6) had K_p values that were 30- to 40-fold lower than the mouse values which suggests that the permeability of ethylene glycol in human skin is not only far lower than its permeability across mouse skin, but also far lower than the permeability of ethanol across the same human skin specimens. While this could certainly have occurred, the fact that such a large ethanol:ethylene glycol K_p differential was not seen in mouse skin raises questions about why human skin should show such a large discrepancy between these related chemicals. The study authors focused on the mouse:human difference in ethylene glycol K_p and did not discuss or even acknowledge this curious ethanol:ethylene glycol K_p difference in human, but not mouse, skin.

Utility (Adequacy) for CERHR Evaluation Process: The utility of the Sun et al. *(40*) study is questionable without further investigation. Given that there is a wide variability in ethylene glycol human skin permeability results across the three available dermal absorption studies *(38-40*), it seems prudent that risk assessment on dermal exposure to ethylene glycol include dermal absorption equations and factors presented in USEPA guidance documents. These equations enable a modeling-based approach for deriving K_p across human skin, which is informed by structure activity relationships (SAR) for dermal penetrability. This approach could be used to help decide which of the reported ethylene glycol K_p values appears to be most consistent with dermal penetration principles and with results for other chemicals. It is clear from the Frantz et al. *(41-43*) series of publications in rats that dermal exposure will yield plasma concentrations of ethylene glycol and metabolites far lower than an equivalent oral (bolus) dose. Therefore, an assumption of much slower dermal penetration in humans than rodents is unnecessary to reach the conclusion that human dermal exposure is unlikely to result in acute poisoning, unless there is an extreme exposure scenario or the skin barrier function has been seriously compromised.

Table 2-1. Results of Skin Absorption Study by Sun et al. *(40)*.

Parameter	Mouse		Human	
	Undiluted Ethylene Glycol	50% Ethylene Glycol	Undiluted Ethylene Glycol	50% Ethylene Glycol
Lag Time to Steady State (hours)	1.02	0.90	3.07	3.10
Steady State Penetration Rate (mg/cm^2/hour)	0.52	0.22	0.013	0.007
Permeability Constant (cm/hour x 10^{-4})	4.68	4.36	0.12	0.14
% Cumulative Absorbed Dose	10.82	4.41	0.14	0.08
% Total Dose Recovery	91.91	75.00	76.48	88.72

2.1.1.2 Animals

2.1.1.2.1 Oral

Studies in rats, mice, dogs, rabbits, and monkeys consistently demonstrated that absorption of ethylene glycol administered by gavage is fast and nearly complete. Gavage administration of a high ethylene glycol dose (~1,000 mg/kg bw) resulted in maximum blood levels of ethylene glycol at 0.2–0.6 hours in mice *(42, 43)*, ~1 hour in rats *(41, 43, 44)* and rabbits *(45)*, 1–2 hours in monkeys *(46)*, and 2 hours in dogs *(47)*. Pottenger et al. *(44)* demonstrated that the absorption rate of ethylene glycol does not differ between non-pregnant and pregnant rats on gestation day (gd) 10. Carney *(35)* noted that blood levels of ethylene glycol increase linearly according to the oral dose administered and are very similar between different species. Table 2-2 outlines blood levels of ethylene glycol in various studies.

Table 2-2. Maximum Levels of Ethylene Glycol in Blood Following Gavage Exposure to Ethylene Glycol.

Sex and Species	Dose (mg/kg bw)[a]	Blood Ethylene Glycol Level (mM)	Reference
Male and Female Sprague-Dawley rats	10 1,000	0.2 21	Frantz et al. *(41, 43)*
Female Sprague-Dawley rats	10 2,500	0.15 45.0	Pottenger et al. *(44)*
Pregnant Female Sprague-Dawley rats	10 150 500 1,000 2,500	0.13 1.4 6.31 14.3 56.8	Pottenger et al. *(44)*
Male Sprague-Dawley rats	2,000	31	Hewlett et al. *(47)*
Female CD-1 mice	10 100 200 400 1,000	0.1 1.6 4.7 7.3 16.4	Frantz et al. *(43)*
Male and female Rhesus monkeys	1,109	20.1	McChesney et al. *(46)*
Male and female mixed-breed dogs	1,000–1,036	29	Hewlett et al. *(47)*

[a]All doses except those reported by Hewlett et al. *(47)* were converted from original units by CERHR. Example calculation for pregnant rats exposed to 2,500 mg/kg bw ethylene glycol in Pottenger et al. *(44)* study: 3,528 µg ethylene glycol/g blood* ~1 g blood/1 mL blood * 1,000 mL/L *1 mg/1,000 µg * 1 mmol ethylene glycol/62.07 mg = 56.8 mM.

A dermal study in rats and mice by Frantz et al. *(41-43)* and an inhalation study in rats by Marshall and Cheng *(48)* were reviewed in detail, since there are so few data by these exposure routes and absorption data is not as well characterized as the oral exposure data.

2.1.1.2.2 Dermal

Frantz et al. *(41-43)* reported that dermal application of a neat (10–1,000 mg/kg bw) or 50% aqueous solution (1,000 mg/kg bw) of ethylene glycol results in slow and incomplete absorption. In two mass balance studies, absorption of ethylene glycol was determined by measurement of radioactivity in body tissues, exhaled air, and excreta. For male and female rats, approximately 32, 29–36, and 22–26% of the 10 mg/kg bw and 1,000 mg/kg bw doses and the 50% solution were absorbed over 96 hours, respectively *(41, 42)*. The respective percentages absorbed in mice treated with 100 and 1,000 mg/kg bw and 50% solution were 43, 51, and 39% over 96 hours *(42)*. The authors concluded that absorption of undiluted and 50% ethylene glycol was greater in mice than rats. Authors noted that the half-life for dermal absorption was about an order of magnitude longer than the half-life for oral absorption. Additional details of these studies and a complete Panel evaluation are included under Section 2.1.3.2.

2.1.1.2.3 Inhalation

Marshall and Cheng *(48)* evaluated the deposition and fate of inhaled ethylene glycol vapor and condensation aerosol in Fischer 344 rats. Two groups of 15 male and female Fischer 344 rats/sex (13–17 weeks old) were exposed to ^{14}C-ethylene glycol (>99% purity) by nose only in the form of vapors (actual concentration=32 mg/m^3) for 30 minutes or aerosols (actual concentration=184 mg/m^3; MMAD=2.3 μm) on Ga_2O_3 particles for 17 minutes. **[The Panel converted the inhalation doses to mg/kg bw values by using the minute volume of rats reported by study authors (0.7–1.3 mL/min/g bw). Exposure to vapor was estimated to be 0.74–1.25 mg/kg bw (e.g., 32 mg/m^3 * 0.47 m^3 inhaled/24 hours * 0.5 hours * 1/0.25 kg, assuming 100% absorption). Exposure to aerosol was estimated at 2.4–4.0 mg/kg bw by using the same equation described above.]** The aerosol dose was based on human studies by Wills et al. *(33)* that demonstrated humans could tolerate an ethylene glycol atmosphere of 188 mg/m^3 for 15 minutes. The vapor concentrations were based on previous observations that 20% of total glycol is present as vapor when aerosols are generated. Deposition of ethylene glycol was determined by measuring radioactivity in different regions of the respiratory tract and other body tissues at intermittent times from 10 minutes to 6 days following exposure. Approximately 60% of the vapor or aerosol inhaled were deposited, largely in the nasal cavity. Between 75 and 80% of the initial body burden was found throughout the body, indicating rapid absorption and distribution following deposition in the nasal cavity. **[The Expert Panel estimated that 60–90% of the inhaled dose was absorbed.]** Excretion patterns observed in this study and Panel critique of this study are included in Section 2.1.3.2.

2.1.2 Distribution

Analyses of tissue, plasma, and urine deposition of ethylene glycol in humans, rats, mice, monkeys, and dogs indicate that it is readily distributed according to total body water *(6, 35)*. The volume of distribution in two patients was estimated to be 0.54–0.56 L/kg bw and the urine-to-plasma ratio in one patient was estimated at 1.0–1.4 *(6)*.

2.1.3 Metabolism

Metabolism of ethylene glycol has been discussed in reviews by ATSDR *(6)*, Carney *(35)*, NTP *(34)*, and Weiner and Richardson *(36)*. The reviews provide generally consistent descriptions of the metabolic process. Figure 2-1 outlines the metabolic pathway of ethylene glycol, which is qualitatively similar in humans, monkeys, dogs, rabbits, rats, and mice. Initially, ethylene glycol is converted to glycolaldehyde by nicotinamide adenine dinucleotide (NAD)-dependent alcohol dehydrogenase (ADH) in a rate-limiting reaction. Reduction of NAD leads to concomitant formation of lactate from pyruvate. **[However, the Panel noted that information regarding the specific ADH enzymes involved remains deficient. The data to date suggest a major role for the class I alcohol dehydrogenase; however, studies to date have not addressed which of the three class I ADH enzymes is most important. The class I ADH locus encodes three enzymes that can homo- or heterodimerize to form the active enzyme, i.e., ADH1A (ADH1 or ADHα), ADH1B (ADH2 or ADHβ), and ADH1C (ADH3 or ADHγ). Further, given the approaches used to implicate ADH, it is difficult to rule out a possible role for CYP2E1, since both pyrazole and 4-methylpyrazole will inhibit both ADH and CYP2E1. Indeed, a recent study by Loeppky and Goelzer *(49)* reported the ability of microsomal suspensions, isolated from isoniazid-treated rats, to oxidize 2 mM ethylene glycol to glycoaldehyde at a rate of 120 nmol/mg/hour. Further oxidation of glycoaldehyde to glyoxal was catalyzed with a V_{max} of 3.2 nmol/min/mg protein and a K_m of 0.32 mM. Both reactions were dependent on the addition of NADPH, offering further evidence for the involvement of cytochrome P450**

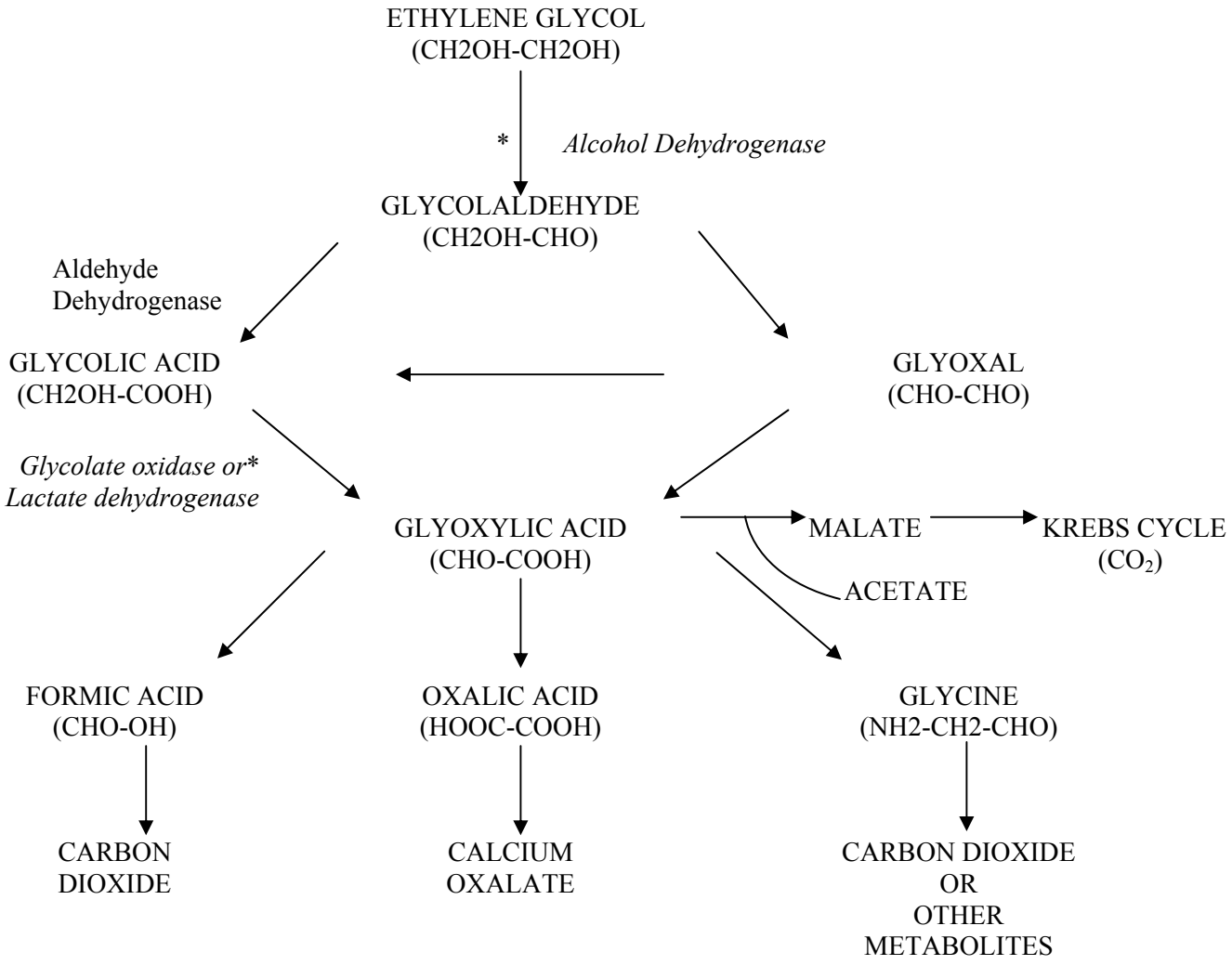
and presumably CYP2E1 in the disposition of ethylene glycol. Studies by Kukielka et al. *(50)* provided evidence of a second pathway where CYP2E1-generated peroxide could facilitate the non-enzymatic oxidation of ethylene glycol to formaldehyde, but did not address the question of whether CYP2E1 could directly participate in the oxidation of ethylene glycol to glycolaldehyde.]

Glycolaldehyde is rapidly converted to glycolate and to a lesser extent glyoxal by cytosolic aldehyde oxidase and aldehyde dehydrogenase. Because glycolaldehyde is rapidly metabolized, very little is found in plasma. Glycolate is a major metabolite in species examined, such as humans, and its potential to accumulate (with a resultant acidosis) is of toxicological significance.

The next major metabolic step is oxidation of glycolate to glyoxylate by glycolate oxidase or lactate dehydrogenase, which is rate-limiting; glyoxal is also converted to glyoxylate. Once formed, glyoxylate is metabolized to formate and then respiratory CO_2, and to urinary oxalate and glycine. While the quantitative importance of the formate versus glycine (or other) pathways in the generation of CO_2 is unclear, a study by Jacobsen *(51)* suggests that the formate pathway is not primary in humans or monkeys. The potential for oxalate to form calcium oxalate crystals is toxicologically significant. As discussed in greater detail below, the major elimination products in rats, rabbits, dogs, and monkeys are CO_2 in expired air and glycolate and unchanged ethylene glycol in urine. Species vary in the amount of other metabolites excreted in urine, including glyoxylic acid, hippurate, and oxalate. A study that found no significant differences in metabolism of ethylene glycol in pregnant versus non-pregnant rats is also discussed below.

Glycolic acid and oxalic acid are also metabolites of proteins and carbohydrates and are thus found in plasma and urine of unexposed, healthy individuals. Background levels of plasma glycolic acid in healthy humans were measured at 0.0044–0.0329 mM *(52-55)*, while oxalic acid levels in plasma were reported at 0.002–0.0233 mM *(52, 53)*. Background levels of glycolic acid in plasma (≤0.03 mM) are well below plasma levels measured in poisoning cases (>10 mM as reported in Section 2.1.3.1). In urine, background levels of glycolic acid and oxalic acid were reported at 0.075–0.790 mM/day and 0.086–0.444 mM/day, respectively *(52-54)*.

Figure 2-1. Metabolism of Ethylene Glycol.



*Rate-limiting steps
Adapted from Carney *(35)* and Jacobsen et al. *(51)*

2.1.3.1 Humans

ATSDR *(6)* notes that case studies of ethylene glycol poisonings in humans reported increased levels of glycolate and lactate in plasma, which were attributed to be the cause of acidosis. Glycolate is a metabolite of ethylene glycol while lactate is generated from pyruvate during the reduction of NAD. Plasma glycolate levels of 12.2–29.3 mM were reported following human poisonings. The detection of calcium oxalate crystals in urine was also reported in case studies *(6)*.

The detection of glycolate anion in several human poisoning cases is important in demonstrating that there is a significant potential for humans to form and retain glycolic acid, similar to what is seen in rodents. For example, Jacobsen et al. *(56)* provide plasma ethylene glycol and glycolate concentrations from six adult male subjects who were admitted to the hospital several hours after ingesting an unspecified quantity of antifreeze on the misconception that it was alcohol. Five of the six subjects were described as chronic alcoholics. All six were treated with ethanol and bicarbonate upon admission to prevent further metabolism of ethylene glycol and to offset the metabolic acidosis. Further, hemodialysis was begun at apparently 4 hours after admission in an effort to remove glycolate.

Data at admission on these six patients indicates a range of glycolate-to-ethylene glycol-plasma concentration ratios: in two subjects the ratio was below 1 (0.62 in both cases); in one subject it was close to unity (1.13), and in the remaining three it was substantially above unity (2.45, 2.95, 4.25). Some of this variability could be due to different times elapsed between ingestion and the sampling of blood as subjects were admitted sometime between 10 and 48 hours post ingestion across the 6 cases. The pre-dialysis elimination of glycolate from plasma was very slow in two patients in which serial sampling was performed for kinetic analysis. These glycolate kinetics are not affected by continued formation from ethylene glycol as ethanol had been administered to block further conversion. The plasma concentration of glycolate in the 6 subjects ranged from 17 to 29.3 mM upon admission, which is 24–41 times higher than the reported K_m, suggesting saturation of glycolate elimination. The acute renal failure seen in each of these patients probably contributed to the prolonged glycolate retention.

The other case report of ethylene glycol poisoning that involved measurement of glycolate in plasma involved three subjects *(57)*. The first was a 2-year-old girl admitted to the hospital 1–1.5 hours after ingesting an unknown amount of antifreeze. The second was a 46-year-old who drank a glassful of antifreeze approximately 6–8 hours prior to admission, while the third was a 14-month-old girl who ingested an unknown quantity of a car wash liquid containing 1% ethylene glycol monobutyl ether. Case 1 showed elevated plasma glycolate (12.2 mM) and ethylene glycol (22.9 mM) for a glycolate:ethylene glycol ratio of 0.53 upon admission. In contrast to the data described above in adults, this elevated concentration of glycolate in a 2-year-old was associated with a readily detectable elimination from plasma ($t_{1/2}$ not reported but apparently in the range of 8 hours from their Figure 2). In contrast, the single adult case in this report had a plasma glycolate concentration of 15.4 mM upon admission, which failed to substantially decrease over the first 8 hours in the hospital but then was rapidly decreased by hemodialysis. In this individual, plasma glycolate was well above ethylene glycol at admission (ratio of 4.97). The third case involved too low an ethylene glycol exposure to produce symptoms or yield measurable glycolate levels in plasma.

These two clinical reports suggest that glycolic acid is a key metabolite with levels in plasma that can readily surpass those of the parent compound in poisoning cases; the reported cases have generally involved glycolate accumulation to levels that exceed metabolic detoxification capability. In cases involving renal failure, glycolate levels in blood may not drop appreciably without hemodialysis. The 2-year-old girl whose serum glycolate levels dropped without dialysis was not reported to have renal failure *(57)*, in contrast to the other cases described above. Based upon this single pediatric case it is impossible to determine whether children are more resistant to ethylene glycol-induced renal failure or can more readily metabolize glycolic acid.

The human data are consistent with the rat data *(44*) that indicate ethylene glycol concentrations above glycolate concentration in blood shortly after dosing, but that this reverses as the metabolite accumulates while parent compound diminishes, a trend that is more evident at the higher ethylene glycol doses used. Thus, the *in vivo* data suggest an ability to form glycolate in humans similar to that in rats.

Strengths/Weaknesses: The human data, while limited, provide data potentially useful for physiologically based toxicokinetic (PBTK) modeling of ethylene glycol metabolism and elimination in humans. The major limitation would be in specifying the amount of ethylene glycol ingested except in the one case in which an estimate could be made from the description that a glassful of antifreeze was ingested approximately 6–8 hours prior to admission *(57*). In the other cases, estimates would have to be made of quantity ingested and amount of time elapsed prior to blood sampling to fit the initial blood ethylene glycol concentration from which the remaining metabolism and elimination steps could be modeled.

Utility (Adequacy) for CERHR Evaluation Process: The human data have utility in demonstrating human metabolites associated with ethylene glycol poisoning and verifying that metabolites are consistent to those observed in animal studies.

One human inhalation study was also reviewed by the Panel. Carstens et al. *(37*) measured ethylene glycol and metabolite levels in the blood and urine of two healthy, non-smoking males (age 54 and 44 years) who inhaled $^{13}C_2$-ethylene glycol (purity of radiolabel: 99 atom% ^{13}C) vapors by mouth from a heated glass vessel for 4 hours. Exhaled air was collected and residual ethylene glycol was measured from the glass vessel. Venous blood was collected before exposure, every 15 minutes during exposure, and up to 4 hours following exposure. Urine was collected before exposure and up to 30 hours following exposure. Samples were analyzed for labeled and unlabeled ethylene glycol, glycolic acid, and oxalic acid using gas chromatography/ mass spectroscopy (GC/MS). On the assumption that ethylene glycol was completely taken up from exhaled air, the authors calculated the amount taken in as the difference between the amount of ethylene glycol placed in the glass inhalation vessel and residual $^{13}C_2$-ethylene glycol in the glass vessel. Data obtained in the study are listed in Table 2-3. Not included in Table 2-3 are plasma oxalic acid levels, which could not be quantified due to non-reproducible recovery from plasma. Kinetic parameters were derived using a one-compartment model and those values are also listed in Table 2-3. Based on kinetic extrapolations of this data, the authors estimated that an 8-hour exposure of these individuals to 10 ppm ethylene glycol (the German maximum allowable concentration (MAC)) would result in an elevation of plasma glycolate levels of 3.1–4.6 μmol/L above background. On a percentage basis, this increase would be only 12.0–16.3% of the background glycolic acid concentration.

Strengths/Weakness: This study is useful in depicting background concentrations of ethylene glycol, glycolic acid, and oxalic acid in urine and glycolic acid in blood. One strength is the analytical separation of dosing related versus background concentrations of ethylene glycol and metabolites by using GC/MS. The results suggest a high absorption rate of ethylene glycol from this dosing medium across the lungs. However, the study has several weaknesses. There were only two subjects analyzed so there is very little information about inter-individual variability. Further, there is only sketchy information on the amount of ethylene glycol dosed and available for inhalation. There were no measurements of ^{13}C-ethylene glycol air concentrations within the inhalation vessel; we only know the nominal amount of ^{13}C-ethylene glycol introduced within the vessel. There is no discussion of the potential for ethylene glycol to degrade within the "warmed" vessel prior to inhalation; this factor would affect the available dose. Carstens et al. do not mention the temperature to which the vessel was warmed. The dose was applied in pulsatile

fashion (16 injections into vessel over 4 hours) rather than as a smooth continuous delivery; the implications of this dosing method on resulting kinetics or relevance to assessing workplace exposures was not discussed by the authors. The authors assumed that absorption was 100% due to the fact that no ethylene glycol was detected in exhaled breath. However, the detection limit for this measurement was not presented so the Panel could not calculate whether this study was a sensitive test of unchanged ethylene glycol exhalation. An accounting of $^{13}CO_2$ exhalation was also missing. These limitations cast some uncertainty over the dose received by the two subjects and also over the claim that there was complete absorption. On the latter point, it is expected that ethylene glycol uptake across the lungs would be high, given its high water solubility and relatively low volatility.

Utility (Adequacy) for CERHR Evaluation Process: An important finding of Carstens et al. *(37*) is that at ethylene glycol inhalation doses that substantially increase the urinary output of ethylene glycol over background (Figure 5 of Carstens et al. study), the amount of glycolic acid formed as measured in plasma and urine is low relative to background. The urinary output data shown in Table 2-3 suggests that only about 10% of ethylene glycol was converted to glycolic acid. This contrasts with much higher glycolate:ethylene glycol ratios in poisoning case studies described above. In those high-dose acute exposures, saturation of glycolic acid removal likely led to a buildup of this metabolite that does not occur under these relatively low and spread-out inhalation exposures. These data, while limited (e.g., n= 2 subjects; exposure dose is somewhat uncertain), have potential for use in calibrating physiologically based toxicokinetic (PBTK) models for doses that are below metabolic saturation.

Table 2-3. Data Obtained from Human Subjects Inhaling Ethylene Glycol Vapors *(37)*.

Parameter	Subject A (54-year-old)	Subject B (55-year-old)
Estimated Total doses of $^{13}C_2$-ethylene glycol (mmol/mg/kg bw/day)	1.43 / 0.96	1.34 / 1.51
Background plasma ethylene glycol (μmol/L)	<7.6	<7.6
$^{13}C_2$-ethylene glycol C_{max} in plasma (μmol/L)	11	15.8
Background plasma glycolic acid (μmol/L)	25.8±3.7	28.3±2.8
$^{13}C_2$-glycolic acid C_{max} in plasma (μmol/L)	0.9	1.8
$T_{1/2}$ for $^{13}C_2$-ethylene glycol in plasma (hours)	2.1	2.6
$T_{1/2}$ for $^{13}C_2$-glycolic acid in plasma (hours)	2.9	2.6
Background ethylene glycol in urine (μmol/24 hours)	18.2	24.5
Background glycolic acid in urine (μmol/24 hours)	274	88
Background oxalic acid in urine (μmol/24 hours)	215	177
$^{13}C_2$-ethylene glycol in urine as percent of inhaled dose within 28 hours after exposure start	6.4	9.3
$^{13}C_2$-glycolic acid in urine as percent of inhaled dose within 28 hours after exposure start	0.70	0.92
$^{13}C_2$-oxalic acid in urine as percent of inhaled dose within 28 hours after exposure start	0.08	0.28
$^{13}C_2$-glycolic acid excreted in urine over 24 hours as percentage of unlabeled glycolic acid	3.7	14.2
$^{13}C_2$-oxalic acid excreted in urine over 24 hours as percentage of unlabeled glycolic acid	0.5	2.1
$^{13}C_2$-ethylene glycol distribution volume (L or L/kg bw)	75 or 0.78	52 or 0.91

2.1.3.2 Animals

Frantz et al. *(41-43)* examined dose-related shifts in metabolism of ethylene glycol in 10–11-week-old male and female Sprague-Dawley rats and 5–6-week-old female CD-1 mice. ^{14}C-ethylene glycol (98–99% purity) was administered by intravenous (IV) infusion in saline, gavage in water, or dermal application of neat or 50% aqueous solution. Oral and IV exposures were administered as a single dose and ranged from 10 to 1,000 mg/kg bw. The highest dose was stated by authors to be double the developmental toxicity NOAEL for rats (500 mg/kg bw/day). Dermal exposures were conducted by applying ^{14}C-ethylene glycol to the shaved backs of animals and occluding for 6 hours, then rinsing with water. Undiluted ethylene glycol was applied at doses of 10 and 1,000 mg/kg bw/day in rats and 100 and 1,000 mg/kg bw/day in mice. Authors attempted to use the same doses as the oral exposure experiments, but application of a neat 10 mg/kg bw

dose to mice was not technically feasible. A 1,000 mg/kg bw dose was also applied as a 50% aqueous solution to simulate automotive antifreeze and deicing formulations. Concentrations of dosing solutions were verified. Blood, tissues, urine, feces, and expired air samples were collected at intervals between 30 minutes and 96 hours post-dosing in 3–4 animals/group/time period and analyzed by GC, high pressure liquid chromatography (HPLC), or liquid scintillation counting.

Toxicokinetic parameters following oral exposure are outlined in Table 2-4 for rats and mice. The authors concluded that plasma kinetics were linear (not dose-dependent) between the 10 and 1,000 mg/kg bw doses in both sexes of rat because mean residence time, area under the concentration versus time curve (AUC), clearance, terminal half-life, and percent dose excreted as ethylene glycol were consistent. In contrast, female mice had inconsistencies between terminal half-life, mean residence time, AUC, and clearance at these same doses, which the authors suggest provided evidence of non-linear (dose-dependent) plasma kinetics. **[The Panel noted that plasma kinetic data needs to be evaluated together with urinary excretion data. See utility statement below.]** Results with IV exposure were consistent with oral exposure results for each species. Following dermal exposure there was no evidence of dose-dependent changes in plasma kinetics or excretion patterns in either species or sex.

Table 2-4. Toxicokinetic Values Reported in Rats and Mice Exposed Orally to Ethylene Glycol by Frantz et al. *(41-43)*.

Parameter	Values for Unmetabolized Ethylene Glycol Doses in mg/kg bw						
	10	**100**	**200**	**400**	**600**	**800**	**1,000**
Female Rat:							
AUC (μg/g·hr)	45.2	NA	NA	NA	NA	NA	4,012
$T_{1/2}^{\beta}$ (hr)	2.5	NA	NA	NA	NA	NA	1.5
MRT_{∞} (hr)	3.8	NA	NA	NA	NA	NA	2.5
$U_{\infty}^{\text{ethylene glycol}}$ (%)	0.2216	NA	NA	NA	NA	NA	0.2490
Cl_{oral} (mL/min/kg)	3.4	NA	NA	NA	NA	NA	3.9
Male Rat:							
AUC (μg/g·hr)	41.3	NA	NA	NA	NA	NA	6,041
$T_{1/2}^{\beta}$ (hr)	1.4	NA	NA	NA	NA	NA	2.0
MRT_{∞} (hr)	2.5	NA	NA	NA	NA	NA	3.6
$U_{\infty}^{\text{ethylene glycol}}$ (%)	0.2278	NA	NA	NA	NA	NA	0.2842
Cl_{total} (mL/min/kg)	4.0	NA	NA	NA	NA	NA	2.8
Female Mouse:							
AUC (μg/g·hr)	5.36	158.4	394.4	719.6	NA	NA	2,501
$T_{1/2}^{\beta}$ (hr)	0.3	0.5	0.5	0.5	NA	NA	1.1
$U_{\infty}^{\text{ethylene glycol}}$ (%)	0.1593	0.3321	0.2733	0.2883	NA	NA	0.3510
MRT_{∞} (hr)	0.6	1.1	1.0	1.2	NA	NA	1.9
Cl_{oral} (mL/min/kg)	7.5	8.8	8.4	9.0	NA	NA	6.7

NA=Not analyzed; AUC=Area under the concentration versus time curve; $t_{1/2}^{\beta}$=Half-life of elimination. MRT_{∞}=mean residence time; $U_{\infty}^{\text{ethylene glycol}}$ =Percent dose excreted as ethylene glycol in urine; Cl_{oral}=clearance after oral dosing; Cl_{total}=total clearance

Excretion patterns observed in mice and rats by Franz et al. *(41-43)* are outlined in Table 2-5 and Table 2-6. Following oral exposure, the primary metabolites eliminated in rats and mice were CO_2 and glycolate. Cumulative ^{14}C excretion patterns over a 96-hour period changed with increasing dose, which led study authors to suggest that oxidative metabolic pathways become saturated with high oral exposures. At the lowest oral dose (10 mg/kg bw), the primary and

secondary routes for elimination of ^{14}C were exhalation of $^{14}CO_2$ and elimination of ^{14}C in urine, respectively (Table 2-5). As dosages increased, urinary elimination of ^{14}C exceeded exhalation of $^{14}CO_2$. Metabolic pathways in mice appeared to become saturated at lower doses than in rats. The study authors noted that the shift to primarily urinary excretion occurred at doses exceeding 400 mg/kg bw in female rats, at 1,000 mg/kg bw in male rats, and at doses exceeding 100 mg/kg bw in female mice. The shift in metabolism at higher doses of ethylene glycol resulted in the accumulation of glycolate. Ethylene glycol and glycolate were the main urinary metabolites detected and the ratio of glycolate to ethylene glycol increased proportional to dose. Percentages of ethylene glycol and metabolites in the urine of male rats are outlined in Table 2-6. Oxalic acid was detected at low levels in the urine of male and female rats, but not mice. The absence of urinary oxalic acid in mice led authors to speculate that mice have a greater capacity than rats to metabolize low doses of ethylene glycol to CO_2.

Frantz et al. *(41-43*) noted that expired $^{14}CO_2$ and urinary ^{14}C were the primary and secondary metabolites, respectively, eliminated over a 96-hour post-dosing period in rats and mice dermally exposed to 10 or 1,000 mg/kg bw (neat or 50% solution) ethylene glycol. Because there was no shift in excretion patterns with increasing dose (Table 2-5), the authors suggested that metabolic pathways do not saturate at high dermal doses due to slow absorption through skin. The majority of radioactivity in urine following dermal exposure was associated with parent compound.

Table 2-5. Excretion Patterns in Rats and Mice Administered ^{14}C-Ethylene Glycol by Oral or Dermal Route in Studies by Frantz et al. *(41, 43*)**.**

Exposure Route:	**Percent disposition of Exhaled CO_2/Urinary ^{14}C at each dose (mg/kg bw)**							
Sex and Species	**10**	**100**	**200**	**400**	**600**	**800**	**1,000**	**1,000 (50% solution)**
Oral:								
Female Rat	48/26	NA	NA	39/38	33/37	32/41	28/35	NA
Male Rat	42/26	NA	NA	39/20	34/26	30/26	27/42	NA
Female Mouse	55/24	42/43	31/44	26/45	NA	NA	22/56	NA
Dermal:								
Female Rat	13/8	NA	NA	NA	NA	NA	11/8	9/4
Male Rat	14/7	NA	NA	NA	NA	NA	14/8	6/5
Female Mouse	NA	10/7	NA	NA	NA	NA	16/12	10/5

NA=Not analyzed

Table 2-6. Urinalysis Results for Ethylene Glycol and Metabolites in Male Rats *(41)*.

Route: Dose Group, mg/kg bw	Percentage Interval Radioactivity Recovery					
	0–12 Hour Interval			12–24 Hour Interval		
	Oxalic acid	Glycolic acid	Ethylene Glycol	Oxalic acid	Glycolic acid	Ethylene Glycol
Oral:						
10	1.7	6.0	92.3	NP	NP	95.6
1,000	NP	25.0	75.0	7.4	37.5	55.1
Dermal-Undiluted:						
10	NP	100	NP	NP	12.8	87.2
1,000	NP	100	NP	NP	2.8	97.2
Dermal- 50% dilution:						
1,000	NP	NP	NP	NP	NP	100 (at 24–36 hours)[a]

NP=No peak detected
[a]First quantifiable interval

Strengths/Weakness/Utility (Adequacy) for CERHR Evaluation Process: The Frantz et al. *(41-43)* studies provide useful toxicokinetic data over a relevant dose range. They provide data showing that ethylene glycol blood levels are nearly linear across a wide range of doses, but these data alone are deceptive because there are underlying non-linearities that are brought to light by the urinary excretion data. The excretion pattern indicates that the percent in urine jumps considerably from 10 to 100 mg/kg in mice, between 10 and 400 mg/kg in the female rat, and at higher doses in the male rat. The combination of ethylene glycol blood level data and the urinary excretion profile suggests that the ethylene glycol oxidative metabolism is saturated but that the excess is excreted renally rather than accumulated in blood or tissue. The pattern also indicates increasing glycolic acid in urine as a percentage of dose over this dose range. Since ethylene glycol oxidation to glycolic acid appears to become saturated, the most plausible mechanism for this excess of urinary glycolic acid is saturated elimination of this metabolite, leading to simultaneous increase in both ethylene glycol and glycolic acid in urine.

Thus, the Frantz et al. data *(41-43)* are important to show the saturation of both ethylene glycol and glycolic acid in rats and mice. The data also test whether bolus oral dosing is necessary for this phenomenon by employing high dermal doses in rats. In limited data (10 and 1,000 mg/kg/day doses only), there does not appear to be any increase in ethylene glycol or glycolic acid in urine, with the vast majority of urinary ^{14}C remaining in the form of unmetabolized ethylene glycol. This suggests efficient removal of glycolic acid under dermal exposure conditions even at doses as high as 1,000 mg/kg. From this, the Panel can conclude that the lower dose rate from dermal exposure does not present a great enough systemic ethylene glycol dose per unit time to saturate the oxidative enzyme systems.

Findings of the Frantz et al. *(41-43)* studies are consistent with results of an older study in which dose-related changes in excretion patterns were seen in male and female Fischer 344 rats

administered ^{14}C-ethylene glycol (>99% purity) in saline via IV at doses of 20, 200, 1,000, or 2,000 mg/kg bw *(58)*.

Pottenger et al. *(44)* compared dose-related pharmacokinetics in adult pregnant (gd 10) versus nonpregnant female Sprague-Dawley rats (n=4–5/group) administered a single gavage dose of $^{13}C_2$-ethylene glycol (96.7% purity) in an aqueous solution. Doses in pregnant rats were 10, 150, 500, 1,000, or 2,500 mg/kg bw, while non-pregnant rats were dosed with 10 or 2,500 mg/kg bw. Doses were at or below levels that produced developmental toxicity in rats. Pregnant rats were treated on gd 10 because this has been shown to be a sensitive period for ethylene glycol-induced developmental toxicity. Blood was collected prior to dosing and at 7 time intervals between 1 and 24 hours after dosing. Total urine eliminated was collected at 12 and 24 hours. Urine and blood samples were examined for ethylene glycol, glycolic acid, and oxalic acid by GC/MS. Table 2-7 lists the primary results for ethylene glycol and glycolic acid.

Table 2-7. Comparison of Ethylene Glycol and Glycolic Acid Toxicokinetics by Pottenger et al. *(44)*.

Parameter	Values for Ethylene Glycol/Glycolic Acid at Each Dose (mg/kg bw)				
	10	**150**	**500**	**1,000**	**2,500**
Pregnant:					
T_{max} (hr)	1/[a]	1/3	1/3	1/3	1/3
C_{max} (μg/g)	7.9/[a]	88.9/20.6	392/131	886/363	3,528/452
AUC (μg/g·hr)	23/[a]	292/84	1,208/641	2,928/1,829	11,638/4,031
$t_{1/2}^{\beta}$ (hr)	1.4/[a]	1.7/1.4	1.7/1	1.8/1.6	1.7/1.5
Total Urinary Elimination (% dose)[b]	14.95/0.88	27.86/1.18	41.92/12.43	39.64/20.13	37.64/32.79
Total $^{13}C_2$-Urinary Elimination (% dose)[c]	15.83	29.71	54.97	60.33	71.09
Non-Pregnant:					
T_{max} (hr)	1/[a]	NA/NA	NA/NA	NA/NA	1/3
C_{max} (μg/g)	9.3/[a]	NA/NA	NA/NA	NA/NA	2,795/432
AUC (μg/g·hr)	27/[a]	NA/NA	NA/NA	NA/NA	11,368/3,807
$t_{1/2}^{\beta}$	1.5/[a]	NA/NA	NA/NA	NA/NA	1.9/1.1
Total Urinary Elimination (% dose)[b]	14.62/1.36	NA/NA	NA/NA	NA/NA	37.63/31.36
Total $^{13}C_2$-Urinary Elimination (% dose)[c]	16.35	NA	NA	NA	69.6

NA=Not analyzed.
[a]Glycolic acid was below the quantifiable limit (2.1 μg/g) in blood at the lowest dose.
[b]Elimination of ethylene glycol/glycolic acid.
[c]Total elimination.

Based on those results, the following conclusions were made by the authors:

- No significant differences in toxicokinetic parameters or urinary excretion profiles were observed between the pregnant (gd 10–11) and non-pregnant rats dosed with 10 or 2,500 mg/kg bw.
- A shift in blood glycolic acid toxicokinetics was noted at doses between 150 and 500 mg/kg bw as evident by C_{max} and AUC values that were not proportionate to increases in dose levels.
- Urinary excretion patterns were dose-dependent. Percentages of total urinary elimination increased with dose from about 16% at the 10 mg/kg bw dose to 70% at the 2,500 mg/kg bw dose. The percentage of glycolic acid excreted in urine was disproportionate to dose starting at 500 mg/kg bw.
- Because shifts in urinary glycolic acid excretion paralleled changes observed in blood, dose-dependent changes in toxicokinetics and urinary excretion were most likely due to saturation of metabolic pathways and not saturation of renal elimination.
- Oxalic acid is not likely involved in the developmental toxicity associated with ethylene glycol since concentrations in blood were usually lower than the quantifiable limit of 4.9 μg/g blood. No dose-response relationship was noted the few times that oxalic acid was detected in blood. **[The Expert Panel did not believe the data were adequate to opine about a possible role of oxalic acid in developmental toxicity, as noted in the strength/weakness/ utility statements below.]** Oxalic acid was excreted in urine at a constant fraction of administered dose (0.36–0.66%), and thus urinary levels increased with dose.

Strengths/Weakness: Significant physiological changes occur during pregnancy that could impact ethylene glycol disposition. The report by Pottenger et al. *(44)* was the first to address the issue of pregnancy and ethylene glycol disposition and, as such, was important. Strengths of the study include a broad dose range that incorporated the NOAEL for developmental toxicity in this species, a thorough pharmacokinetic analysis of both the parent compound and the two recognized major metabolites (glycolic acid and oxalic acid), and where expected, the fact that the data are in agreement with previous findings. A limitation of the study is that since exhaled breath was not collected, there is no attempt at mass balance. While it appears from the urinary data that there is a dose-dependent saturation of ethylene glycol and glycolic acid metabolism, the lack of exhaled CO_2 data and mass balance creates some uncertainty in this data set (e.g., changes in urinary metabolite levels may have been due to altered ethylene glycol bioavailability with increasing dose or due to measurement error).

Utility (Adequacy) for CERHR Evaluation Process: The utility of the Pottenger et al. *(44)* study for assessing human fetal exposure is uncertain. The study was limited to a narrow window of gestation, gd 10–11, a sensitive time frame for ethylene glycol developmental toxicity in the Sprague-Dawley rat. A literature search has not revealed a reliable assessment of whether ADH or other ethylene glycol metabolizing enzymes might change in the mother during pregnancy. Further, it is unclear whether a later point in gestation would have yielded substantially different toxicokinetic results due to the physiological changes that accompany later stages of pregnancy. If it is assumed that no changes occur, then it may be possible to conclude that pregnancy would not have any profound impact on maternal ethylene glycol disposition. Maternal toxicokinetic handling of ethylene glycol and metabolites is critical in determining dose to the fetus, but ontological development of ADH in the fetus may be different across species, which leads to the uncertainty that rat maternal toxicokinetic data may not extrapolate well for describing dosimetry to the human fetus. Given the differences in the developmental expression of the ADH enzymes between the rat and humans, combined with uncertainty about the specific enzymes involved in human ethylene glycol metabolism, it is difficult to assess how well these data might extrapolate to the human situation; the authors make no attempt to do so. There also are concerns about the lack of concentration dependence for ethylene glycol and glycolic acid half-lives, which is

incompatible with a zero order process being a rate-determining step. However, the sensitivity of β-elimination rate calculations to metabolic saturation is limited by the rapid elimination of ethylene glycol and glycolic acid by other pathways (renal) and by the fact that key points in the glycolic acid blood-decay curve were near the limit of detection. Finally, the reported blood oxalate data were at or near the limits of detection for the assay and were independent of dose. While the blood oxalate data are questionable, the urinary oxalate data show a linear increase in oxalate excretion over the entire dose profile, suggesting no limitation on the production of oxalate. This may have been difficult to detect in blood due to rapid elimination into urine and/or due to calcium oxalate precipitation in blood.

The Pottenger et al. study provides important confirmation of the saturation in glycolic acid oxidation in the rat that was suggested by Frantz et al. *(41-43*). In this case there is a well-defined dose range (ethylene glycol dose of 150–500 mg/kg bw) in which saturation takes place. While the Frantz et al. *(41-43*) data sets generally support the concept that percent urinary excretion increases with increasing ethylene glycol dose, the Frantz et al. data show a less marked trend than that suggested by the Pottenger et al. data. The combination of the Frantz et al. and Pottenger et al. data sets supports the concept that developmental toxicity in rats (NOAEL 500 mg/kg bw; LOAEL 1,000 mg/kg bw) appears to occur under conditions in which glycolic acid removal is saturated. At non-saturating doses (low oral doses, dermal exposure), the glycolic acid that is formed from ethylene glycol appears to be rapidly converted to further oxidation products and eliminated primarily as exhaled CO_2. At oral doses as low as 150–500 mg/kg bw, glycolic acid oxidation appears to become saturated, leading to the potential for its accumulation in blood. Given the evidence for increasing glycolic acid and oxalate exposure with dose throughout the dose range tested by Pottenger et al., this study cannot distinguish (nor was it designed to do so) which of these metabolites may be more important for developmental effects.

A satellite study *(59*) conducted in conjunction with a subchronic toxicity study *(60*) measured levels of ethylene glycol, glycolic acid, and oxalic acid in blood, urine, and kidneys of male F344 and Wistar rats exposed to ethylene glycol through diet at doses of 0, 150, 500, and 1,000 mg/kg bw/day for 1 or 16 weeks. The satellite study is discussed in detail in Section 2.2.2.1. An interesting finding of the study is that oxalic acid levels in kidney greatly exceeded levels in blood in both strains of rats (See Tables 2-12 and 2-13). As noted by Corley et al. *(59*), background oxalic acid blood levels are typically measured at 1–10 μg/g and blood levels in treated animals rarely exceeded 20 μg/g due to solubility limits. Because blood and urinary concentrations of oxalic acid were not indicative of renal levels, Corley et al. concluded that they are not appropriate biomarkers for renal exposure. **[The Expert Panel noted that Corley et al. *(59*) provides experimental proof that oxalic acid precipitates out of blood when its solubility limit is exceeded and forms microcrystals that eventually lodge in the kidney. High variability in blood oxalic acid measurements suggests a heterogenous distribution of microcrystals and resulting randomness of sample collected.]**

Marshall and Cheng *(48*) demonstrated that rat excretion patterns following inhalation exposure are similar to those following oral exposure. Two groups of 15 male and female Fischer 344 rats/sex (13–17 weeks old) were exposed to ^{14}C-ethylene glycol (>99% purity) by nose only in the form of vapors (32 mg/m^3) for 30 minutes or aerosols (184 mg/m^3; MMAD=2.3 μm) on Ga_2O_3 particles for 17 minutes. **[The Expert Panel estimated exposure to vapor at 0.74–1.25 mg/kg bw and exposure to aerosol at 2.4–4.0 mg/kg bw. See Section 2.1.1.2.3 for calculation.]** Within the first 4 days following exposure, 63 and 20% of the ethylene glycol from the vapor exposure was eliminated as expired $^{14}CO_2$ and as urinary ^{14}C, respectively. For rats exposed to ethylene glycol aerosol, percentages of ethylene glycol eliminated through exhaled air

and urinary excretion were 70 and 11%, respectively. For vapor and aerosol exposure, 80 and 56% of urinary excretion, respectively, occurred during the first 24 hours and were comprised entirely of ethylene glycol. Metabolites found in urine at later time periods were not reported, although it appears that urine was analyzed for ethylene glycol metabolites. Information about absorption is included under Section 2.1.1.2.3.

Strengths/Weaknesses: The strength of the Marshall and Cheng *(48)* paper is that it utilized high-dose inhalation exposure to probe ethylene glycol fate in a relevant animal model and it used sufficient numbers of animals (n=15 per dose group). Its main weakness is the limited identification of radiocarbon in urine, with no specific mention of assay for glycolic acid. Further, the radiocarbon data in blood and tissues may be confounded by the metabolism of ethylene glycol to formate and its subsequent utilization and labeling of tissues via the one carbon pool.

Utility (Adequacy) for CERHR Evaluation Process: The Marshall and Cheng *(48)* study provides perspective on inhalation exposures. The doses used in the study are far lower than the oral doses needed to demonstrate metabolic saturation and glycolic acid buildup, which comports with the authors' findings of relatively complete metabolism of ethylene glycol. The actual amount inhaled may be lower since high concentrations of ethylene glycol may be irritating and result in a reflexive decrease in respiratory rate. Given that Wills et al. *(33)* found that concentrations higher than 200 mg/m^3 were highly irritating and poorly tolerated by human volunteers, it seems that the warning properties of ethylene glycol would prevent sufficiently high inhalation exposures that result in saturating conditions.

The effect of dose rate on ethylene glycol and glycolic acid blood levels in rats were studied by Carney et al. *(61)*. For this study, rats were dosed with ethylene glycol at 0, 1,000, or 2,000 mg/kg bw/day by either bolus subcutaneous (SC) injection or slow, continuous administration by an SC infusion pump. Rats receiving the bolus injections had higher mean blood levels of ethylene glycol (9.5 and 21.9 mM at each respective dose) and glycolic acid (3.3 and 6.3 mM at each respective dose) than rats receiving ethylene glycol by continuous infusions (ethylene glycol levels: 2.3 and 4.3 mM, respectively; glycolic acid levels: 0.1 and 1.0 mM, respectively). Complete details of this study and an evaluation of developmental toxicity through each exposure method are discussed in Section 3.2.4. **[The Expert Panel notes that since very little glycolic acid was found in plasma after the 1,000 mg/kg bw/day pump infusion dose, it would appear that glycolic acid was readily metabolized and thus metabolism was not saturated. However, after 2,000 mg/kg bw/day via infusion, there is evidence that glycolic acid did accumulate in plasma as the doubling of infusion dose led to a 10-fold increase in glycolate plasma levels. While this dose rate appears to have saturated glycolic acid removal, it yielded blood levels that were still lower than the glycolate levels seen after bolus dosing. However, this conclusion is based upon single time points after bolus (3 hours post exposure on gd 7, 9, 12, and 15) and constant infusion (samples sometime on days gd 7, 9, 12, and 15) dosing. The Panel noted that no estimate of AUC dose of ethylene glycol or glycolic acid was provided. The AUC dose of ethylene glycol would have been beneficial for calculations that demonstrate the percent bioavailability of ethylene glycol from each dosing method. AUC calculations are useful for confirming that observed differences in toxicity are attributed to dose rate and are not partially due to bioavailability differences between dosing methods. The AUC dose of glycolic acid is an important dose metric for testing how glycolic acid is related to developmental toxicity. Although an AUC dose for ethylene glycol was not provided, it can be estimated based on findings in gavage studies, with the caveat that ethylene glycol dosing through gavage versus bolus SC dosing are similar but not exactly equivalent *(62)*. In the rats receiving 1,000 mg/kg bw/day by continuous infusions, the**

average blood ethylene glycol level was 140 μg/g; multiplying this value by 24 hours results in an estimated 24-hour AUC dose of 3,360 μg/g·hr. In the Pottenger et al. *(44)* study, an AUC value of 2,928 μg/g·hr was calculated for rats gavage dosed with 1,000 mg/kg bw. Therefore, it appears that bioavailability of ethylene glycol was similar between rats receiving 1,000 mg/kg bw ethylene glycol through bolus or continuous SC dosing. The implication from this paper is that a peak concentration of glycolic acid of at least 3 mM is needed for developmental toxicity. With slow infusion of ethylene glycol, a glycolic acid peak or spike is not obtained, but the long-term cumulative (AUC) dose is the more relevant dose metric. The natural supposition from this paper is that the AUC doses for an equivalent ethylene glycol applied dose from the two dosing methods were roughly equivalent, but that the peak dose was much higher from bolus dosing, which would explain the greater toxicity from bolus dosing. That appears to be the case from AUC estimates; however, AUC was not evaluated in this study. The paper does suggest that saturation of glycolic acid metabolism occurs somewhere between a constant infusion dose of 1,000 and 2,000 mg/kg bw/day. In contrast, Pottenger et al. *(44)* shows saturation from bolus dosing (gavage, not SC, in this case) occurs in the ethylene glycol dose range of 150–500 mg/kg bw. This would suggest that continuous dosing is approximately 3–10 fold less efficient at saturating glycolic acid metabolism when compared to bolus dosing.]

A companion study to Carney et al. *(61)* was conducted to examine further the effects of dose rate in Sprague-Dawley rats gavage treated with 100 or 1,000 mg/kg bw ethylene glycol in a water vehicle on gd 11 *(62)*. **[The Expert Panel reviewed the data for the study and agreed with author conclusions that high-dose bolus gavage dosing at 1,000 mg/kg bw results in much higher maternal blood levels of ethylene glycol and glycolic acid than were observed with slow continuous dosing in the Carney et al. study. Half-life of ethylene glycol and glycolic acid in blood and changes in urinary excretion patterns from 100 to 1,000 mg/kg bw were similar to values obtained in previous studies by Pottenger et al. *(44)* and Frantz et al. *(41-43)*. AUC, half-lives, and levels of ethylene glycol, glycolic acid, and oxalic acid were measured in maternal blood, maternal kidneys, extraembryonic fluid, and embryos. The Panel noted that although this is a well-conducted study, the data were obtained from small numbers of samples (n≤3)/time period. Additionally, there was a great deal of variability in tissue levels of the metabolites, and many of the oxalic acid levels were near the detection limit of the method. However, the study is useful in confirming and extending some of the findings from the companion study reviewed above. Once again, a superlinear increase in glycolic acid blood concentration is shown when going from a continuous infusion of 1,000 to 2,000 mg/kg bw/day, which is in contrast to bolus dosing in which the increase in blood glycolic acid is approximately 2-fold across these doses. This makes the dose rate comparison (bolus versus continuous) less extreme at the 2,000 mg/kg bw/day exposure level than at the 1,000 mg/kg bw/day level. Also of interest is the finding that ethylene glycol and glycolic acid are consistently higher in the embryo relative to in maternal blood. In particular, glycolic acid concentrations were generally 2- to 4-fold higher in embryos than in maternal blood. While this study's purpose did not involve explaining the basis for this phenomenon, these authors speculate that it may be caused by pH differential between the embryonic and maternal compartment leading to glycolate ion trapping in the embryonic compartment. It may also be possible that metabolism of ethylene glycol to glycolic acid occurs in the embryo and thus contributes to the greater internal dose of glycolic acid in the conceptus. Given that this study focused upon a narrow range of gestation (gd 11), it is unclear how the maternal/offspring ratio might change across different developmental stages as embryonic/fetal pH and metabolic function changes. Thus, this study supports the concept that metabolic saturation of glycolic acid removal occurs more readily under bolus**

dosing as opposed to low-dose rate scenarios. It shows that the difference in glycolic acid internal dose across methods of administration (bolus vs continuous infusion) can vary depending upon the total daily dose applied. Finally, this study provides evidence that glycolic acid is higher on the embryonic side of the placenta, suggesting a concentrating mechanism that is poorly understood but that is highly relevant to risk assessment.]

Some limited studies in non-rodent species were briefly summarized in order to compare the findings with rodent studies.

Limited data in a series of studies conducted by McChesney et al. *(46)* demonstrated that monkeys produce the same metabolites as mice and rats. Two female Rhesus monkeys (3.3 kg) IV treated with 139 mg/kg bw ^{14}C-ethylene glycol in saline excreted ^{14}C primarily in urine and then expired air at 1 and 4 hours post-dosing; the temporal differences in sampling time do not permit direct comparisons with the cumulative values obtained in rodents over a period of days in the studies by Frantz et al. *(41-43)*. In two separate experiments by McChesney et al. *(46)*, the percentage of dose excreted in urine as ^{14}C and ethylene glycol at 0–48 hours post-dosing by gavage with ^{14}C ethylene glycol at 1 mL/kg bw **[1,109 mg/kg bw]** ranged from 34.2 to 54.4% and 17.1 to 25.9%, respectively, in 4 female monkeys (5–7 kg). In 1 of the monkeys, it was determined that the percentage of dose excreted in urine as glycolic acid was 11.5% and as oxalic acid was 0.27% at 0–48 hours post dosing. Unidentified compounds represented 5.5% of the dose.

McChesney et al. *(46)* also describe a preliminary experiment conducted in 2 chimpanzees exposed to ^{14}C-ethylene glycol by IV at 2 mL/kg bw **[2,218 mg/kg bw]** or 1 mL/kg **[1,109 mg/kg bw].** At 9 hours post dosing, the animal exposed to the higher dose excreted 4% of the administered ^{14}C, 40% of this being in the form of ethylene glycol. The chimpanzee exposed to the lower dose excreted 28% of the administered ^{14}C in the first 8 hours, 30% in the form of ethylene glycol. In the 8–24 hour period it excreted an additional 11.3% of the administered ^{14}C, 17% as ethylene glycol. Expired $^{14}CO_2$ was not measured.

Strengths/Weakness/Utility (Adequacy) for CERHR Evaluation Process: McChesney et al. *(46)* provide limited data on ethylene glycol disposition in rats and Rhesus monkeys. The rat studies provide useful tissue distribution and mass balance information following an IV dose of 139 mg/kg bw of radiolabeled ethylene glycol. However, only one rat was followed long enough (24 hours) to understand total metabolic disposition. In this one rat, only 14.4% of the dose was eliminated in expired air and 46.5% was excreted in urine. These values are considerably lower for exhaled dose and higher for urinary elimination than expected at this low dose based upon Frantz et al. *(41-43)*, who used oral dosing. It is possible that since IV administration does not involve first-pass hepatic metabolism, a greater percentage of administered dose is eliminated as unmetabolized ethylene glycol in urine rather than becoming completely metabolized and appearing in exhaled breath as CO_2. However, this is speculative since the data are from only a single rat.

The limited data in both rats and monkeys fall short of providing useful cross-species pharmacokinetic comparisons. Regarding monkeys, mass balance tissue and excretory data are provided only for two monkeys and only through 4 hours post-dosing (Table 2, Fig. 2 of the study). Plasma half-life data from two experiments in monkeys (n=3–4 per time point in each experiment) are useful in showing one-compartment decay kinetics with a half-life range of 2.7–3.7 hours, with the longer half-lives seen in monkeys that were 1 year older than monkeys in the first experiment with the shorter half-life. The dose in these 2 experiments was 1 mL/kg (1,109 mg/kg bw) by the oral route. The disposition of ethylene glycol in these animals is not well

described, with no attempt at mass balance and only the amount of ethylene glycol in urine for 3 monkeys (16–20% of the dose) shown. Follow up studies in 3 monkeys found that on average, 52% of the urinary elimination of a 1 mL/kg bw oral dose was in the form of ethylene glycol with the remainder being other metabolites. Urinary metabolism was further characterized in a single monkey dosed orally with 1 mL/kg bw. The urinary mass balance for this monkey again indicated that 50% of radiolabel in urine was parent compound, with approximately one-third appearing as glycolic acid (11.5% of total dose).

Overall, the variety of experiments reported by McChesney et al. *(46)* have the weaknesses of low numbers of animals tested, incomplete information reported for any given experiment, and no attempt at mass balance except in two experiments that looked primarily at short-term disposition in rats and monkeys. The most useful information is the reported half-life in monkeys (2.7–3.7 hours), which suggests somewhat slower clearance in monkeys than in rats given a similarly high dose of ethylene glycol [1.5–2 hours; Frantz et al. *(41-43)*]. The evidence for glycolic acid excretion in a single monkey at the rate of 11.5% of the administered dose suggests that monkeys may eliminate less ethylene glycol as glycolic acid than seen in rats (Pottenger et al. *(44)* showed 20% eliminated as glycolic acid after a similar oral dose). However, the monkey data are too limited (only one animal, one dose, no mass balance) to provide useful quantitative information regarding glycolic acid excretion or to make cross-species comparisons. The utility of this excretion data is simply that it provides evidence for substantiative glycolic acid production and elimination in monkeys.

Regarding the McChesney et al. chimpanzee data, the high-dose animal died after 9 hours so only very limited toxicokinetics data are available from this animal (no blood data, no mass balance, only 4% of the dose accounted for in urine). Urine was collected from the other chimpanzee until 24 hours post dosing, at which point only 39% of the dose was accounted for in urine, leading to the possibility of extensive metabolism and ethylene glycol elimination as CO_2 for the majority of the dose. This would be interesting given that at this high dose (1,109 mg/kg bw) less elimination through exhalation (and thus less complete metabolism) and more urinary elimination are expected based upon the rat studies *(44)*. However, there is data from only one chimpanzee tested at only one dose, and there was no collection of exhaled radiolabel for this animal. Thus, these data cannot be relied upon.

Some limited information on the metabolism of ethylene glycol in rabbits and a comparison to other species is available from a study by Gessner et al. *(63)*. In that study, ^{14}C-ethylene glycol (98±2% purity) in water was administered orally or by SC injection to chinchilla rabbits, albino rats, guinea pigs, and cats **[sex not specified]**. In rabbits (n=1/dose/time period) administered oral doses of 124–2,000 mg/kg bw, approximately 20–30% of the radioactivity was excreted in the urine within 2 days. At the lowest dose (124 mg/kg bw), a large proportion of the ^{14}C was eliminated in the expired air as carbon dioxide, 42% within 1 day and 60% within 3 days. **[The percentage of ^{14}C eliminated in expired air was not reported for the remaining doses; it was not clear if the values were not measured or were below limits of detection.]** Ethylene glycol was found to be the major urinary metabolite at 24 hours following oral administration of ethylene glycol at 25 mg/kg bw in 2 rabbits and 125 mg/kg bw in 1 rabbit. Ethylene glycol represented 6–15 and 10% of the 25 mg/kg bw and 125 mg/kg bw doses, respectively. Trace levels of oxalic acid (0.01–0.11% of dose) were also present. No other ethylene glycol metabolites were detected including glycolic acid. Excretion of oxalate in urine appears to vary between cats, rats, rabbits, and guinea pigs. Following administration of ethylene glycol by an unspecified route at doses of 100–1,000 mg/kg bw/day, cats were found to have the highest levels of urinary oxalate (0.7–3.7% of dose); rabbits and guinea pigs had the lowest levels of oxalate in urine (~0.05% of dose); and rats had intermediate levels (0.5–1.1% of dose).

Strengths/Weakness: The Gessner et al. *(63)* study is limited by the small numbers tested at any dose level, the lack of mass balance information (including no CO_2 measurements for most dose groups), and the potential method-detection limit issues that may have prevented the finding of glycolic acid and other metabolites in urine. However, the study does offer the suggestion of metabolic saturation of ethylene glycol in several species as evidenced by a trend towards increasing urinary excretion with increasing dose.

Utility (Adequacy) for CERHR Evaluation Process: The toxicokinetics evaluations in rats and rabbits were useful in that a broad range of doses were evaluated, but only one animal was tested at any given dose level. The results suggest an increasing amount of radiolabel in urine and less in expired air with increasing dose that is expected from other studies as well. Analysis of radiolabel in urine suggests that ethylene glycol constituted the majority of urinary excretion in 2 rabbits dosed with 25 mg/kg bw and 45% of urinary excretion in 1 rabbit dosed with 125 mg/kg bw. While trace levels of oxalic acid were detected in all 3 rabbits, other urinary metabolites (e.g., glycolic acid) were not detectable. This may be related to high detection limits for the analytical method for these metabolites; the minimum detection limits were not stated but appear to be based upon melting point determinations following a series of metabolite derivatizations; the sensitivity and percent recovery of these methods was not reported and is uncertain.

Hewlett et al. *(47)* studied excretion patterns in rats and dogs administered ethylene glycol in water by gavage. Twenty-four male Sprague-Dawley rats (3/time point) were dosed with 2,000 mg/kg bw and 1 male and 1 female mixed-breed dog were dosed with 1,000 and 1,036 mg/kg bw, respectively. Ethylene glycol and glycolate levels in blood and urine were measured at various time intervals between 1 and 24 hours by GC or HPLC. Glycolate levels peaked between 4 and 6 hours post-dosing in both species. Renal excretion of ethylene glycol in rats was almost complete in the first 6 hours, while glycolate excretion required another 6 hours. In rats and dogs respectively, about 20 and 30% of the dose was excreted as ethylene glycol and 5 and 4% as glycolate within 24–30 hours. The study authors concluded that toxicokinetics were similar in both species. **[These results and those of a case reporting ethylene glycol poisoning in a human without renal failure *(64)* suggest that renal clearance is one important pathway of ethylene glycol elimination.]**

Strengths/Weakness/Utility (Adequacy) for CERHR Evaluation Process: While Hewlett et al. *(47)* concluded that ethylene glycol kinetics are similar between rats and dogs, Figure 1 of the study shows essentially the same peak ethylene glycol plasma level in both species despite the fact that rats received approximately twice the dose given to dogs. This suggests a significant interspecies difference. The shorter half-life in rats provides at least a partial possible explanation for the similarity in plasma ethylene glycol levels across species (i.e., more rapid elimination in rats would tend to curtail peak blood levels relative to dogs). The use of only a single dose per species and the lack of measurement of exhaled breath or mass balance limit the utility of the rat data for risk assessment. Added to these limitations, the use of only two dogs limits the conclusions that can be made concerning ethylene glycol kinetics in this species. However, an important data set contained within this paper is the evidence of ethylene glycol-induced diuresis in rats immediately after the 2,000 mg/kg bw dose followed by a rapid decline in urine output to below control levels. This suggests impaired renal function by 8–12 hours post-dosing. The implications of this impairment in renal function at this relatively late point in the time course of ethylene glycol disposition is not clear, but it may compound the risks from high-dose ethylene glycol exposure.

Blood glycolate levels in ethylene glycol-treated animals were measured in a limited number of studies and are presented below in Table 2-8. Table 2-8 demonstrates that high bolus doses of ethylene glycol produce relatively high glycolic acid levels in blood (5–13 mM). One study *(44)* suggests that from a dose-response perspective, there is a super-linear increase with dose in rats. Consistent with the blood trend data was a marked increase in urinary glycolic acid excretion at doses higher than 150 mg/kg bw. Levels of blood glycolic acid observed in human poisonings are outlined in Section 2.1.3.1.

Table 2-8. Maximum Levels of Glycolic Acid in Blood Following Gavage Exposure to Ethylene Glycol.

Sex and Species (Exposure Route)	Ethylene Glycol Dose (mg/kg bw)[a]	Blood Glycolic Acid Level (mM)	Reference
Female Sprague-Dawley rats (gavage)	2,500	5.7	Pottenger et al. *(44)*
Pregnant Sprague-Dawley rats (gavage)	150 500 1,000 2,500	0.27 1.7 4.8 5.9	Pottenger et al. *(44)*
Pregnant Sprague-Dawley rats (gavage)	2,500	8.8	Carney et al. *(65)*
Pregnant Sprague-Dawley rats (bolus SC injection)	1,000 2,000	3.3[b] 6.3[b]	Carney et al. *(61)*
Pregnant Sprague-Dawley rats (slow, continuous SC infusion)	1,000 2,000	0.1[b] 1.0[b]	Carney et al. *(61)*
Male Sprague-Dawley rats (gavage)	2,000	13	Hewlett et al. *(47)*
Male and female mixed-breed dogs (gavage)	1,000–1,036	8	Hewlett et al. *(47)*

[a]**[Doses in Pottenger et al. *(44)* study converted from original units using a glycolic acid molecular weight of 76.05 and by assuming that the specific gravity of blood is ≅1. Example calculation for 2,500 mg/kg bw exposure in pregnant rats: 452 μg glycolic acid/g blood x 1 g blood/1 mL x 1,000 mL/1L x 1 mg/1,000 μg x 1 mmol glycolic acid/76.05 g = 5.9 mM.]**

[b]These were not necessarily peak glycolic acid levels.

Two studies provide evidence of a possible second metabolic pathway for ethylene glycol in the rat. Kukielka and Cederbaum *(66)* found evidence that in the rat "… ethylene glycol is oxidized to formaldehyde by an oxidant derived from H_2O_2 and nonheme iron, and that cytochrome P-450 may function to generate the H_2O_2 and to catalyze reduction of the nonheme iron." Based on increased levels of radical adducts in urine and bile of rats treated with ethylene glycol and 4-methyl pyrazole, Kadiiska and Mason *(67)* suggested there may be a second minor pathway for ethylene glycol that may compete with alcohol dehydrogenase.

2.1.3.3 In Vitro Metabolism Studies

One study evaluated ethylene glycol and glycolic acid metabolism in rat and human liver samples *in vitro (68)*. The study utilized liver homogenate 9,000g supernatant (S-9) from Sprague-Dawley rats and from 10 female human donors (age not specified). The S-9 samples were pooled across

all individuals so that only a single result was available for rats or humans. Incubations were run across a range of protein, substrate, and cofactor concentrations in order to optimize conditions for the turnover of ^{13}C-ethylene glycol or ^{13}C-glycolic acid. Metabolites were measured by GC/MS. ^{13}C-ethylene glycol metabolism was very slow under the variety of conditions tried, which prevented the calculation of metabolic rate constants. In contrast, the turnover of ^{13}C-glycolic acid was readily apparent. An optimized incubation system was then used for determining the kinetic constants for ^{13}C-glycolic metabolism. The optimized conditions for ^{13}C-glycolic acid turnover (e.g., involving cytosol rather than S-9 and effects following ethylenediaminetetraacetic acid (EDTA) addition) are consistent with glycolate oxidase, which is present in cytosol and is inhibited by metal ions such as copper. Incubations across a broad range of ^{13}C-glycolic acid concentrations yielded the V_{max} and K_m values shown in Table 2-9.

Table 2-9. Michaelis-Menten Constants for the Metabolism of ^{13}C-Glycolic Acid in Liver Cytosol Samples from Rats and Humans *(68)*.

Species	V_{max} (μmole/hr/mg)	K_m (mM)	V_{max}/K_m
Rat	0.54	0.79	0.68
Human	0.41	0.19	2.16

These data indicate that human cytosol was approximately three times more efficient at metabolizing glycolic acid due to its 4-fold lower K_m (higher affinity). This also indicates that the human enzyme would saturate at a 4-fold lower substrate concentration than would the rat enzyme.

Strengths/Weaknesses: This study by Bartels *(68)* provides important information for cross-species comparisons of the saturation of glycolic acid metabolism. The glycolic acid rat metabolic data appear to be valid and, in fact, correspond fairly well to data on the saturation point for rats *in vivo*. Pottenger et al. *(44)* reported that the maximal blood glycolic acid concentration was 20.6 mg/L at the non-saturating dose of 150 mg/kg bw and was 131 μg/mL at the saturating dose of 500 mg/kg bw. This converts to a glycolic acid concentration of 0.27–1.72 mM for the dose range where saturation of glycolic acid metabolism begins to occur in gavaged rats. The fact that the *in vitro* K_m occurs in this range suggests this experiment provides results useful for *in vivo* extrapolation. The study is limited by the use of a single pooled human sample. This prevents an understanding of the degree of human inter-individual variability possible in glycolic acid metabolism. The study was unable to assess ^{13}C-ethylene glycol turnover. The failure in this regard is puzzling in light of the integrity of the tissue preparations as evidenced by their ready turnover of ^{13}C-glycolic acid. The study authors had no explanation for this.

Utility (Adequacy) for CERHR Evaluation Process: The Michaelis-Menten constants provided in this study *(68)* for glycolic acid metabolism appear to be reliable for the rat based upon comparison with *in vivo* data. The cross-species comparison with humans suggests that humans are approximately three times more efficient in glycolic acid metabolism but that this reaction would saturate at a 4-fold lower concentration in humans. This information is critical for cross-species extrapolation of ethylene glycol metabolism and internal dosimetry of metabolites of developmental concern. The implication is that if saturation of rat glycolic acid metabolism is clearly evident at an ethylene glycol bolus dose of 500 mg/kg bw, then the human saturation point may be 4-fold lower, or 125 mg/kg bw. The *in vitro* data also suggest that at lower, non-saturating doses, humans would be 3-fold more efficient at glycolic acid metabolism than rats. However,

there are a number of caveats that need to be placed on this assessment. First, the ethylene glycol dose that will saturate glycolic acid metabolism depends upon the function of enzymes in addition to glycolate oxidase (ADH, ALDH). Cross-species comparisons of the kinetics of the initial steps in ethylene glycol metabolism are not currently possible. Further, the emphasis placed on the Bartels and Clark results should be limited due to the fact that the data come from a single *in vitro* study and this study used a single pooled human liver sample. Further, there are no *in vivo* data to be brought to bear on the question of what the saturation point might be for ethylene glycol and glycolic acid metabolism in humans. The available human *in vivo* data are from either very low exposure doses well below saturation *(37*) or very high overdose datasets *(56, 57*). Therefore, the issue of the saturation point for glycolic acid metabolism in humans is informed by the Bartels and Clark study, but additional data are needed to confirm this finding and provide cross-species comparisons of the initial steps in ethylene glycol metabolism.

2.1.3.4 Developmental- and Species-Specific Variations in Metabolism and Enzyme Activities

Because metabolites of ethylene glycol are responsible for the systemic toxicity observed with high exposures, varying activities of metabolic enzymes such as alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) may affect toxicity. A number of studies examined ADH or ALDH activity in human placenta, age-related activity of the enzymes, and species-specific differences in humans and rats. Though the focus of most studies was ethanol, the studies are relevant to ethylene glycol since both chemicals are substrates of ADH and ALDH. Therefore, a brief review of the data was conducted by the Panel.

2.1.3.4.1 Placental Metabolic Capacity

Studies in humans and rodents suggest that the placenta has extremely limited capacity to metabolize ethylene glycol and its metabolites. Pares et al. *(69*) isolated Class III ADH from full-term human placenta and found it had low activity for ethanol and a K_m value for octanol that was 100 times higher than the Class I ADH enzyme found in human liver. Zorzano and Herrera *(70*) found that ALDH from full-term human placentas had a lower activity and V_{max} and a higher K_m value than ALDH isoenzymes from liver.

In rats, placenta was found to have no ADH activity and ALDH activity in placenta was found to be 4–7% of liver ALDH activity *(71*). The findings of the study were consistent with several older studies referenced in the introduction and discussion.

2.1.3.4.2 Developmental Aspects of Metabolic Capacity

Activity of ADH and ALDH was found to vary with developmental stage. Pikkarainen and Raiha *(72*) measured *in vitro* ADH activity in the livers of human fetuses, children, and adults (n=1–3/age group) using ethanol as a substrate. The ADH activity in 2-month-old fetal livers was about 3–4% that of adults. In 4–5-month-old fetuses, ADH activity was roughly 10% that of adults, and in infancy, activity was about 20% that of adults. ADH activity increased in children with age and at 5 years of age, reached a level that was within the ranges noted for adults. Great variation was noted in adult ADH activity. Somewhat different results were reported subsequently by Smith et al. *(73*) who examined liver ADH activity using ethanol as a substrate and also examined the ontogeny of individual ADH class I isoforms. They reported total ADH activity in human fetal liver (at 9–22 weeks gestation) that was 30% of adult values; in premature infants and children less than 1 year of age, activity was 50% of adult values. Individual enzyme activity was

determined using starch gel electrophoresis with an *in situ* assay. A total of 222 liver samples were assayed, 56 from fetuses (9–22 weeks gestation), 37 from premature infants and infants less than 1 year of age, and 129 from adults more than 20 years of age. In fetal liver samples with a mean gestational age of 11 weeks, only the ADH1A enzyme was detectable. By 17 weeks, both ADH1A and ADH1B were measurable, although ADH1A predominated. By 19 weeks, products from all three loci were observed, with ADH1A greater than ADH1B, and ADH1B greater than ADH1C. At 30 weeks, ADH1A and ADH1B levels were equivalent, but still greater than ADH1C, but by 36 weeks, ADH1B expression dominated. In the adult, hepatic ADH1A expression was nondetectable, whereas expression from the *ADH1B* and *ADH1C* loci were equivalent. Interestingly, this progressive change in expression was tissue-specific. In the lung, there were no observed differences between the fetal and adult samples and only ADH1C was detectable. ADH expression in the intestine and kidney was low and did not change appreciably with age.

Sjoblom et al. *(71)* found that in Wistar rats ADH activity in the liver was low before birth, being 5 and 16 % of adult activity on gd 15 and 20, respectively. There was a rapid increase at birth with 53% of adult levels on pnd 1, and a continued gradual increase with age to 82% of adult activity on pnd 47. Similar developmental patterns were noted for ALDH in rat liver.

[It appears that human liver ADH is expressed much earlier in development and may well contribute to ethylene glycol metabolic disposition. However, given the paucity of knowledge regarding isoform specificity towards ethylene glycol, it is uncertain how these data on ethanol metabolism might be extrapolated. Assuming that the enzyme most active in ethanol metabolism, ADH1B, also is most active in ethylene glycol metabolism, then significant fetal metabolism is not predicted until later in gestational development (20–36 weeks).]

Studies also have been done on the developmental expression of CYP2E1. In the liver, this enzyme is detectable as early as the second trimester, but at levels at 10–30% of adults *(74)*. Hepatic levels increase rapidly after the neonatal period, reaching adult levels by 6 months to 1 year of age *(75)*.

The ontogeny of glycolate oxidase in humans has not been studied. In rodents, there is some evidence that hepatic glycolate oxidase activity in juvenile (1-month-old) Wistar rats is 3–4-fold lower than in more mature rats *(76)*. The lack of human data for this enzyme during development is an important uncertainty due to its role in clearing glycolic acid.

2.1.3.4.3 Hepatic Metabolic Capacity in Humans Versus Rats

Zorzano and Herrera *(77, 78)* found different ADH isoenzymes in liver homogenates from humans (class I ADH) and rats (ADH-3), which differed greatly in kinetic properties. Using ethanol as a substrate at a pH of 10.5, activity, K_m, and V_{max} in humans was measured at 6.24 Units/g tissue, 2.10 mM, and 7.70 Units/g tissue, respectively, while activity, K_m, and V_{max} in rats was measured at 2.72 Units/g tissue, 1.02 mM, and 2.96 Units/g tissue, respectively. Two different low K_m ALDH isoenzymes were found in humans and rats but they had similar activities using acetaldehyde as the substrate at pH 8.8 (humans: K_m=9 μM and V_{max}=0.85 Units/g tissue; rats: K_m=10 μM and V_{max}=0.87 Units/g tissue).

2.1.3.4.4 Inter-Individual Variability Due to Genetic Polymorphisms and Hereditary Metabolic Disorders

Reviews by Agarwal *(79*), Bosron and Li *(80*), Pietruszko *(81*), and Burnell et al. *(82*) discussed genetic polymorphisms for ADH and ALDH in humans. Class I ADH, the primary ADH in human liver, is a dimer composed of randomly associated polypeptide units encoded by 3 loci (ADH1A, ADH1B, and ADH1C). Polymorphisms resulting in altered phenotypes are observed at the ADH1B (ADH1B*2 and ADH1B*3) and ADH1C (ADH1C*2) loci. The ADH1B*2 allele is estimated to occur in 15% of Caucasians of European descent, 85% of Asians, but less than 5% of African Americans. Fifteen percent of African Americans have the ADH1B*3 allele, while this variant is essentially absent in other ethnic groups. Both the ADH1B*2 and ADH1B*3 enzymes have V_{max} values for ethanol that are 100-fold higher than those exhibited by ADH1B*1. The ADH1B*2 and ADH1B*3 differ in their affinities for ethanol, which are approximately 20- and 70-fold lower than ADH1B*1, respectively

There are two primary ALDH isoenzymes in human liver, ALDH2 (also referred to as E_2, ALDHI, or $ALDH_2$) and ALDH1 (also referred to as E_1, ALDHII, or $ALDH_1$) *(79-81*). About 50% of Japanese and Chinese carry a phenotypically null variant of the ALDH2 enzyme.

Although less well defined, considerable variability in human CYP2E1 expression, resulting from both environmental and genetic influences, has also been observed *(83*).

There are several disease conditions which could, in theory, affect glycolic acid clearance. Disorders of intermediary metabolism involving disruption of mitochondrial oxidative phosphorylation can produce extreme lactic acidosis. This has been associated with elevated glycolic acid in one case study *(84*) with the suggestion that the elevation in glycolic acid is due to lactate competing with glycolic acid for glycolate oxidase. However, no other reports of elevated glycolic acid have been found in such disorders and the ability of high lactate levels to retard glycolic acid oxidation has not been studied. This leads to an uncertainty regarding the ability to clear glycolic acid in individuals who have very high lactate levels, a condition which can occur due to several different metabolic defects. Another metabolic disorder, primary hyperoxaluria, is associated with elevated glycolic acid in serum. This appears to occur due to a defect in glyoxylate elimination (conversion to glycine), which leads to excess glyoxylate available both for oxidation to oxalic acid and for back conversion (via LDH) to glycolic acid *(85-87*). The prevalence of this disorder is not well known, but following ethylene glycol exposure, such individuals could plausibly receive higher internal glycolic acid doses due to this impaired removal of glyoxalate.

[The Expert Panel concluded that given the roles for ADH, ALDH, glycolate oxidase, and potential CYP2E1 in the disposition of ethylene glycol, the issues of species differences, developmental differences, and genetic variability are of significant relevance to conducting risk assessment and/or to the development of PBPK models.]

2.1.4 Elimination

2.1.4.1 Humans

The half-life for elimination of ethylene glycol in humans is reported as 2.5–8.4 hours, and after 24–48 hours, little-to-no ethylene glycol can be detected in urine or tissue *(6*). Evaluation of human ethylene glycol elimination data are typically complicated by treatment with ethanol to prevent further metabolism of ethylene glycol to glycolic acid *(56, 57, 64*). This prevents a direct

comparison of ethylene glycol half-life in rats and humans. A recent study demonstrated that at much lower inhalation doses of ethylene glycol, the plasma half-lives of both parent compound and glycolic acid were 2–3 hours in two male subjects *(37)*.

Cheng et al. *(64)* measured renal clearance in a 25-year-old man who drank about 280 mL of antifreeze containing 95% ethylene glycol and was treated with ethanol and hemodialysis. It was found that the normally functioning kidney greatly contributes to the excretion of ethylene glycol. Mean renal clearance was measured at 27.5 mL/minute, fractional excretion was about 20%, and the half-life for renal clearance of ethylene glycol was estimated at 18 hours. The findings were in contrast to renal clearance rates of 1–4 mL/minute reported for patients with acute renal failure. In another case study, post-ethanol treatment serum toxicokinetics suggested a half-life of approximately 15 hours *(57)*, a value that is consistent with the 18-hour half-life for renal elimination reported by Cheng et al. *(64)*. In human cases before dialysis was employed, glycolic acid clearance was generally very slow *(56)*. These slow clearances may be due to a combination of high blood glycolate levels which saturated metabolism and impaired renal function. In one case involving overdose in a 2-year-old girl, glycolate levels were high, but the decline from serum (without dialysis) suggests a half-life of approximately 8 hours *(57)*.

2.1.4.2 Animals

Ethylene glycol is rapidly cleared from blood with elimination half-lives reported at 0.3–1.1 hours in mice *(43)*, 1–3 hours in rats *(41, 43, 44)*, and about 2.7–3.7 hours in monkeys *(46)* and dogs *(47)*. The half-lives of both ethylene glycol and glycolic acid are dose-independent *(41-44)*, such that even high glycolic acid concentrations in blood that were likely in excess of the K_m for glycolic acid metabolisms were rapidly cleared. Rapid clearance under saturating conditions with limited metabolic removal suggests that renal elimination can effectively remove both parent compound and metabolite. This, however, may not be the case in subjects with renal impairment, which could result from calcium oxalate formation; this could compound the reproductive/developmental toxicity from ethylene glycol. As discussed in greater detail in Section 2.1.3.2, ethylene glycol is primarily eliminated in urine as parent compound or glycolic acid and in exhaled air as CO_2; the percentage of each elimination product is highly dependent on dose rate and route of exposure *(41-44)*.

2.2 General Toxicity

2.2.1 Human Data

2.2.1.1 Acute Exposure

Acute ethylene glycol toxicity in humans is well characterized and numerous case studies have addressed the topic. Such case studies contribute little to the understanding of developmental and reproductive effects. Therefore this section is derived primarily from reviews conducted by LaKind et al. *(88)*, ATSDR *(6)*, NTP *(34)*, and Carney *(35)*. Numerous human deaths resulting from intentional or accidental ingestion of ethylene glycol have been documented. The lethal oral dose for humans has been estimated at 1,400–1,600 mg/kg bw. However, the estimation of acute lethal doses in humans is uncertain because the exact quantity ingested cannot be quantified. Toxicity associated with acute oral exposure to ethylene glycol is characterized by at least three distinct stages that can overlap. Death can occur during any of the stages. Stage I occurs within 30 minutes to 12 hours following intake and primary symptoms include central nervous system (CNS) depression and gastrointestinal upset. Individuals in Stage I appear to be drunk and

depending on the dose, CNS symptoms can include ataxia, slurred speech, somnolence, and convulsions. Metabolic acidosis is said to occur during Stage I or Stage II. Stage II occurs at 12–72 hours following ingestion and is characterized by cardiopulmonary toxicity. Observed at this stage is severe metabolic acidosis characterized by reductions in blood pH and bicarbonate levels. Severe serum hyperosmolality and increased anionic gap can also occur. Cardiopulmonary symptoms during this stage may include tachypnea, hypernea, tachycardia, cyanosis, pulmonary edema, or cardiac failure. Metabolic acidosis is thought to be the cause of these symptoms. Another possible cause of symptoms is hypocalcemia that can occur as oxalate binds with calcium. Stage III, that occurs at 24–72 hours following ingestion, is characterized by renal toxicity. Calcium oxalate crystal deposition within kidneys is thought to be the major contributing factor to renal failure. Additional symptoms that can occur during Stage III include flank pain and polyuria later followed by oliguria. Histological examination of kidneys reveals both tubular necrosis and oxalate crystals. Neurological symptoms that uncommonly occur 6 or more days after ethylene glycol ingestion suggest that there may be a fourth stage of toxicity involving cranial nerves. The symptoms include deafness, facial paralysis, and other neurologic sequelae. Autopsy material from a person in this newly discovered fourth stage revealed dense refractile crystal deposition along portions of the seventh and eighth cranial nerves *(89*).

Data on acute ethylene glycol toxicity resulting from inhalation or dermal exposure are very limited.

In a controlled study, nasal and throat irritation were noted following short-term inhalation exposure to ethylene glycol at concentrations exceeding 140 mg/m^3. A concentration of 188 mg/m^3 could only be tolerated for 15 minutes. Ethylene glycol levels above 200 mg/m^3 were intolerable *(33*).

ATSDR *(6*) stated that acute dermal exposure to ethylene glycol is most likely to occur through products such as antifreeze but is not likely to lead to toxic effects. LaKind et al. *(88*) reported that ethylene glycol appears to be a mild skin irritant but not a skin sensitizer.

2.2.1.2 Repeated Exposures

The data on repeat exposure to ethylene glycol in humans are limited and not as extensively covered in the literature as acute toxicity data. Therefore, original studies were reviewed to evaluate human toxicity in controlled *(33*) and occupational studies *(11, 30*). This section was not broken down according to exposure routes since exposures likely occurred through multiple routes.

In a controlled study by Wills et al. *(33*), 19 male prisoners were continuously exposed to aerosolized ethylene glycol for 20–22 hours/day for 30 days. The study was conducted in a prison ward hospital that was converted to an exposure chamber. Ethylene glycol mists were generated using three air conditioning units. Air concentrations were measured at five locations within the chamber by analyzing ethylene glycol levels in air collected from evacuated polyethylene bottles. Mean daily concentrations of ethylene glycol were 3–67 mg/m^3 and mean weekly concentrations were 17–49 mg/m^3. The diameter of mist droplets was 1–5 microns. The control group consisted of 14 male prisoners. Ten of those men were never exposed to ethylene glycol, while four were just exposed to ethylene glycol for 7 days in a pilot study. Neurobehavioral effects were measured and electrocardiographs and electroencephalographs were conducted prior to exposure and following 2 and 4 weeks of exposure. Blood samples were collected before exposure and approximately every 2–3 days during exposure. Urine samples were collected daily to check for

oxalate crystals, and twice weekly for urinalysis. **[Statistical analysis was not described.]** Exposure resulted in individual ethylene glycol levels of 0.094–0.18 mg/mL in serum and 0.021–0.077 mg/mL in urine. Ethylene glycol levels were similar in unexposed controls (0.09–0.21 mg/mL in serum and 0.017–0.077 mg/mL in urine). Exposure resulted in no significant changes in urinalysis, hematological, or blood chemistry (including urea nitrogen, creatinine, and plasma pH) parameters, or in neurobehavioral, heart, or brain function. Subjects did occasionally complain of headaches and lower back pain. **[The Expert Panel noted several limitations regarding analytical methods used for this study. Those limitations are discussed in Section 2.1.1.1.2.]**

Gerin et al. *(11)* conducted a study to measure ethylene glycol exposure and kidney function in 33 male Canadian aviation workers (21–52-years-old) exposed to ethylene glycol-based deicing fluid. Details about the exposure portion of the study are included in Section 1.2.4.2. The study was conducted in Quebec from January to March of 1992. Personal exposures to ethylene glycol vapors and mists were measured at <2.5–22 mg/m^3 and <17–190 mg/m^3, respectively. Post-shift levels of ethylene glycol in urine ranged from <5–129 mmol/mol creatinine. Diethylene glycol was sometimes detected in air or urine samples at levels that were about one-tenth the ethylene glycol concentrations. Some of the workers wore paper masks that offered some protection against mists but not vapors. Possible confounding factors considered included demographics, work activities, health problems, analgesic intake, smoking habits, alcohol intake, and non-occupational exposures to solvents and ethylene glycol. T-tests were conducted to analyze time-related (temporal) data, such as urine levels before and after the shift. Subgroup exposure (dose-response) data were analyzed by analysis of variance (ANOVA), and Fisher's exact test. Kidney function was assessed by measuring pre- and post-shift urinary levels of β-N-acetyl-glucosaminidase, albumin, β-2-microglobulin, and retinol-binding protein. There were some significant associations between kidney function parameters and ethylene glycol exposures. However, no consistent effects were observed and most values were within normal limits. The authors concluded that there was no evidence of acute or chronic renal toxicity related to ethylene glycol exposure in this study. They also noted that the statistical power of the study may have been limited due to small sample size and wide variations in the renal function parameters examined.

Laitinen et al. *(30)* examined exposure to ethylene and propylene glycol and possible indicators of biochemical renal effects in Finnish motor servicing workers. Details about the exposure part of the study are discussed in Section 1.2.4.2. Ten male mechanics from five different garages participated in the study. The only skin protection used by some workers was leather gloves. Ten age-matched office workers served as controls. **[Ages of subjects and possible confounding factors were not discussed.]** Urine samples were collected after the work shift and analyses results were compared to controls. Differences between groups were evaluated by Student's t-test. As discussed in Section 1.2.4.2, urinary ethylene glycol levels were significantly higher in mechanics (7.3 versus 1.7 mmol/mol creatinine, respectively). Urinary oxalic acid levels were slightly higher in mechanics (47 versus 36 mmol/mol creatinine in controls), but differences between controls did not reach statistical significance. Of the biochemical parameters examined in urine, glycosaminoglycans levels were significantly lower in controls. Urinary calcium concentration and succinate dehydrogenase activity were marginally reduced in mechanics, but the effects were not statistically significant. Urinary levels of ammonia were higher in exposed workers. **[The effect was said to be significant in the text but not in the table.]** According to the study authors, increased ammonia excretion is typically observed with chronic acidosis. **[As discussed in Section 1.2.4.2, the Expert Panel noted several limitations regarding air**

measurements, sample size, and reporting of analytical details. Due to the limitations, the Panel concluded that this study should be considered preliminary.]

LaKind et al. *(88)* noted a case study that reported nystagmus and decreased blood cell counts in 38 women exposed to unknown concentrations of ethylene glycol vapors from a heated ethylene glycol-containing mixture. LaKind et al. *(88)* noted that exposures were not known and other occupational conditions were not considered.

2.2.2 Experimental Animal Data

2.2.2.1 Oral Exposure

The majority of acute toxicity studies provide no information about ethylene glycol effects on reproductive organs. For that reason, much of the information in this section was obtained from reviews by LaKind et al. *(88)*, ATSDR *(6)*, and Carney *(35)*. Based on reported minimum lethal doses (MLD), it has long been assumed that most laboratory animals are less sensitive than humans to acute ethylene glycol exposure. For example, the minimum lethal oral dose in rats, 3,800 mg/kg bw, is much higher than the MLD in humans, 1,400–1,600 mg/kg bw. However, the assumption of increased human sensitivity has recently been questioned due to an inability to determine accurately doses received by humans in accidental or intentional poisoning exposures *(90)*. Table 2-10 outlines LC_{50}s and MLDs reported in various animal species.

Table 2-10. Comparison of Oral Minimum Lethal Doses (MLD) and LD_{50}s in Animals.

Species	Oral MLD (mg/kg bw/day)	Oral LD_{50} (mg/kg bw)
Rat	3,800[b]	4,000–8,790[a]
Mouse	Unknown	8,400–15,400[a]
Guinea Pig	Unknown	6,610–8,200[a]
Rabbit	Unknown	5,000[b]
Dog	6,700–7,400[c]	>8,000[b]

[a]LaKind et al. *(88)*
[b]Carney *(35)*
[C]NTP *(34)*

Toxic effects induced by ethylene glycol exposure in animals are similar to those observed in humans. Like humans, toxicity in animals proceeds in three stages that include: CNS effects; metabolic acidosis and cardiopulmonary effects; and renal toxicity. Repeated exposures in animals also result in the same types of symptoms. Table 2-11 lists some common symptoms of ethylene glycol toxicity in humans and the animal species for which each symptom has also been observed under either acute or repeated exposure conditions.

Table 2-11. Symptoms of Acute Ethylene Glycol Toxicity Noted in Various Animal Species.

Symptom	Species With Symptom
Central Nervous System Toxicity	Rat, Mouse, guinea pig, Rabbit, Dog
Metabolic Acidosis	Dog, Primate, Rat
Pulmonary Toxicity	Dog, Mouse, Rat, guinea pig
Cardiac Toxicity	Dog
Calcium Oxalate Crystals Formation	Cat, Dog, Rat, Monkey, Rabbit, Pig
Renal Lesions	Dog, Rat, Mouse, guinea pig
Renal Failure	Dog, Cat, Rabbit

Numerous repeat-dose studies have examined toxicity of ethylene glycol in various species. The studies consistently demonstrate the kidney as the cardinal target organ. The liver has also been noted as a target organ in some studies *(34)*. The Expert Panel did not conduct an exhaustive review of repeat-dose toxicity studies since the majority of the studies did not include a histological examination of reproductive organs. However, an NTP *(34)* report included some interesting observations about species and sex-specific aspects of ethylene glycol exposure that warrant discussion.

Male rats were found to be more susceptible than female rats to ethylene glycol-induced toxicity. Subchronic *(91-93)* and chronic *(94, 95)* dietary studies in male and female rats have consistently demonstrated a higher mortality rate, greater severity of kidney lesions and oxalate crystal deposition, and higher levels of blood urea nitrogen (BUN) and creatinine in males. For example, in subchronic studies, kidney lesions were noted in males fed a diet with 25,000 ppm and females fed a diet with 50,000 ppm ethylene glycol. Health Canada *(19)* and ACGIH *(96)* noted studies that demonstrated reduced kidney stone formation and urinary oxalic acid in castrated versus intact male rats administered 0.5% ethylene glycol in drinking water for 28 days; administration of testosterone to castrated rats resulted in a reversal of effects.

Sensitivity was found to vary widely among different species. Two chronic studies conducted in $B6C3F_1$ or CD-1 mice found no or less severe lesions in mice compared to male rats *(34, 95)*. All of these studies are reviewed in detail below. Variability in species toxicity was also noted in developmental toxicity studies conducted in mice, rats, and rabbits. Tyl et al. *(97)* noted that more than 40% of rabbits died following gavage treatment with 2,000 mg/kg bw/day, while no rats or mice died following gavage dosing with 5,000 and 3,000 mg/kg bw/day, respectively *(98)*. In a developmental toxicity screening study, there was a 10% death rate in mice gavage treated with 11,090 mg/kg/day of ethylene glycol *(99)*. These data indicate that rabbits respond somewhat differently to ethylene glycol than do rats and mice. A paucity of information on the physiologic and metabolic response to ethylene glycol in rabbits allows only speculation about potential mechanisms. Renal oxalate deposition is certainly a striking finding in rabbits treated with high levels of ethylene glycol and is a plausible mechanism of toxicity. However, a cause and effect relationship for oxalate deposition and toxicity has yet to be established *(56)*. Also, it is not known if rabbits develop severe metabolic acidosis as do most other species *(47)*.

CERHR reviewed repeat dose oral exposure studies conducted in rodents by Melnick *(91)*, NTP *(34)*, and Gaunt et al. *(92)* and in monkeys by Blood et al. *(100)* since they included histological examinations of reproductive organs. A subchronic rat study conducted by Robinson et al. *(93)* was also reviewed since it is one of the key studies examining kidney effects. Some chronic studies conducted to evaluate carcinogenicity *(34, 95)* are reviewed in Section 2.4.2. These rodent

subchronic and chronic studies represent the key systemic toxicity studies that were presented in reviews by ATSDR *(6)* and Health Canada *(19)*. A report by Mertens *(60)* was also reviewed since it was released subsequent to the reviews.

Melnick *(91)* and NTP *(34)* reported the results of a 13-week study in male and female B6C3F$_1$ mice (age 63 days) exposed to ethylene glycol (>99% pure) through their diet. The purpose of the study, conducted according to GLP was to determine doses to be used in a 2-year study described under Section 2.4.2. Ten animals/sex/group were exposed to 0, 3,200, 6,300, 12,500, 25,000, or 50,000 ppm ethylene glycol in food. **[CERHR estimated doses based on actual mean body weights of animals at the end of the experiment (~31 g for males and ~25 g for females, as reported in Table D1 of NTP study) and food intake rate (~8 g/day, as noted in Table F1 in the NTP report) measured during weeks 1–13 of the 2-year NTP *(34)* study. The CERHR dose estimates were 830, 1,630, 3,230, 6,450, and 12,900 mg/kg bw/day in males and 1,020, 2,020, 4,000, 8,000, and 16,000 mg/kg bw/day in females from the low- to high-dose groups, respectively.]** Doses were selected based on effects reported in the literature. Observed endpoints included survival, body and organ weight, clinical signs, necropsy, hematology, blood chemistry, urinalysis, and histopathology. Incidence data were evaluated by logistic regression, the Fisher exact test, the Cochran-Armitage trend test, pairwise comparisons, and determining overall dose response trends. Continuous data were analyzed by the Williams', Dunnett's, or Jonkheere's test. There were no differences in terminal body weight, organ weights, or clinical findings. No lesions in reproductive tissues (ovary, uterus, prostate, testis preserved in 10% formalin) were reported following examination of primarily control and high-dose animals. Mild treatment-related lesions were observed in the liver (centrilobular hepatocellular hyaline degeneration) and kidneys (nephropathy) of male mice in the 25,000 ppm and 50,000 ppm groups.

Subchronic toxicity of ethylene glycol in Fischer 344/N rats was reported by Melnick *(91)*. Groups of 9–10 7-week-old male and female rats/sex/group were fed diets containing 0, 0.32, 0.63, 1.25, 2.5, or 5.0% ethylene glycol (>99% purity) for 13 weeks. The study authors estimated that the 1.25% concentration was equivalent to 600–1,000 mg/kg bw/day in males and the 2.5% concentration was equivalent to 1,000–1,500 mg/kg bw/day in females. **[Based on the 2 doses estimated by study authors, CERHR estimated that intakes were approximately 150–250, 300–500, 600–1,000, 1,200–2,000, and 2,400–4,000 mg/kg bw/day in males and 125–188, 250–375, 500–750, 1,000–1,500, and 2,000–3,000 mg/kg bw/day in females from the low- to high-dose groups, respectively].** It was determined that ethylene glycol was stable in feed for 2 weeks. Dose selections were based on effects reported in the literature. Parameters evaluated included body and organ weights, blood chemistry, urinalysis, and histopathology. **[There was no discussion of statistical analyses.]** Four males in the 5% group died and body weight gain was significantly reduced in males from the 2.5 and 5% groups. Significant organ weight effects included increased relative kidney weights in males and females of the 2.5 and 5.0% groups and decreased relative thymus weight in males of the 5.0% group. Organs were fixed in 10% formalin and a histopathological evaluation was conducted in control and high-dose animals, and organs from lower dose groups if gross lesions were observed or if effects were noted in organs of the high-dose group. Histopathological observations in kidneys from the 2.5 and 5% group males included toxic nephrosis and deposition of crystals that appeared to be calcium oxalate. Calcium oxalate-like crystals were also detected in the urinary bladder, urethra, and brain of males from the 5% groups. Less severe, multifocal tubular lesions were seen in kidneys of the 5% group females, but there were no oxalate crystals present. No lesions were reported in testes, prostate, ovaries, or uterus. The only treatment-related blood chemistry effects were significantly increased BUN and creatinine levels in males of the 2.5 and 5.0% groups. Treatment had no effect on urinalysis parameters. The authors concluded that ethylene glycol appears to produce no renal toxicity at doses of 1.25% (600–1,000 mg/kg bw/day) in males and 2.5% (1,000–1,500 mg/kg

bw/day) in females.

Gaunt et al. *(92)* also examined subchronic toxicity of ethylene glycol exposure in rats. Twenty-five male and female weanling Wistar rats were fed diets containing 0, 0.05, 0.1, 0.25, or 1.0% ethylene glycol (98.5% purity) for 2, 6, or 16 weeks. Doses were equivalent to 0, 35, 71, 180, and 715 mg/kg bw/day in males and 0, 38, 85, 185, and 1,128 mg/kg bw/day in females. The aim of dose selection was to obtain no effects at the lower dose levels and renal toxicity at the higher dose levels. **[There was no verification of ethylene glycol levels in food.]** During treatment, rats were monitored for clinical signs, food and water intake, weight gain, and renal function. Five rats/sex/group were sacrificed at 2 and 6 weeks and 15 rats/sex/group were sacrificed at 16 weeks. At sacrifice, hematological, serum chemistry, and urinalysis parameters were examined. The kidney, uterus, ovaries, testes, prostate, and seminal vesicles were among the organs that were fixed in 10% neutral buffered formalin, embedded in paraffin wax, and examined histologically in all exposure groups. Methods of statistical analyses, which included Student's t-test and Chi-Square test, were not discussed in detail but were referenced. This summary of results focuses on the group treated for 16 weeks; similar effects were seen in the groups treated for 2 or 6 weeks. There were no clinical signs of toxicity or adverse effects on body weight gain, food intake, serum chemistry, or hematology. Significantly increased water intake in females was not dose-related and authors noted that significance likely resulted from low water intake by controls. Tables in the study list the incidence of testicular atrophy and cystic uterus and ovary and there appear to be no changes related to ethylene glycol treatment. **[The table did not report severity of effects.]** The kidney was the only organ with histological changes attributed to ethylene glycol treatment by the authors. Incidence of males with crystals and lesions in the kidney was significantly elevated in the 0.25 and 1.0% dose groups. An increased number of females in the 1.0% group had kidney lesions but statistical significance was not obtained. Excretion of oxalic acid was significantly increased in both sexes in the 1.0% group; in treated males, oxalic acid levels were 100–500% of control levels while the 1.0% females had oxalic acid values that were 30–100% of control values. Also noted in males of the 1.0% group were significant increases in absolute kidney weight, oxalic acid crystals in urine, and secretion of a larger volume of urine with a lower specific gravity. A "no-untoward-effect level" of 0.1% (71 and 85 mg/kg bw/day in males and females, respectively) was identified by study authors.

Robinson et al. *(93)* dosed 10 male and female Sprague-Dawley rats/sex/group (85 days old at start of study) with ethylene glycol (100% purity) in drinking water for 10 or 90 days. Only the 90-day portion of this study is described here. Doses in drinking water were 0, 0.25, 0.50, 1.0, or 2.0% in males (205, 407, 947, or 3,134 mg/kg bw/day) and 0.50, 1.0, 2.0, or 4.0% in females (597, 1,145, 3,087, or 5,744 mg/kg bw/day). Dose selection was based upon results of the 10-day study and concentrations in drinking water were confirmed. All animals were necropsied at the end of exposure and parameters evaluated included hematology and clinical chemistry. A histopathological examination was conducted in kidneys of all animals. For other organs, histopathology was examined in five control animals per sex and all surviving animals in the high-dose group. **[Although a histopathological examination was conducted in male and female reproductive organs from the control and high-dose group, the results were not reported.]** Statistical analyses included Tukey's multiple comparison procedure, Kruskal-Wallis Rank Sum Test, one-factor ANOVA, Fisher's Exact Test, and Pearson's Correlation Coefficient and Correlation Analysis. Death occurred in 8/10 females in the 4.0% group and 2/10 males in the 2.0% group. Body weight gain was significantly reduced in males of the 2.0% group. The only hematological effect was a reduction in leukocyte numbers in females of the 0.5, 2.0, and 4.0% dose groups. The only significant clinical chemistry findings that appeared to be dose-related included increased creatinine levels in males dosed with ≥1% and increased BUN and phosphorus in males of the 2% group. No significant organ weight effects occurred in females. Significant

absolute organ weight changes that occurred in males included increased kidney weight at ≥1%, increased brain and gonads weight at 2%, and decreased heart, liver, and lung weight at 2%. **[It does not appear that organ to body weight ratios were statistically analyzed.]** Histopathology was only reported for kidneys. Significant increases in incidence and severity of kidney lesions and birefringent crystal deposition occurred in the males exposed to ≥1% and females exposed to ≥2%. Lesions in males occurred with greater frequency and severity compared to females.

Mertens *(60)* conducted a GLP study to compare sensitivity in F344 and Wistar male rats exposed to ethylene glycol. Six-week-old CDF(F-344)/CrlBR and CRL: WI(Glx/BRL/Han)IGS BR male rats (10/strain/group) were randomly assigned to groups receiving ethylene glycol (99.99% pure) at doses of 0, 50, 150, 500, and 1,000 mg/kg bw/day through diet for 16 weeks. **[Rationale for dose selection was not discussed by study authors but CERHR notes that doses were within ranges previously examined in Wistar rats by Gaunt et al.** *(92)***.]** Diets were analyzed to verify homogeneity and stability of ethylene glycol. Animals were observed daily with detailed physical examinations conducted weekly. Body weights and food intake were recorded weekly. During the 24 hours prior to sacrifice, water intake was recorded and urine was collected for urinalysis. Following sacrifice, all animals were necropsied. Kidneys were weighed and prepared for histological evaluation and immunostaining for alpha 2-μ-globulin detection. Statistical analyses consisted of one-way ANOVA followed by Dunnett's test if the ANOVA revealed statistical significance ($p<0.05$). Death occurred in 2/10 Wistar rats in the 1,000 mg/kg bw/day group; clinical signs in those rats included emaciation and/or dermal atonia. One of the animals also had a dark red discoloration in the right eye and small seminal vesicles. Body weight gain was significantly lower in Wistar rats of the 500 and 1,000 mg/kg bw/day group; food intake was reduced in those groups of Wistar rats and reached statistical significance at the 1,000 mg/kg bw/day level. Water intake was significantly increased in Wistar rats exposed to ≥500 mg/kg bw/day and F344 rats exposed to 1,000 mg/kg bw/day. Urinalysis revealed significantly lower specific gravity, increased urine volume, and increased occurrence of white blood cells in Wistar rats exposed to ≥500 mg/kg bw/day and F344 rats exposed to 1,000 mg/kg bw/day. Treatment-related increases in the incidence of calcium oxalate crystals were observed in Wistar and F344 rats exposed to ≥150 mg/kg bw/day. Incidence of calcium oxalate crystals was 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 exposed to 0, 50, 150, 500, or 1,000 mg/kg bw/day, respectively. Because oxalic acid was not deposited in the kidney but excreted as crystals in urine in the 150 mg/kg bw/day groups, the study authors considered the effect to be a detoxification process and not an adverse effect. Absolute and relative (to body weight) kidney weights were significantly higher in Wistar rats of the 500 mg/kg bw/day group and Wistar and F344 rats of the 1,000 mg/kg bw/day group. Nephropathy associated with crystalluria was noted in Wistar and F344 rats of the 500 and 1,000 mg/kg bw/day groups, with greater severity in the Wistar rats. There were no treatment-related increases in alpha 2-μ-globulin in either strain of rat. The study authors identified a NOEL **[NOAEL[1]]** of 150 mg/kg bw/day for both strains of rats, but noted that Wistar rats are twice as sensitive to ethylene glycol induced nephrotoxicity as are F344 rats.

A satellite study *(59)* conducted in conjunction with the Mertens *(60)* study examined levels of ethylene glycol, glycolic acid, and oxalic acid in blood, urine, and kidneys of F344 and Wistar rats exposed to 0, 150, 500, and 1,000 mg/kg bw/day ethylene glycol in diet for 1 or 16 weeks. Five rats/strain/dose were examined in each time period. Tables 2-12 and 2-13 outline the results of this study. The dosimetry of ethylene glycol and its metabolites, especially oxalic acid, differed

[1] Since the Expert Panel is considering only adverse effects in the selection of effect levels, the terminology of NOAEL will be used throughout this document.

between Wistar and F344 rats, leading authors to opine that dosimetry differences are a factor in the varying sensitivity between the two strains. Urinary clearance of ethylene glycol and metabolites was reduced in Wistar rats of the 500 and 1,000 mg/kg bw/day group at 16 weeks and study authors attributed the change in kinetics to increased renal toxicity. Wistar rats in the 500 and 1,000 mg/kg bw/day groups had higher levels of glycolic acid and especially oxalic acid in kidneys than F344 rats. Study authors concluded that differing sensitivities in renal toxicity between the two rat strains was clearly associated with oxalic acid. In both strains of rats in the 500 and 1,000 mg/kg bw/day groups, renal levels of oxalic acid greatly exceeded blood levels.

Table 2-12. Ethylene Glycol (EG) and Metabolite Levels in F344 Rats *(59)*.

SAMPLE	**Dose (mg/kg bw/day)**			
Weeks Exposure: Analyte (μg/g±SD)[a]	**0**	**150**	**500**	**1000**
BLOOD				
1 week:				
EG	<0.2	5.90±1.51	22.00±5.19	57.36±12.54
GA	<0.6	<0.6	2.73±0.40	39.98±10.46
OA	0.85±0.29	0.19±0.19	0.35±0.15	0.09±0.11
16 weeks:				
EG	<0.2	8.51±2.42	30.53±8.51	132.9±22.8
GA	0.64±0.23	1.63±0.29	5.55±1.86	118.8±7.2
OA	7.21±0.05	6.18±2.48	5.50±1.67	10.20±1.96
KIDNEY				
1 week:				
EG	2.09±1.98	5.85±2.17	16.55±3.98	44.55±12.36
GA	2.01±0.25	2.57±0.42	4.00±0.60	46.42±11.02
OA	8.15±1.79	2.81±1.72	1.70±0.73	30.84±13.03
16 weeks:				
EG	<0.8	9.81±3.05	34.63±9.66	159.0±19.5
GA	1.10±0.49	2.41±0.60	5.96±1.91	140.4±15.3
OA	<0.6	13.56±6.72	32.92±12.03	20,616±19,857
URINE				
1 week:				
EG	<2.4	5,129±601	14,508±847	32,033±1604
GA	85.37±9.97	357±50	1,210±197.5	8,554±2,622
OA	250.9±64.7	272.4±109.1	1,161±586	764±487
16 weeks:				
EG	14.56±7.78	10,340±1,626	35,427±8,872	59,877±3642
GA	<0.5	186±74	870±296	5,621±1,590
OA	301±183	361±153	1,364±533	1,205±931

[a]Urine values expressed in units of μg
EG = ethylene glycol
GA = glycolic acid
OA = oxalic acid

Table 2-13. Ethylene Glycol (EG) and Metabolite Levels in Wistar Rats *(59)*.

SAMPLE Weeks Exposure: Analyte (µg/g±SD) [a]	Dose (mg/kg bw/day) 0	150	500	1000
BLOOD				
1 week:				
EG	<0.2	11.76±2.43	30.89±16.11	78.76±39.77
GA	<0.6	1.71±1.05	6.69±5.89	48.76±31.89
OA	<0.1	0.86±1.17	1.13±0.40	1.38±0.68
16 weeks:				
EG	<0.2	1.65±1.27	15.02±8.48	45.12±40.26
GA	1.40±1.27	0.57±0.33	12.67±15.42	84.27±58.45
OA	6.49±1.79	3.07±0.17	5.15±1.42	17.96±4.38
KIDNEY				
1 week:				
EG	<0.8	3.65±1.01	20.11±14.04	72.31±36.73
GA	0.89±0.29	1.37±0.28	7.39±5.77	53.31±36.37
OA	2.86±1.39	1.77±0.45	15.43±5.82	1,972±3615
16 weeks:				
EG	2.63±1.88	6.34±2.87	18.41±12.04	58.08±51.23
GA	0.57±0.46	1.25±0.43	35.36±29.34	232.5±29.6
OA	5.38±3.76	32.60±32.43	33,108±46,787	100,812±31,899
URINE				
1 week:				
EG	<2.4	5,553±895	18,247±5985	24,810±14,749
GA	97.80±58.08	474.8±101.7	2,530±1,199	9,710±5,516
OA	131.8±63.6	631.4±251.9	2,344±1,844	5,614±2,897
16 weeks:				
EG	93.4±50.1	8,233±1,193	8,285±7,090	4,854±3,164
GA	<0.5	226.2±147.2	341.3±349.4	2,850±971
OA	<20.6	577.8±427.1	63.69±34.72	84.30±63.51

[a]Urine values expressed in units of µg
EG = ethylene glycol
GA = glycolic acid
OA = oxalic acid

Blood et al. *(100)* fed two male Rhesus monkeys diets containing 0.2% ethylene glycol **[purity not specified]** and one female Rhesus monkey a diet containing 0.5% ethylene glycol for 3 years. **[The ages of the monkeys were not specified and there were no control animals.]** No abnormal calcium deposits were observed in x-rays that were taken every 3 months during the study. Following sacrifice, a histopathological evaluation **[methods not specified]** was conducted in heart, esophagus, stomach, intestine, liver pancreas, urogenital system (kidneys, ureters, bladder, testes, ovaries, and uterus), spleen, lymph nodes, thyroids, parathyroids, adrenal, pituitary, and bone marrow. The only histopathological effect observed in the kidney of one male was a few scattered glomeruli that were sclerotic and had thickened Bowman's capsules,

eosinophilic material in tubules, and mononuclear cells in the interstitium. The authors concluded that no toxic effects were observed in the monkeys.

Histopathological findings in reproductive organs were not reported for a monkey study conducted by Roberts and Seibold *(101)*. Renal toxicity was reported for males exposed to >15 mL/kg bw (17 mg/kg bw) ethylene glycol in drinking water for 6–157 days. The study is limited because only one monkey was exposed for most time durations and some monkeys were exposed to multiple dose concentrations.

2.2.2.2 Inhalation Exposure

Inhalation data are very limited. Because of the scarcity of inhalation data, the Expert Panel evaluated one inhalation study, even though reproductive organs were not examined. Coon et al. *(102)* exposed male and female Sprague-Dawley and Long-Evans rats (n=15/dose), male and female Princeton-derived guinea pigs (n=15/dose), male New Zealand rabbits (n=3/dose), male beagle dogs (n=2/dose), and male squirrel monkeys (n=2/dose) 8 hours a day, 5 days a week for 6 weeks to reagent-grade ethylene glycol at concentrations of 0, 10, or 57 mg/m^3. Continuous 90-day exposures to 12 mg/m^3 were also conducted. Concentrations within exposure chambers were monitored. Serum biochemistry and hematology were examined before and after exposure. Organs that were evaluated histologically in at least half the animals included heart, lung, liver, kidney, and spleen. Authors reported normal hematology and blood chemistry values. Mild histopathological changes in liver were sometimes noted in rats, guinea pigs, and monkeys but authors did not consider the effects treatment related. Eye effects noted with continuous exposure to 12 mg/m^3 included moderate to severe irritation in rabbits and corneal opacity and possible blindness in 2 rats. Deaths were reported for 1 rabbit, 3 guinea pigs, and 1 rat exposed continuously to 12 mg/m^3, but the animals did not display any other types of toxicity. **[The Expert Panel noted that this study is limited by insufficient reporting of protocol details and no discussion of statistical analyses.]**

2.2.2.3 Dermal Exposure

In a prenatal toxicity study, no renal lesions, clinical signs of toxicity, or changes in organ or body weight were observed in CD-1 mice treated dermally with up to 3,549 mg/kg bw/day ethylene glycol for 6 hours/day on gd 6–15 *(103)*. Complete details of this study are included in Section 3.2.3.

2.3 Genetic Toxicity

Because the NTP *(34)* and ATSDR *(6)* already conducted thorough reviews of genetic toxicity information, the Expert Panel summarized the main findings of the reviews in Tables 2-14 and 2-15. The majority of findings were negative, but NTP noted positive results in a chromosomal aberration and dominant lethal mutation assay in rats. NTP questioned the validity of the two positive studies due to limitations such as lack of chemical purity information, no reporting of primary data, and unacceptably low control values. ATSDR *(6)* stated that “because of the information available in *in vitro* culture and animals, it is reasonable to conclude that exposure to ethylene glycol poses minimal risk of causing genotoxic effects (GTE) in exposed persons."

Table 2-14 *In Vivo* Genotoxicity Results.

Species or Assay Type	Dose	Endpoint	Result	Referenced In
F344 rat	400–1,000 mg/kg bw	Dominant Lethal Mutation	Negative	ATSDR *(6)* NTP *(34)* DePass et al. *(104)*
Rat	120 or 1,200 mg/kg bw	Dominant Lethal Mutation	Positive	NTP *(34)*
Rat	1,200 mg/kg bw	Chromosomal Aberration	Positive	NTP *(34)*
Bracon hebetor (parasitic wasp)	Not specified	Dominant Lethal Mutation	Negative	NTP *(34)*

Table 2-15. *In Vitro* Genotoxicity Results.

Species (strain)	Concentration	Endpoint	Result Without Activation	Result With Activation	Reference
Salmonella typhimurium (Total of five studies)	≤10,000 μg/plate in one study	Mutation	Negative	Negative	ATSDR *(6)* NTP *(34)*
Escherichia coli	NS	DNA Damage	Negative	Negative	ATSDR *(6)* NTP *(34)*
Schizosaccharomyces pombe	NS	Mutation	*	*	ATSDR *(6)* NTP *(34)*
Neurospora crassa	NS	Aneuploidy	*	*	ATSDR *(6)* NTP *(34)*
Mouse lymphoma L5178Y Cells	≤5,000 μg/mL	TFT resistance	Negative	Negative	NTP *(34)*
Chinese Hamster Ovary Cells	≤5,000 μg/mL	Chromosomal Aberration And SCE	Negative	Negative	NTP *(34)*
Human Embryonic Fibroblasts	NS	Chromosomal Aberration	Negative	No Data	NTP *(34)*

*Negative results were obtained but it was not specified if it was in the presence or absence of metabolic activation.
NS=not specified
SCE=Sister chromatid exchange
TFT=Trifluorothymidine

2.4 Carcinogenicity

2.4.1 Human Data

In a study comparing 26 workers with renal cancer to 190 controls in a Texas Chemical plant, no association was found between renal cancer and ethylene glycol exposure *(105)*. ATSDR *(6)* noted that the sample size was small.

2.4.2 Experimental Animal Data

The following two carcinogenicity studies were evaluated in detail since the studies included a histological evaluation of reproductive organs.

The NTP *(34)* conducted a GLP, 2-year carcinogenicity assay in $B6C3F_1$ mice (age 55–63 days). Sixty animals/sex/group were exposed to ethylene glycol through diet at doses of 0, 6,250, 12,500, and 25,000 ppm for males and 0, 12,500, 25,000, and 50,000 ppm for females. Based on measured dietary intakes, the authors estimated these exposure groups to correspond to approximate average doses of 1,500, 3,000, and 6,000 mg/kg bw/day in males and 3,000, 6,000, and 12,000 mg/kg bw/day in females. Doses for this study were based on results of the NTP 13-week study described under Section 2.2.2.1. The parameters observed included survival, body and organ weights, clinical signs, necropsy, hematology, blood chemistry, and histopathology (organs preserved in 10% formalin). Statistical analyses are discussed under the summary for the 13-week study in Section 2.2.2.1. At a 15-month interim sacrifice, 6–10 animals/sex were examined in each group. The number of surviving animals was 23–37/group/sex by the end of the study; there was no difference in survival between treated and control groups. For most organs including reproductive organs, histopathology was examined only in control and high-dose groups, in animals that died before study completion, and in tissues with masses or lesions. No significant ($p<0.05$) increases in incidence of neoplastic or nonneoplastic lesions were seen in reproductive organs of treated males (testes, seminal vesicle, prostate, preputial gland, penis, epididymis, ductus deferens, coagulating gland) or females (ovary, oviduct, uterus). The primary effects noted in the 2-year study were: significant hepatocyte hyaline degeneration in the liver of males at the 2 highest doses (3,000 and 6,000 mg/kg bw/day) and in females at the highest dose (12,000 mg/kg bw/day); and medial hyperplasia of the pulmonary arterioles in females at all doses (3,000, 6,000, and 12,000 mg/kg bw/day). Significant treatment-related nephropathic effects were not observed. A few oxalate-like crystals were seen in renal tubules, urethrae, and/or urinary bladders of high-dose males (6,000 mg/kg bw/day). The authors concluded that the study provided no evidence of carcinogenicity in mice.

DePass et al. *(95)* treated groups of 80 male and female Crl: CD-1 mice/sex/group and 130 Fischer 344 rats/sex/group for 2 years with ethylene glycol (99.9% pure) in diet at concentrations resulting in doses of 0, 40, 200 or 1,000 mg/kg bw/day. The doses were based on findings of preliminary studies that demonstrated mild renal toxicity in male rats treated for 40 days with a dose similar to the highest dose in this study. The stability of ethylene glycol in diet was verified. Mice were 42 days old and rats were 38 days old at study initiation. In addition to terminal sacrifice, interim sacrifices were as follows: 20 mice/sex/group at 80 weeks, 10 rats/sex/group at 6 and 12 months and 20 rats/sex/group at 18 months. Observed endpoints in rats included survival, body and organ weight, clinical signs, necropsy, hematology, blood chemistry, and urinalysis. Organs were preserved in 10% formalin and histopathology was conducted in control and high-dose animals, all tissue with gross lesions, and target tissues. Reproductive organs examined histologically included ovaries, uterus, epididymides, testes, and prostate. Histopathology was the only parameter examined in mice. Statistical analyses included life-table

techniques for incidence data, and Duncan's and Bartlett's tests, t-test, and ANOVA for continuous data. All male rats in the 1,000 mg/kg bw/day group died or were euthanized before 18 months of treatment. Significant effects in male rats of the high-dose group (1,000 mg/kg bw/day) at 12 months into the study included decreased body weight gain, increased water intake, increased BUN and creatinine, decreased erythrocytes count, reduced hematocrit and hemoglobin levels, increased neutrophil numbers, increased urine volume, and decreased urine specific gravity and pH. The authors did not consider changes in any of these parameters to be treatment-related in female rats. Increased kidney weights and urinary calcium oxalate crystals were present in male and female rats of the high-dose group. In addition, uric acid crystals in urine were also observed in 1,000 mg/kg bw/day females following 18–24 months of treatment. Significant histopathologic lesions in the 1,000 mg/kg bw/day males included tubular dilation, peritubular nephritis, parathyroid hyperplasia, and generalized soft tissue mineralization. Females in the 200 and 1,000 mg/kg bw/day groups experienced fatty changes in liver that reached statistical significance at 1,000 mg/kg bw/day. No treatment-related effects on rat reproductive tissues were reported. There was no evidence of carcinogenicity in rats. No significant increases in treatment-related non-neoplastic lesions were seen in mice. **[Because tables listing histopathological effects in mice were not available, it is not possible to verify a lack of lesions in reproductive organs.]** No evidence for carcinogenicity in mice was found, other than a significant increase in time-adjusted lymphosarcoma in high-dose female mice; the majority of statistical analyses conducted determined that the effect was not statistically significant. The authors concluded that the study provided no evidence of oncogenic effects in rodents.

Health Canada *(19*) considered the DePass et al. *(95*) study to be inadequate for a dose-response evaluation due to inconsistent histological assessment of the onset and progression of non-cancer lesions, complete mortality of male rats in the highest dose group, a dosing regimen that resulted in a 100% lesion incidence at the highest dose group (1,000 mg/kg bw/day), and minimal effects at the next lower dose (200 mg/kg bw/day). The ACC *(90*) disagreed with the Health Canada evaluation and stated that the DePass et al. study is acceptable for a dose-response assessment. According to the ACC, the two pathologist who reviewed the histology slides at different sacrifice intervals stated that they consulted with each other and used consistent criteria and terminology to assess kidney histology. Furthermore, the ACC noted that the dosing regimen is standard for toxicology studies and the large spacing between doses results in a conservative risk assessment.

ATSDR *(6*) concluded that "Studies in both humans and animals indicate that there is little carcinogenic risk after ethylene glycol exposure, although the data are scanty."

2.5 Potentially Sensitive Subpopulations

There are no known subpopulations with increased susceptibility to toxicity associated with ethylene glycol exposures. Sensitivity to any chemical exposure could be increased by factors such as compromised organ function, genetic variation, developmental stage, and dietary deficiencies resulting in reduced detoxification and excretion capability *(6*).

ATSDR *(6*) also points out that the sweet taste of ethylene glycol, common improper storage and disposal, and the undeveloped reasoning skills of children result in a potentially hazardous situation for small children. In 2001, 847 cases of ethylene glycol poisoning were reported in children younger than 6 years of age *(25*).

2.6 Summary

2.6.1 Toxicokinetics and Metabolism

2.6.1.1 General Toxicokinetics and Metabolism

Humans can be exposed to ethylene glycol through the oral, dermal, and inhalation routes. Oral intake of ethylene glycol results in rapid and near complete absorption in numerous species including humans, mice, rats, dogs, rabbits, and monkeys *(6, 35, 41-43, 46, 47*). In contrast to oral absorption, dermal absorption was found to be slow in rats and mice *(41-43*). There are no controlled studies of *in vivo* dermal absorption in humans but one *in vitro* study suggested that absorption of ethylene glycol is slower in human compared to mouse skin *(40*). The study provided only qualitative information due to questionable discrepancies in permeability constants between the control ethanol compound and ethylene glycol in humans and mice. For instance, the permeability constant for ethanol was approximately equal in human and mouse skin while the permeability constant for ethylene glycol was 30–40 times lower in humans than mice. However, the human and animal data set suggests that dermal exposure to ethylene glycol is unlikely to result in human poisonings unless there is an extreme exposure scenario or the skin barrier function is seriously compromised. One study conducted in two male volunteers suggests that ethylene glycol is absorbed through inhalation *(37*). A study in rats suggests that up to 60–90% of inhaled ethylene glycol vapors or mists may be absorbed from the respiratory tract *(48*). From the limited data available in rats *(48*) and humans *(37*) and on the theoretical grounds that high water solubility and low volatility lead to a high blood:air partition coefficient, it appears that ethylene glycol aerosol or vapor uptake from the respiratory tract is high, perhaps as high as 90%. However, irritating properties of ethylene glycol may limit the amount available for absorption from the respiratory tract due to its warning properties and reflexive decreases in respiratory rate.

Once absorbed through any exposure route, ethylene glycol is readily distributed according to total body water in humans, rats, mice, monkeys, and dogs *(6, 35*).

Metabolism of ethylene glycol is qualitatively similar in humans, monkeys, dogs, rabbits, rats, and mice *(6, 34-36*). Figure 2-1 outlines the metabolism of ethylene glycol. In a rate limiting reaction, ethylene glycol is converted to glycolaldehyde by alcohol dehydrogenase and possibly also CYP2E1. Glycolaldehyde is quickly metabolized to glycolate and, to a minor extent, glyoxal by cytosolic aldehyde oxidase and aldehyde dehydrogenase (ALDH). Glycolate is a major metabolite that tends to accumulate following high-dose ethylene glycol exposure in humans and animals. Human poisoning case studies demonstrated that blood levels of glycolate can exceed levels of ethylene glycol *(56, 57*). Glycolate is converted to glyoxylate by glycolate oxidase or lactate dehydrogenase in the second rate limiting step. Glyoxylate is metabolized to formate, oxalate, glycine, and CO_2 through various pathways. Glycolic acid and oxalic acid are also metabolic products of proteins and carbohydrates and are thus found in plasma and urine of unexposed, healthy individuals. However, background plasma levels of glycolic acid (≤0.03 mM) *(52-55*) are well below plasma levels measured in poisoning cases (>10 mM) *(6*).

Although the general aspects of ethylene glycol metabolism are well characterized, the Panel noted that there remain some questions regarding the more detailed aspects of metabolism. Class I ADH, the primary ADH in human liver, is a dimer composed of randomly associated polypeptide units encoded by 3 loci (ADH1A, ADH1B, and ADH1C). It is not known which of the three forms of human class I ADH is most important in metabolism. In addition, the methods used to study metabolism do not rule out a possible role for CYP2E1. Pyrazole and 4-methylpyrazole, which were used to inhibit ADH activity in metabolic studies, also inhibit

CYP2E1. Studies have suggested a possible role of CYP2E1in the oxidation of ethylene glycol *(49, 50*).

The major ethylene glycol elimination products in humans and animals include carbon dioxide in exhaled air and ethylene glycol and glycolate in urine. The half-life for ethylene glycol elimination in humans is 2.5–8.4 hours with little remaining in tissues and urine after 24–48 hours *(6*). The half-life in humans as assessed in poisoning cases is complicated by antidotal treatment with ethanol to prevent additional metabolism; this prevents a direct comparison of half-lives in humans versus animals. Further, the half-life in human poisoning cases is likely affected by renal impairment that appears to decrease the renal clearance of both ethylene glycol and glycolic acid. However, the recent data of Carstens et al. *(37*) demonstrated that at much lower inhalation ethylene glycol doses, the plasma half-lives of both parent compound and glycolate were 2–3 hours in two human subjects. These half-lives are consistent with half-lives found in rats of 1–3 hours *(41-44*) over a wide dose range and of 2.7–3.7 hours in monkeys *(46*). However, the ethylene glycol half-life in mice would appear to be shorter (≤1 hour) *(41-43*), while no plasma glycolic acid data are available in mice. Half-lives for both ethylene glycol and glycolic acid were independent of dose in rats, but this does not reflect the underlying saturation of metabolic clearance because the terminal elimination half-life is measured at times when blood levels have declined below the saturation point *(44*). In addition, renal clearance compensates somewhat for the metabolic overload so that its effect on overall half-life is not readily seen *(41-44*).

2.6.1.2 Exposure Route and Dose Rate Effects on Metabolic Saturation

A series of studies examined metabolism of ethylene glycol following oral gavage exposure in rats and mice *(41-43*). The studies demonstrated that at low doses, ^{14}C-ethylene glycol is primarily excreted as $^{14}CO_2$ in expired air, and to a lower extent, as ^{14}C in urine. As doses increase, urinary elimination exceeds excretion in breath. Patterns of parent compound versus metabolites in urine also change according to dose with percent urinary glycolate increasing according to dose. The changes in excretion pattern suggest saturation of metabolism that occur at doses between 10 and 100 mg/kg bw/day in mice, 10 and 400 mg/kg bw in female rats, and doses exceeding 1,000 mg/kg bw in male rats. Pottenger et al. *(44*) also observed indications of saturated metabolism in female rats, as noted by elevations in total urinary elimination (excretion in breath not measured) and percent urinary glycolic acid excretion with increasing dose. The shift in metabolism was seen at doses between 150–500 mg/kg bw. The Panel noted that half-lives of ethylene glycol and glycolic acid reported by Pottenger et al. *(44*) were independent of concentration and thus incompatible with a zero order process. However the Panel stated that the ß-elimination rate calculations may have been insensitive to identifying saturating conditions due to possible rapid elimination by other pathways (e.g., renal elimination) and several key glycolic acid data points that were at or near the detection limit. Together the data from Frantz et al. *(41-43*) and Pottenger et al. *(44*) suggest that saturation of ethylene glycol metabolism and potential glycolate accumulation in blood occur between doses of 150–500 mg/kg bw in rats.

Frantz et al. *(41-43*) also studied excretion patterns in mice and rats exposed dermally to neat or 50% aqueous ^{14}C-ethylene glycol. Dermal exposure to 10 or 1,000 mg/kg bw/day resulted in excretion occurring primarily through $^{14}CO_2$ in breath and secondarily through ^{14}C in urine. Dermal exposure to 1,000 versus 10 mg/kg bw did not appear to increase ethylene glycol or glycolate levels in urine and the majority of ^{14}C was found as unmetabolized ethylene glycol. The finding suggests that in rats, removal of glycolic acid is efficient following dermal exposure with

up to 1,000 mg/kg bw/day for 6 hours and that saturation of metabolic enzymes does not occur under those conditions.

Excretion occurring primarily through $^{14}CO_2$ exhalation and secondarily through urinary ^{14}C excretion suggested lack of metabolic saturation in rats inhaling ethylene glycol at 184 mg/m^3 (2.4–4.0 mg/kg bw) as aerosol for 17 minutes or 32 mg/m^3 (0.74–1.25 mg/kg bw) as vapors for 30 minutes *(48)*. Exposure concentrations were based on aerosol levels found to be irritating in humans *(33)* and the observation that 20% of total ethylene glycol is present as vapor during the generation of an aerosol. The study demonstrates that exposure to an irritating concentration of ethylene glycol will likely result in a systemic dose that is well below oral doses resulting in metabolic saturation and glycolate accumulation. Therefore, the warning properties of ethylene glycol should prevent exposures resulting in metabolic saturation.

Carney et al. *(61)* measured ethylene glycol and glycolate levels in the blood of rats exposed to 1,000–2,000 mg/kg bw/day ethylene glycol by bolus SC injection or slow, continuous SC infusion. Blood ethylene glycol and glycolate levels were lower following SC infusion versus bolus dosing, but infusion did lead to a 10-fold increase in glycolate plasma levels at 2,000 mg/kg bw/day. It therefore appears that saturation occurred somewhere between 1,000 and 2,000 mg/kg bw/day with continuous dosing. In contrast, Pottenger et al. *(44)* reported saturation between 150–500 mg/kg bw/day following bolus gavage dosing. Therefore, it appears that continuous dosing is 3–10-fold less efficient at saturating glycolate metabolism than bolus dosing.

There is limited information from a single *in vitro* study *(68)* to suggest that human metabolism of glycolic acid is more efficient than rat. Based upon a comparison of the K_ms reported in this study, it also appears that the human enzyme would saturate at a 4-fold lower concentration than the rat enzyme. The kinetic constants in that study seem to pertain to glycolate oxidase based upon the incubation conditions used. There is concordance between the rat K_m measured *in vitro* and the blood glycolic acid concentration seen *in vivo* where saturation begins to occur. The weight placed on this study is limited by the fact that it is from a single pooled liver sample. Additionally, cross-species comparisons regarding the initial steps in ethylene glycol metabolism (ADH, ALDH) are not possible due to a lack of data. The data do not provide any information on inter-individual variability in glycolic acid metabolism. The *in vitro* data are useful to provide a screening level estimate for cross-species differences in the saturation of glycolic acid metabolism and for exploratory models of ethylene glycol/glycolic acid human toxicokinetics.

2.6.1.3 Toxicokinetic and Metabolic Issues Related to Pregnancy

The rat study conducted by Pottenger et al. *(44)* examined two issues related to pregnancy: possible changes in toxicokinetics and metabolism of ethylene glycol related to pregnancy and possible evidence of metabolic saturation at doses that produce teratogenicity in rodents (see Section 3.2.1.1.2). The study compared toxicokinetic parameters and excretion patterns in pregnant (gd 10–11) versus non-pregnant rats and found no significant differences between the two groups. As noted by the Panel, gd 10–11 represents a sensitive time period for ethylene glycol-induced developmental toxicity, but is limited to a narrow window of gestation. It is not known if the physiological changes that occur in later stages of pregnancy would have resulted in significantly different toxicokinetic results. In addition, extrapolation of the findings to humans is unclear due to differences in ontological developmental of ADH enzymes between rats and humans and uncertainty regarding specific human enzymes that metabolize ethylene glycol. Such factors lend uncertainty to the extrapolation of rat maternal toxicokinetic data to estimate dosimetry in the human fetus.

As discussed above, the Panel noted evidence of saturated glycolic acid metabolism at ethylene glycol gavage doses between 150 and 500 mg/kg bw/day in the Pottenger et al. *(44)* study. When dosing occurred by continuous infusion rather than bolus dosing, a higher daily dose was required to exhibit saturation (1,000–2,000 mg/kg bw/day). Developmental toxicity studies conducted by bolus dosing in rats (see Section 3.2.1.1.2) identify a NOAEL of 500 mg/kg bw/day and LOAEL of 1,000 mg/kg bw/day, suggesting that developmental toxicity occurs under conditions of saturated glycolic acid removal.

Metabolic capacity of ADH and ALDH enzymes in placenta was studied in humans and rats using ethanol as a substrate *(69-71)*. The studies found that isoenzymes in placenta of rats and humans had very low activity, thus suggesting that the placenta has limited capacity to metabolize ethylene glycol and its metabolites.

2.6.1.4 Development of ADH, ALDH, CYP2E1 and Glycolate Oxidase

ADH activity in livers of fetuses, children, and adults has been compared using ethanol as a substrate. One study examined ADH activity in livers from 56 fetuses, 37 premature infants and infants less than 1 year, and 129 adults, and reported that ADH activity was 30% of adult activity in 9–22-week-old fetuses and 50% of adult activity in infants younger than 1 year of age, including premature infants *(73)*. Qualitatively similar results were obtained in a second study using 1–3 liver samples/age group; the study reported that ADH reached adult activity levels at 5 years of age *(72)*. Similar patterns of development were seen for ADH and ALDH enzymes in rat studies *(71)*. Ontogeny of ADH class I isoforms in humans was found to change according to developmental stage *(73)*. In fetuses with a mean gestational age of 11 weeks, only the ADH1A enzyme was detected from liver samples. As gestational age advanced, expression of the ADH1B and ADH1C enzymes began to increase with the two isoforms first detected at 17 and 19 weeks, respectively. In adult liver samples, ADH1B and ADH1C expression were equal and expression of ADH1A was not detectable.

The Expert Panel noted that extrapolation of the ethanol metabolism data is uncertain due to the paucity of knowledge regarding isoform specificity towards ethylene glycol. If it is assumed that the enzyme most active in ethanol metabolism, ADH1B, is also most active in ethylene glycol metabolism, then it would be predicted that significant fetal metabolism would not occur until 20–36 weeks of gestation.

Glycolate oxidase activity may also be lower in early life as suggested by data in juvenile rats which indicated 3–4 times lower activity than in more mature rats *(76)*. The Panel is not aware of any data on the ontogeny of glycolate oxidase in humans. Given the importance of this enzyme in eliminating glycolate oxidase, this is an important data gap.

2.6.1.5 Inter-individual Variability due to Genetic Polymorphisms and Metabolic Disorders

Polymorphisms resulting in altered phenotypes are observed in the ADH1B (ADH1B*2 and ADH1B*3) and ADH1C (ADH1C*2) loci *(79-82)*. The ADH1B*2 allele occurs in an estimated 15% of Caucasians, 85% of Asians, and less than 5% of African Americans. An estimated 15% of African Americans have the ADH1B*3 allele, which is virtually absent in other ethnic groups. Ethanol V_{max} values for the ADH1B*2 and ADH1B*3 enzymes are 100-fold higher than the value reported for the ADH1B*1 enzyme. Ethanol affinities for ADH1B*2 and ADH1B*3 are about 20- and 70-fold lower than the affinity for ADH1B*1, respectively.

While ADH is generally recognized as being responsible for the initial oxidation of ethylene glycol, there is evidence that a microsomal enzyme, possibly CYP2E1, also contributes to this metabolism *(49)*. Data on the relative importance of ADH versus the microsomal oxidative pathway are lacking. However, if CYP2E1 does make a substantial contribution to ethylene glycol metabolism, factors such as *in utero* and postnatal development of enzyme function, as well as genetic polymorphisms, can lead to inter-individual variability in the internal dose of ethylene glycol and metabolites.

ALDH2 (also called E_2, ALDHI, or $ALDH_2$) and ALDH1 (also called E_1, ALDHII, or $ALDH_1$) are the primary ALDH isoenzymes in human liver *(79-81)*. About 50% of Asians carry a phenotypically null variant of the ALDH2 enzyme.

Another source of inter-individual variability in ethylene glycol toxicokinetics is the potential for glycolate oxidase function to vary across individuals. This is the key enzyme in the removal of glycolic acid formed from ethylene glycol oxidation. Sources of variability in the regulation and function of this enzyme have not been extensively studied. However, it appears that high lactate levels that occur in those having disorders of intermediary metabolism may compete with glycolic acid for glycolate oxidase *(84)*. Further, elevated glycolic acid levels are possible in those who have disorders in glyoxylate metabolism, such as those that occur in primary hyperoxaluria *(85-87)*. Individuals with these conditions might be at greater risk from ethylene glycol due to the potential for greater accumulation of glycolic acid. However, it is unknown how prevalent these disorders are or whether other disorders may also promote glycolic acid accumulation under basal or ethylene glycol exposure conditions.

2.6.2 General Toxicity

2.6.2.1 Human Data

Acute effects associated with ingestion of ethylene glycol are well characterized *(6, 34, 35, 88)*. CNS depression and gastrointestinal upset are common symptoms occurring between 30 minutes and 12 hours following ingestion. Metabolic acidosis can occur following that time period and is characterized by reduced blood pH and bicarbonate levels; serum hyperosmolality and increased anionic gap can also occur. Metabolic acidosis is possibly the cause of cardiopulmonary and renal toxicity that are often observed with ethylene glycol poisonings. Renal toxicity is characterized by oxalate crystal deposition and tubular necrosis. Symptoms associated with renal toxicity include polyuria followed by oliguria and flank pain. Calcium oxalate deposition is thought to be a major factor in renal failure. The human lethal oral dose has been estimated at 1,400–1,600 mg/kg bw but there is considerable uncertainty associated with that estimate since human intake is difficult to quantify. In survivors of ethylene glycol poisoning, neurological symptoms possibly involving cranial nerves are infrequently seen 6 or more days following exposure.

Acute exposure to 140 mg/m^3 ethylene glycol caused nasal and throat irritation while levels above 200 mg/m^3 were intolerable *(33)*. It has been noted that dermal exposure to ethylene glycol is not likely to result in toxicity *(6)*. Ethylene glycol appears to be a mild skin irritant but not sensitizer *(88)*.

There is a limited amount of data on repeated human exposures to ethylene glycol. In a controlled study where 19 men were continuously exposed to 3–67 mg/m^3 ethylene glycol for 20–22 hours/day for 30 days, ethylene glycol levels in blood and urine were similar to 10 unexposed controls *(33)*; there were no effects on heart, brain, or neurobehavioral function, urinalysis, hematological, or blood chemistry parameters (including urea nitrogen, creatinine, and plasma pH). A study of 33 male aviation workers exposed to <2.5–22 mg/m^3 and <17–190 mg/m^3 ethylene glycol vapors and mists, respectively, over a 3-month period found no evidence of ethylene glycol-induced acute or chronic renal toxicity (measured by urinary β-N-acetyl-glucosaminidase, albumin, β-2-microglobulin, and retinol-binding protein) *(11)*; however, the study authors noted that the study may have had limited statistical power due to small sample size and wide variations in renal function parameters. Compared to unexposed controls, ten male mechanics had higher levels of ethylene glycol in urine and a significant reduction in urinary glycosaminoglycans level and increase in urinary ammonia *(30)*. Slight but not significant increases in urinary oxalic acid levels and decreases in urinary calcium concentration and succinate dehydrogenase activity were also noted for the exposed mechanics. The study in mechanics was somewhat limited by insufficient reporting of details, as described in Section 1.2.4.2.

2.6.2.2 Experimental Animal Studies

Toxic effects observed in acute or repeat-dose studies in animals are similar to those observed in humans and include CNS effects followed by metabolic acidosis, cardiopulmonary effects, and then renal toxicity *(6, 35, 88)*. Such symptoms have been observed in standard laboratory species such as rats, mice, guinea pigs, and rabbits. Other species such as dogs, cats, and non-human primates also exhibit similar manifestations of toxicity. Table 2-10 outlines MLDs and LD_{50}s observed in various animal species. In general, there are consistent toxic effects in the species examined, including humans.

Systemic effects observed in repeat-dose chronic and subchronic rat and mouse studies are outlined in Tables 2-16 and 2-17, respectively. In repeat-dose rodent subchronic and chronic toxicity studies, the kidney was consistently shown to be a target of ethylene glycol toxicity. Exposure to high concentrations of ethylene glycol was shown to produce kidney lesions, oxalate crystal deposition, and other indications of renal toxicity such as increased BUN and creatinine levels. Renal toxicity was found to vary according to sex and species. In dietary or drinking water exposure studies conducted for 13–16 weeks, renal crystal deposition and/or lesions were noted in male rats treated with ≥180–2,000 mg/kg bw/day ethylene glycol *(60, 91-93)*; in those same studies, kidney effects in female rats were less severe and occurred at higher dose levels (1,128–3,087 mg/kg bw/day). In a 2-year dietary study in rats, exposure to 1,000 mg/kg bw/day ethylene glycol resulted in renal oxalate crystal deposition in males and females with males also experiencing increases in BUN, severe lesions, and death *(95)*.

Mice were found to be less sensitive to ethylene glycol-induced renal toxicity. In a 13-week and 2-year dietary study, mild renal nephropathy or a few oxalate-like crystals were observed in males at doses of ≥6,000 mg/kg bw/day ethylene glycol *(34, 91)*. In those same studies, no renal

crystals or lesions were noted in female mice at doses ≥12,000 mg/kg bw/day. Renal toxicity was reported in monkeys given ethylene glycol in drinking water but dose limitations preclude the identification of an effect concentration *(101*).

Oxalate crystal deposition and a 40% death rate was noted in pregnant rabbits gavage dosed with 2,000 mg/kg bw/day ethylene glycol on gd 6–19 *(97*), while no overt toxicity was noted in rats or mice gavage dosed with 5,000 and 3,000 mg/kg bw/day, respectively, during gestation *(98*). Therefore, it appears that rabbits are affected differently by ethylene glycol from rats or mice.

As noted in Tables 2-16 and 2-17, liver lesions were also noted in the rodent studies but not as consistently as kidney effects.

2.6.3 Genetic Toxicity

Negative results were reported for the majority of *in vivo* and *in vitro* genetic toxicity tests that examined dominant lethal mutations, chromosomal aberrations, mutations, aneuploidy, and DNA damage *(6, 34*). Positive results were obtained in an *in vivo* chromosomal aberration assay and dominant lethal mutation assay in rats, but the NTP questioned the validity of the studies based on study limitations *(34*). ATSDR stated that, "Because of the information available in *in vitro* culture and animals, it is reasonable to conclude that exposure to ethylene glycol poses minimal risk of causing genotoxic effects in exposed persons."

2.6.4 Carcinogenicity

A limited human study found no association between renal cancer and ethylene glycol exposure in 26 workers and 190 controls from a Texas chemical plant *(6, 105*).

Two chronic cancer studies in mice and 1 in rats found no evidence of carcinogenicity following dietary exposure to up to 6,000 mg/kg bw/day in male mice, 12,000 mg/kg bw/day in female mice, and 1,000 mg/kg bw/day in male and female rats for 2 years *(34, 95*).

Table 2-16. Summary of Key Subchronic and Chronic Toxicity Studies in the Rat.

Dose (mg/kg bw/day	Exposure Regimen	Species/ Strain	Dose (mg/kg bw/day): Effect	Reference
M: 150–250, 300–500, 600–1,000, 1,200–2,000, 2,400–4,000[a] **f:** 125–188, 250–375, 500–750, 1,000–1,500, 2,000–3,000[a]	13-week dietary exposure	F344/N rat	**m – 1,200–2,000:** ↓bw gain, ↑relative kidney weight, renal toxic nephrosis and crystal deposition , ↑BUN and creatinine **m – 2,400–4,000:** Death in 4 males, ↓bw gain , ↑relative kidney weight, ↓relative thymus weight, renal toxic nephrosis and crystal deposition, crystals in bladder, urethra, and brain, ↑BUN and creatinine **f – 1,000–1,500:** ↑Relative kidney weight **f – 2,000–3,000:** ↑Relative kidney weight, renal lesions but no crystal deposition No histopathology in reproductive organs	Melnick et al. *(91)* NTP *(34)*
M: 35, 71, 180, 715 **f:** 38, 85, 185, 1,128	16-week dietary exposure	Wistar rat	**m – 180:** ↑Renal lesions and crystal deposition **m – 715:** ↑Renal lesions and crystal deposition, ↑oxalate excretion and crystals in urine, ↑kidney weight, ↑urine volume, ↓ urine specific gravity **f – 1,128:** Non-significant ↑renal lesions, ↑oxalate excretion No treatment-related reproductive organ histopathology, bw gain, or hematology	Gaunt et al. *(92)*
M: 50, 150, 500, and 1,000	16-week dietary exposure	Wistar and F344 Rat	**Wistar – 500:** ↓bw gain and food intake, ↑water intake, ↓urine specific gravity, ↑urine volume, ↑white blood cells and calcium oxalate in urine, ↑absolute and relative kidney weight, ↑nephropathy associated with crystalluria **Wistar – 1,000:** Death in 2/10, ↓bw gain and food intake, ↑water intake, ↓urine specific gravity, ↑urine volume, ↑white blood cells and calcium oxalate in urine, ↑absolute and relative kidney weight, ↑nephropathy associated with crystalluria **F344 – 500:** ↑Calcium oxalate in urine, ↑nephropathy associated with crystalluria **F344 – 1,000:** ↑Water intake, ↑calcium oxalate in urine, ↓urine specific gravity, ↑urine volume, ↑white blood cells and calcium oxalate in urine, ↑absolute and relative kidney weight, ↑nephropathy associated with crystalluria	Mertens *(60)*

Dose (mg/kg bw/day	Exposure Regimen	Species/ Strain	Dose (mg/kg bw/day): Effect	Reference
m: 205, 407, 947, 3,134 **f:** 597, 1,145, 3,087, 5,744	90-day drinking water exposure	SD Rat	**m – 947:** ↑Creatinine, ↑kidney weight, ↑renal lesions and crystal deposition **m - 3,134:** Death in 2/10, ↓bw gain, ↑creatinine, ↑BUN and phosphorus, ↑kidney, brain, and gonad weight, ↓heart, liver, and lung weight, ↑renal lesions and crystal deposition **f – 597:** ↓Leukocyte numbers **f – 3,087:** ↓Leukocyte numbers, ↑renal lesions and crystal deposition **f - 5,744:** Death in 8/10, ↓leukocyte numbers, ↑renal lesions and crystal deposition	Robinson et al. *(93)*
40 200 1,000	2-year dietary study	F344 Rats	**m – 1,000:** ↑ Death, ↓bw gain, ↑water intake, ↑BUN and creatinine, ↓erythrocytes, hematocrit, and hemoglobin, ↑neutrophils, ↑urine volume, ↓urine specific gravity and pH, ↑kidney weight, ↑urinary oxalate crystals, ↑renal lesions **f – 1,000:** ↑Kidney weight, ↑urinary oxalate crystals, ↑uric acid crystals, ↑liver fatty changes No lesions in reproductive system and no evidence of carcinogenicity	DePass et al. *(95)*

[a]Doses estimated by CERHR

Table 2-17. Summary of Key Subchronic and Chronic Toxicity Studies in the Mouse.

Dose (mg/kg bw/day	Exposure Regimen	Species/ Strain	Dose (mg/kg bw/day): Effect	Reference
m: 830, 1,630, 3,230, 6,450, 12,900[a] **f:** 1,020, 2,020, 4,000, 8,000, 16,000 [a]	13-week dietary exposure	$B6C3F_1$ Mouse	**m – 6,450:** Mild liver lesions and nephropathy **m - 12,900:** Mild liver lesions and nephropathy No effects on survival, body or organ weight, hematology, blood chemistry, urinalysis, or reproductive organ histopathology	Melnick et al. *(91*) NTP *(34*)
m: 1,500, 3,000, 6,000 **f:** 3,000, 6,000, 12,000	2-year dietary study	$B6C3F_1$ Mouse	**m – 3,000**: ↑Liver lesions **m – 6,000**: ↑Liver lesions, few oxalate-like crystals **f – 3,000**: ↑Arteriole hyperplasia **f – 6,000**: ↑Arteriole hyperplasia **f – 12,000**: ↑Liver lesions, ↑arteriole hyperplasia No neoplastic or non-neoplastic lesions in kidney or reproductive organs No effects on survival, bw gain, hematology, or blood chemistry	NTP *(34*)
40 200 1,000	2-year dietary study	CD-1 mouse	No treatment-related histopathology	DePass et al. *(95*)

[a]Doses estimated by CERHR

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

No human developmental toxicity data were identified.

3.2 Experimental Animal Data

3.2.1 Oral Exposure

3.2.1.1 Prenatal Toxicity Studies

3.2.1.1.1 Mouse

Price et al. *(98*) [also*(106*)] looked at prenatal developmental toxicity associated with oral exposure to ethylene glycol in COBS Crl:CD-1 mice in a study conducted according to GLP. On gd 6–15, timed-mated mice received gavage doses of ethylene glycol (>99% purity) in distilled water at 0, 750, 1,500, or 3,000 mg/kg bw/day. Doses were based on results of preliminary experiments and dosing solutions were verified to be within 10% of theoretical concentrations. At scheduled sacrifice on gd 17, confirmed pregnant females, 23–25 per group, were evaluated. The gravid uterus was weighed and the status of uterine implants recorded. Each live fetus was examined for external, visceral, and skeletal malformations. Viscera were examined using the Staples fresh tissue dissection technique and the skeleton was examined by staining with Alizarin Red S stain. The heads of half the fetuses were fixed in Bouin's solution and examined. Statistical analyses were conducted by the General Linear Model procedure and included tests for linear trends, ANOVA, Williams Multiple Comparison Test, Dunnett's Test, Chi-Square test, or Fisher Exact Probability Test.

Incidences for statistically significant findings in the Price et al. *(98*) [also *(106*)] study are summarized in Table 3-1. The adjusted maternal weight gain (excluding gravid uterine weight) was not different from the controls in any treated group, but weight gain during treatment and gestation was significantly reduced in the 1,500 and 3,000 mg/kg bw/day groups. Absolute but not relative liver weight was decreased in the 1,500 and 3,000 mg/kg bw/day dose groups; there was no histological evaluation. The percent non-live implants per litter exhibited a dose-related increase; however, no dose was significantly different from controls in pair-wise comparisons. A significant reduction in live fetuses/litter was noted in the 3,000 mg/kg bw/day group. Fetal weight was significantly decreased at all doses. The proportion of live litters with malformed live fetuses and the percentage of malformed fetuses per litter was significantly greater than controls in all treated groups. **[This was the only malformation data presented as fetuses affected per litter.]** The number of litters containing fetuses with external and visceral malformations was significantly increased at the 3,000 mg/kg bw/day dose, while numbers of litters containing fetuses with skeletal malformations were significantly increased at all dose levels. The most common malformations involved neural tube closure defects and craniofacial and axial skeletal dysmorphogenesis. The study authors concluded that severe developmental toxicity occurred at doses that did not produce serious maternal toxicity. **[The Expert Panel concluded that a developmental toxicity NOAEL was not established in this study. A maternal NOAEL of 750 mg/kg bw/day was selected by the Expert Panel.]**

Strengths/Weaknesses: The Price et al. *(98)* study is GLP-compliant with adequate numbers of animals per group and a design that permits evaluation of dose-response relationships. Its primary shortcomings are the use of high dose levels and the resultant outcome that compound-related effects were observed in offspring at all dose levels, prohibiting the establishment of a NOAEL for effects in offspring.

Utility (Adequacy) for CERHR Evaluation Process: While Price et al. *(98)* is not useful for risk extrapolation purposes, it does provide background regarding the types of developmental effects that may be observed at high to very high doses. It is noted that gavage administration delivers high amounts of agent over a brief time period, which is unlikely to mirror expected human exposure.

Table 3-1. Prenatal Toxicity Study of Ethylene Glycol in Mice by Price et al. *(98)* [also *(106)*].

Effect	**Doses (mg/kg bw/day)**			
	0	**750**	**1,500**	**3,000**
Maternal body weight gain on gd 6–15 (g)	12.40	11.58	8.54**	8.42**
Maternal liver weight (g)	2.72	2.63	2.49**	2.47**
No. live fetuses/litter	11.88	11.50	10.41	9.83*
Fetal body weight/litter (g)	0.974	0.882**	0.787**	0.712**
% Live malformed fetuses/litter	0.25	10.00**	37.77**	56.54**
No. litters with malformed fetuses/no. examined (%)	1/25 (4.00)	16/24*** (66.67***)	18/22*** (81.82***)	22/23*** (95.65***)
No. litters with external malformations/no. examined [%]	0/25 [0]	3/24 [12.50]	2/22 [9.09]	8/23** [34.78]
No. litters with visceral malformations/no. examined [%]	0/25 [0]	0/24 [0]	2/22 [9.09]	7/23** [30.4]
No. litters with skeletal malformations/no. examined [%]	1/25 [4.00]	15/24*** [62.50]	17/22*** [77.27]	22/23*** [95.65]
NOAEL		Maternal		

Protocol: CD-1 mice were exposed to ethylene glycol by gavage on gd 6–15. Dams were sacrificed on gd 17 and fetuses from 22–25 litters/group were evaluated for prenatal developmental toxicity.
Notes:
*= p<0.05, **=p<0.01, ***= p<0.001
( )=Values calculated by authors; [ ]=Values calculated by CERHR
There were no effects on corrected maternal weight gain.

Neeper-Bradley et al. *(107)* [also Tyl and Frank *(108)*] studied the effects of oral exposure to ethylene glycol on mouse prenatal developmental toxicity in a study conducted according to GLP. Timed-pregnant Crl:CD-1 (ICR) BR mice (30/dose group) received a daily gavage dose of ethylene glycol (100% purity) in deionized water at 0, 50, 150, 500 or 1,500 mg/kg bw/day on gd 6–15. Doses were selected to be at or below the mid- and low-dose levels of the Price et al. *(98)* study. Concentrations of dosing solutions were verified. At scheduled sacrifice on gd 18, the adult mice (19–24/treatment group) were weighed and kidneys were retained for subsequent microscopic examination. The gravid uterus was weighed and examined and the status of uterine implants recorded. Each live fetus was examined for external, visceral, and skeletal malformations. Visceral effects were evaluated using the Staples method and skeletal effects were examined by staining with Alizarin Red S. The litter was the statistical unit of comparison. Statistical analyses for continuous variables included Levene's test for equal variances, ANOVA, or t-tests with Bonferroni probabilities. Nonparametric data were evaluated with the Kruskal-Wallis test followed by the Mann-Whitney U test and incidence data were compared with Fisher's Exact Test. For all statistical tests, the fiducial limit of 0.05 (two-tailed) was used as the criterion for significance.

Incidences of statistically significant effects observed in the Neeper-Bradley et al. *(107)* study are listed in Table 3-2. No chemical-related maternal toxicity, including effects on body weight, water intake, and liver and kidney weight, was observed at any dose level. No significant effects were noted on the number of corpora lutea per dam or the number of total nonviable or viable implants per litter. Fetal body weights per litter were significantly reduced at the 1,500 mg/kg bw/day dose group. There were no significant increases in the incidence of individual or total external or visceral malformations. The incidence of total malformations in litters was significantly increased in the 500 mg/kg bw/day group, but no individual type of malformation was reported to be statistically significant at that dose level. Total skeletal malformations were significantly increased in litters of the 1,500 mg/kg bw/day dose groups. Skeletal malformations included fused or extra ribs and fused thoracic or lumbar arches. The incidences of one individual skeletal variation (extra lumbar rib) in litters from the 500 mg/kg bw/day group and 23 individual skeletal variations (i.e., poorly ossified thoracic and lumbar centra, extra lumbar ribs) in litters of the 1,500 mg/kg bw/day group were significantly increased; total skeletal variations were not significantly increased in any dose group. The study authors identified a maternal and fetal NOEL **[NOAEL[2]]** of 1,500 and 150 mg/kg bw/day, respectively, under the conditions of this study. **[The Expert Panel agreed with the authors' interpretation.]**

Strengths/Weaknesses: The Neeper-Bradley et al. *(107)* study is GLP-compliant with adequate numbers of animals per group and a design that permits evaluation of dose-response relationships. The doses selected included doses below those reported by Price et al. *(98)*, permitting the identification of a NOAEL for effects in offspring following gavage exposure.

Utility (Adequacy) for CERHR Evaluation Process: This study by Neeper-Bradley et al. *(107)* is useful for risk extrapolation purposes, with the caveat that exposures were delivered as bolus doses by means of gavage administration, which are unlikely to mirror expected human exposure.

[2] Since the Expert Panel is considering only adverse effects in the selection of effect levels, the terminology of NOAEL will be used throughout this document.

Table 3-2. Developmental Toxicity Study of Ethylene Glycol in CD-1 Mice by Neeper-Bradley et al. *(107)* [also Tyl and Frank *(108)*].

Effect	Doses (mg/kg bw/day)				
	0	**50**	**150**	**500**	**1,500**
Fetal body weight per litter on gd 6–15 (g)	1.325	1.369	1.330	1.285	1.156**
No. litters with skeletal malformations/no. examined (%)	2/19 (10.5)	3/20 (15.0)	1/24 (4.2)	5/24 (20.8)	17/21** (81.0)
No. litters with malformations/ no. examined (%)	3/19 (15.8)	7/20 (35.0)	5/24 (20.8)	12/24* (50.0)	17/21** (81.0)
Skeletal variations				[a]	[a]
NOAELs			Fetal		Maternal

Protocol: CD-1 mice were exposed to ethylene glycol by gavage on gd 6–15. Dams were sacrificed on gd 18 and fetuses from 19–24 litters/group were evaluated for prenatal developmental toxicity.
Notes:
*=p<0.05, **=p<0.01, ***=p<0.001
[a]See text for description of statistically significant increases in variations.
There were no effects on maternal weight gain, water intake, liver or kidney weight, number of corpora lutea and implantation sites, and external or visceral malformations.

3.2.1.1.2 Rat

Price et al. *(98)* examined rat prenatal toxicity in a study conducted according to GLP. Timed-pregnant CD rats were dosed by gavage with ethylene glycol (>99% purity) in distilled water at 0, 1,250, 2,500, or 5,000 mg/kg bw/day on gd 6–15. Doses were based on results of preliminary experiments and dosing solutions were verified to be within 10% of theoretical concentrations. At scheduled sacrifice on gd 20, maternal liver weight, kidney weights, and gravid uterus weight were determined for each dose group (27–29 rats/group). Number of implantation sites, resorptions, dead fetuses, and live fetuses were recorded. Each live fetus was weighed and examined for external, visceral, and skeletal defects. Viscera were examined using the Staples fresh tissue dissection technique and the skeleton was examined by staining with Alizarin Red S stain. The heads of half the fetuses were fixed in Bouin's solution and examined. Statistical analyses were conducted by the General Linear Model procedure and included tests for linear trends, ANOVA, Williams Multiple Comparison Test, Dunnett's Test, Chi-Square test, or Fisher Exact Probability Test.

Incidences for statistically significant findings in the Price et al. *(98)* study are summarized in Table 3-3. No clinical signs were observed in the treated pregnant rats except for piloerection. No effect on corrected maternal weight gain (excluding gravid uterine weight) was observed (data not shown in Table 3-3); however, the body weight gains among all treated groups were significantly reduced during the treatment period. Body weight gains for the 2,500 and 5,000 mg/kg bw/day groups were significantly reduced for the entire gestational period (data not shown in Table 3-3). Maternal water consumption was increased throughout the treatment and post-treatment period in a dose-related manner with significantly more water consumed in the 2,500 and 5,000 mg/kg bw/day groups. The only organ weight effects were significantly decreased absolute liver weight in the 5,000 mg/kg bw/day group and increased relative kidney weight in

the 2,500 and 5,000 mg/kg bw/day groups; no histopathology was conducted. A statistically significant increase in post implantation loss was observed in the 5,000 mg/kg bw/day group. Live litter size was significantly reduced at the 2,500 and 5,000 mg/kg bw/day dose levels. Fetal body weight was decreased at these same dose levels. There was a significant increase in the percentage of fetuses malformed per litter at the 2,500 and 5,000 mg/kg bw/day dose level. **[This was the only malformation data presented as fetuses affected per litter.]** A significant increase in the percentage of litters with malformed fetuses was observed in all treated groups. Significant increases were noted for the number of litters containing fetuses with external malformations (5,000 mg/kg bw/day), visceral malformations (1,250 and 5,000 mg/kg bw/day), and skeletal malformations (2,500 and 5,000 mg/kg bw/day). The most common malformations were neural tube closure defects and craniofacial and axial skeletal dysmorphogenesis. The study authors concluded that severe developmental toxicity occurred at doses that did not produce serious maternal toxicity. **[The Expert Panel noted that the claim for increased visceral malformations in the 1,250 mg/kg bw/day was based on 7 cases of hydroureter, 3 cases of hydronephrosis, and 2 great artery anomalies. The Panel disagreed with the classification of these effects as malformations and stated that the effects should be classified as variations. In addition, none of the findings were repeated in a subsequent study by Neeper-Bradley et al. *(107)* described below. The Expert Panel concluded that the data in this study appear to support a NOAEL of 1,250 mg/kg bw/day; however, the ensuing study by Neeper-Bradley et al. *(107)* identified a developmental NOEL [NOAEL[3]] of 500 mg/kg/day.]**

Strengths/Weaknesses: Price et al. *(98)* is GLP-compliant with adequate numbers of animals per group and a design that permits evaluation of dose-response relationships. The day of sacrifice (gd 20) is earlier than that of the ensuing studies, which used gd 21. This makes direct comparison of some of the data difficult.

Utility (Adequacy) for CERHR Evaluation Process: The Price et al. *(98)* study could be useful for risk extrapolation purposes, with the caveats that exposures were delivered as bolus doses by means of gavage administration, which is unlikely to mirror expected human exposure and ensuing studies established a lower NOAEL.

[3] Since the Expert Panel is considering only adverse effects in the selection of effect levels, the terminology of NOAEL will be used throughout this document.

Table 3-3. Developmental Toxicity Study of Ethylene Glycol in CD Rats by Price et al. *(98)*.

Effect	Doses (mg/kg bw/day)			
	0	**1,250**	**2,500**	**5,000**
Maternal body weight gain on gd 6–15 (g)	42.03 ± 1.96	34.81 ± 1.73**	29.45 ± 1.38**	20.68 ± 1.93**
Maternal liver weight (g)	15.47 ± 0.26	15.01 ± 0.28	14.95 ± 0.27	13.70 ± 0.35*
Relative maternal kidney weight (% body weight)	0.517 ± 0.012	0.531 ± 0.007	0.573 ± 0.008**	0.615 ± 0.021**
Maternal water consumption on gd 6–15 (g)	130.3 ± 4.3	128.4 ± 3.0	154.4 ± 4.0**	165.0 ± 3.8**
% Postimplantation loss/litter	4.70 ± 1.23	6.35 ± 1.85	6.27 ± 1.35	21.34 ± 5.24*
No. live fetuses/litter	13.54 ± 0.28	12.75 ± 0.38	11.90 ± 0.60*	11.04 ± 0.79*
Fetal body weight/litter (g)	3.404 ± 0.052	3.312 ± 0.058	2.916 ± 0.056**	2.388 ± 0.089**
% Live fetuses malformed/litter	1.37 ± 0.97	6.65 ± 2.04	25.11 ± 4.84**	73.53 ± 6.42**
No. litters with malformed live fetuses/no. examined [%]	2/28 (7.14)	11/28** (39.29**)	20/29*** (68.97***)	25/26*** (96.15***)
No. litters with external malformations/no. examined [%]	0/28 [0]	0/28 [0]	4/29 [13.79]	15/26*** [57.69]
No. litters with visceral malformations/no. examined [%]	0/28 [0]	6/28* [21.43]	2/29 [6.90]	8/26** [30.77]
No. litters with skeletal malformations/no. examined [%]	2/28 [7.14]	6/28 [21.43]	19/29*** [65.52]	24/26*** [92.31]
NOAEL		Fetal Maternal		

Protocol: CD rats were exposed to ethylene glycol by gavage on gd 6–15. Dams were sacrificed on gd 20 and fetuses from 26–29 litters/group were evaluated for prenatal developmental toxicity.
Notes:
*= p<0.05, **=p<0.01, ***= p<0.001
( )=Values calculated by authors.
[ ]=Values calculated by CERHR.
There were no effects on maternal corrected weight gain or clinical signs.

Neeper-Bradley et al. *(107)* [also *(109)*] investigated the prenatal developmental toxicity of ethylene glycol administered by gavage to pregnant rats in a study conducted according to GLP. On gd 6–15, timed-pregnant Crl:CD (Sprague-Dawley) rats (25/dose group) received daily doses of ethylene glycol (99.9% purity) in deionized water at 0, 150, 500, 1,000, or 2,500 mg/kg bw/day. Concentrations of dose solutions were analytically verified. **[No rationale was provided for dose selection.]** At scheduled sacrifice on gd 21, 22–25 dams/group were evaluated for body, liver, and kidney weight. The gravid uterus was weighed and examined for status of implantation

sites. A total of 21–24 litters/group were examined. Live fetuses were weighed, sexed, and examined for external abnormalities. All fetuses were examined for visceral malformations by the Staple's method and for skeletal malformations and variations by staining with Alizarin Red S. Heads from one-half of the fetuses were fixed in Bouin's solution and examined for soft tissue malformations. The litter was the unit of comparison in statistical analyses. Continuous variables were analyzed by Levene's test for equal variances, ANOVA, and/or t-tests with Bonferroni probabilities. Non-parametric data were analyzed by the Kruskal-Wallis test, Mann-Whitney U test and/or Fisher's Exact Test. For all statistical tests, a probability value of $P<0.05$ (two-tailed) was used as the critical level of significance.

Incidences of statistically significant findings are outlined in Table 3-4. No treatment-related maternal deaths, abortions, or early deliveries occurred. Maternal weight gain was significantly decreased in the 2,500 mg/kg bw/day group, but corrected weight gain was unaffected. Maternal water consumption significantly increased among rats receiving 2,500 mg/kg bw/day. Significant organ weight changes included increased relative liver weight at 1,000 and 2,500 mg/kg bw/day and increased relative and absolute kidney weight at 2,500 mg/kg bw/day. Microscopic evaluation of kidneys from high-dose dams revealed no treatment-related lesions; livers were not examined. There were no observed effects on gestational parameters that included number of corpora lutea, total number of implantations/litter, or on sex ratio. Significant developmental effects in the 1,000 mg/kg bw/day dose group included reduced fetal body weight and increased incidences of litters containing fetuses with two skeletal malformations (missing thoracic arch and missing ribs). At 2,500 mg/kg bw/day, significantly increased frequencies of litters containing fetuses with visceral, skeletal, external, and total malformations were observed. Defects observed in the high-dose group included gastroschisis, hydrocephaly, lateral ventricle dilation, umbilical hernia, and malformations of the ribs and vertebrae. A significantly increased incidence of skeletal variants (primarily involving delayed ossification) were also observed in litters from the 1,000 and 2,500 dose groups. The author-reported NOELs **[NOAELs[4]]** for maternal and developmental toxicity were 1,000 and 500 mg/kg bw/day, respectively.

Strengths/Weaknesses: The Neeper-Bradley et al. *(107)* study is GLP-compliant with adequate numbers of animals per group and a design that permits evaluation of dose-response relationships. The day of termination (gd 21) is later than that of the previous studies, which used gd 20. This makes direct comparison of some of the data difficult.

Utility (Adequacy) for CERHR Evaluation Process: This study by Neeper-Bradley et al. *(107)* should be useful for risk extrapolation purposes, with the caveat that exposures were delivered as bolus doses by means of gavage administration, which are unlikely to mirror expected human exposure.

[4] Since the Expert Panel is considering only adverse effects in the selection of effect levels, the terminology of NOAEL will be used throughout this document.

Table 3-4. Prenatal Toxicity Study of Ethylene Glycol in CD Rats by Neeper-Bradley et al. *(107)* [also *(109)*].

Effect	Doses (mg/kg bw/day)				
	0	**150**	**500**	**1,000**	**2,500**
Maternal body weight gain on gd 6–15 (g)	35.53 ± 12.2	40.79 ± 8.6	40.08 ± 9.1	39.23 ± 6.4	26.46 ± 11.3**
Maternal water intake on gd 6–15 (g/rat/day)	34.02 ± 4.4	34.32 ± 4.7	36.38 ± 10.7	34.87 ± 6.0	43.73 ± 7.2**
Maternal kidney weight (g)	1.799 ± 0.16	1.833 ± 0.15	1.837 ± 0.20	1.906 ± 0.20	1.967 ± 0.19**
Relative maternal kidney weight (% body weight)	0.634 ± 0.06	0.631 ± 0.04	0.637 ± 0.07	0.656 ± 0.05	0.698 ± 0.05**
Relative maternal liver weight (% body weight)	4.577 ± 0.35	4.829 ± 0.31	4.621 ± 0.28	4.867 ± 0.40*	4.881 ± 0.38**
Fetal body weight/litter (g)	5.245 ± 0.26	5.408 ± 0.22	5.217 ± 0.30	4.981 ± 0.31*	4.033 ± 0.40**
No. litters with external malformations/no. examined (%)	0/24 (0)	1/22 (4.5)	0/22 (0)	2/23 (8.7)	8/21** (38.1)
No. litters with soft tissue malformations/no. examined (%)	6/24 (25.0)	9/22 (40.9)	9/22 (40.9)	9/23 (39.1)	17/21** (81.0)
No. litters with skeletal malformations/no. examined (%)	0/24 (0)	0/22 (0)	1/22 (4.5)	10/23** (43.5)	21/21** (100.0)
Skeletal variations				[a]	[a]
NOAELs			Fetal	Maternal	

Protocol: CD rats were exposed to ethylene glycol by gavage on gd 6–15. Dams were sacrificed on gd 21 and fetuses from 21–24 litters were evaluated for prenatal developmental toxicity.
Notes:
*= p<0.05, **=p<0.01, ***= p<0.001
[a] See text for description of statistically significant increases in variations.
There were no effects on maternal corrected weight gain, kidney lesions, deaths, abortions, early deliveries, or numbers of implantation sites and corpora lutea.

Maronpot et al. *(110)* treated groups of more than 20 pregnant Fischer 344 rats (100 days old) with ethylene glycol (99.9% pure) in the diet (target doses were 0, 40, 200, and 1,000 mg/kg bw/day) from gd 6 to15. Doses corresponded to those of a concurrent 2-year assay that demonstrated toxicity at the highest dose. A positive control group received 500 mg/kg bw hydroxyurea in saline intraperitoneally (IP) on gd 11. Pregnant females were sacrificed on gd 21, and the fetuses from each group were randomly allocated to either a visceral and head examination group or a skeletal examination (by maceration and staining) group. Statistical analysis included F test for continuous data, Fisher's Exact Test for binomial data, multiple sum

of ranks test for non-parametric data, and other paired tests for significant differences (Student's t-test, Cochran's t-test) ($p<0.05$). At least 20 litters or 164–190 fetuses/group were examined. Evaluation of maternal toxicity was limited to clinical signs and body weight gain.

The authors stated there were no clinical signs or significant differences between control and treated group corrected maternal body weight gains when examined on gd 6, 11, or 21 **[data not shown]**. Fetal data were shown in tables, and no significant effects on fetal length, weight, litter size, or total implantations were reported. The positive controls exhibited numerous major malformations (e.g., tail malformation, twisted limbs, skeletal and heart malformations). These effects were not seen in ethylene glycol-treated groups. A statistically significant ($p<0.001$) increase in incidence of poorly ossified and unossified vertebral centra in fetuses from the 1,000 mg/kg bw/day group (Table 3-5) was described by the study authors as evidence of delayed fetal maturation and suggestive of minimal embryotoxicity. Reduced ossification was not reported as statistically significant in litters. Authors stated that the incidence of major malformations was not increased in treated rats. In the conclusion, the study authors state that the absence of major malformations is interpreted as a preliminary indication of lack of teratogenicity of this chemical. **[The Expert Panel selected a fetal NOAEL of 1,000 mg/kg bw/day for this study. Although reduced ossification was noted at the 1,000 mg/kg bw/day dose, the Panel questioned whether a dose that results in reduced ossification but an otherwise normal vertebrae should be identified as a LOAEL. The Panel noted that current thinking on reduced ossification suggests that three or more vertebrae from the same fetus should be affected before the dose producing the effect should be identified as a LOAEL. The data as presented in the paper do not provide that level of detail. However, the lack of other findings (e.g., no change in body weights, no other consistent alterations in skeletal integrity), suggest that the dietary NOAEL is 1,000 mg/kg bw/day.]**

Strengths/Weaknesses: The design of the Maronpot et al. *(110)* study permits evaluation of dose-response relationships and starts with adequate numbers of animals per group, although the pregnancy rate (~80% in all groups) is surprisingly, but uniformly, low. The dietary mode of administration provides exposure over a longer period of time than gavage dosing. This may more adequately model likely human exposure patterns than the previous studies with bolus dosing.

Utility (Adequacy) for CERHR Evaluation Process: The Maronpot et al. *(110)* study should be useful for risk extrapolation purposes.

Table 3-5. Prenatal Toxicity Study of Ethylene Glycol in Rats by Maronpot et al. *(110)*.

Effect	Doses (mg/kg bw/day)			
	0	**40**	**200**	**1,000**
No. fetuses with poorly ossified vertebrae/no. examined (%)	3/167 (1.8)	1/190 (0.5)	4/164 (2.4)	24/169 (14.2)***
No. fetuses with unossified vertebrae/number examined (%)	19/167 (11.4)	33/190 (17.4)	31/164 (18.9)	44/169 (26.0)***
NOAELs:				Maternal Fetal[a]
Protocol: Fischer 344 rats were exposed to ethylene glycol in diet on gd 6–15. Dams were sacrificed on gd 21 and fetuses from 20–21 litters/group were evaluated for prenatal developmental toxicity. **Notes:** ***=p<0.001, significance obtained when analyzed for fetuses but not litters affected. [a]Expert Panel's selection of a fetal NOAEL was higher than the study authors. There were no effects on corrected maternal body weight gain or clinical signs, malformations, fetal length or body weight, litter size, or total implantations at any dose. The 500 mg/kg bw hydroxyurea positive control showed increased visceral and skeletal malformations.				

The Expert Panel is aware of a Chinese manuscript by Yin et al. (released around the late 1980s) for a study of ethylene glycol prenatal toxicity in rats. It is not known if the manuscript was ever published. A literature search revealed that the information was published in an abstract *(111)*. The Expert Panel notes that the majority of results from that study appear to be qualitatively consistent with results from the rat prenatal studies described above.

3.2.1.1.3 Rabbits

Tyl et al. *(97)* studied the prenatal developmental toxicity of ethylene glycol in 5-month-old New Zealand White rabbits in a study conducted according to GLP. Artificially inseminated does, 23–24 per group, received ethylene glycol (98% purity) in deionized/distilled water at doses of 0, 100, 500, 1,000, or 2,000 mg/kg bw/day by gavage on gd 6–19. Ethylene glycol concentrations in dosing solutions were verified. **[The rationale for dose selection was not presented.]** At scheduled necropsy on gd 30, maternal liver, kidney, and gravid uterus weights were recorded. Ovarian corpora lutea were counted and uterine implantation sites were recorded. Kidneys were examined histologically in 10–17 dams/group. All live fetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations and variations. Viscera were examined according to the Staples method and the skeleton was stained with Alcian Blue/Alizarin Red S. Heads from half the fetuses were fixed in Bouin's solution and examined. The litters were considered the experimental unit. Data were analyzed with the General Linear Trend Models procedures for ANOVA, Bartlett's test for homogeneity of variance, Williams' and Dunnett's multiple comparison tests, and/or Fisher's Exact Probability Test.

At necropsy, 20–22 dams and litters/group were evaluated in the control and 3 lowest dose groups; 9 does and litters were evaluated in the highest dose group due to a high mortality rate. At 2,000 mg/kg bw/day, 42% of the does died, three delivered early, and one aborted. Kidney weights were slightly increased in the 2,000 mg/kg bw/day group (not statistically significant), and necropsy revealed renal toxicity including tubule dilatation and degeneration, epithelial necrosis, and intraluminal oxalate crystal deposition. There were no effects on maternal weight gain or water intake. No statistically significant ($p < 0.05$) effects on pre- or postimplantation loss, number of fetuses, fetal body weight, or sex ratio per litter were observed at any of the doses

tested. There was no evidence of teratogenicity. Findings of this study are summarized in Table 3-6. The study authors identified NOAELs of 1,000 mg/kg bw/day for maternal toxicity and at least 2,000 mg/kg bw/day for developmental toxicity. **[The Expert Panel concurs with the authors' identification of NOAELs. Very few anomalies were observed among the small number of pups that could be examined from the high-dose group. The fact that reduced number of pups at the high dose resulted from maternal deaths and whole litter loss suggests that pups were not so severely affected that they died.]**

Strengths/Weaknesses: The design of this GLP-compliant study by Tyl et al. *(97)* permits evaluation of dose-response relationships and starts with adequate numbers of animals per group, although survival to term among the high-dose animals was low.

Utility (Adequacy) for CERHR Evaluation Process: The Tyl et al. *(97)* study should be useful for risk extrapolation purposes, with the caveat that exposures were delivered as bolus doses by means of gavage administration, which are unlikely to mirror expected human exposure.

Table 3-6. Prenatal Toxicity Study of Ethylene Glycol in Rabbits by Tyl et al. *(97)*.

Effects	Doses				
	0	**100**	**500**	**1,000**	**2,000**
Early delivery/no. examined	1/23	1/24	1/24	1/22	3/22
No. maternal deaths/no. examined	0/22	0/23	0/23	0/21	8/19
No. dams with renal lesions/no. examined	4/10	5/10	6/10	5/10	14/17
No. dams with renal crystals/no. examined	0/10	0/10	0/10	0/10	8/17
NOAELs				Maternal	Fetal

Protocol: New Zealand White rabbits were exposed to ethylene glycol by gavage on gd 6–19. Dams were sacrificed on gd 30 and fetuses from 9–22 litters/group were evaluated for prenatal developmental toxicity.
Notes:
There were no effects on maternal water intake and weight gain, fetal malformations, implantation loss, litter size, fetal body weight, or sex ratio at any dose.

3.2.1.2 Postnatal Toxicity Studies

Price et al. *(112)* investigated the effects of prenatally administered ethylene glycol on the postnatal development of rats. In a study conducted according to GLP, timed-mated Crl:COBS CD (Sprague-Dawley) BR rats received gavage doses of ethylene glycol (99.6% purity) in distilled water at 0, 250, 1,250, or 2,250 mg/kg bw/day during gd 6–20. The doses were based on findings of previous studies conducted in the laboratory and concentrations of dose solutions were verified. Thirty-eight to 49 dams/group delivered litters and were sacrificed on postnatal day (pnd) 1. Pups from 33–42 litters/group were fostered to untreated control dams on pnd 1. The pups were monitored for growth and viability, developmental landmarks, sexual maturation, locomotor activity, and performance on a complex learning task. Pups were intermittently sacrificed to evaluate external and visceral malformations (pnd 1, 4, 22, and 63), skeletal malformations (pnd 22), and liver, kidney and brain histopathology (pnd 4, 22, and 63). Data were analyzed using General Linear Models together with Bartlett's test for homogeneity of variance, Dunnett's and Williams tests, ANOVA, Chi-Square test, and/or Fisher's Exact Probability Test.

Incidences of statistically significant findings in the Price et al. *(112*) study are listed in Table 3-7. Maternal body weight gain was reduced in the 2,250 mg/kg bw/day group; **[corrected body weights were not reported].** Absolute and relative uterus weight were reduced in the 2,250 mg/kg bw/day group. Absolute and relative maternal kidney weight were significantly increased among rats treated with 2,250 mg/kg bw/day. **[Tables indicate an increase in absolute and relative kidney weight, but the abstract and discussion state that absolute and relative kidney weights were decreased.]** Histological evaluation revealed treatment-related renal pathology (tubular dilation and regeneration) in dams treated with 2,250 or 1,250 mg/kg bw/day. Gestational length was significantly prolonged in dams of the 2,250 and 1,250 mg/kg bw/day groups. Treatment had no effect on the numbers of implantation sites. On pnd 1–4, there were significant increases in pup cumulative mortality/litter and significant reductions in litter size and pup weight gain in the 2,250 mg/kg bw/day group. The authors noted that a total of 9 pups in the 2,250 mg/kg bw/day group (males and females sacrificed on pnd 1, 4, 22, 27, and 63) had hydrocephaly versus no pups in the control group. **[The statistical significance of this effect was not discussed.]** In pups sacrificed on pnd 22, there was a significantly increased incidence of skeletal malformations (defects in ribs, sternebrae, and vertebrae) at the 2,250 mg/kg bw/day dose level. Absolute kidney weights were reduced in pups of the 2,250 mg/kg bw/day group on pnd 22. Significant decreases in kidney weight (absolute in males and relative in females) were observed in the 2,250 and 1,250 mg/kg bw/day groups on pnd 63; there were no kidney or liver lesions in offspring from the highest dose group. Absolute brain weights were significantly reduced in males and females of the 2,250 mg/kg bw/day dose group on pnd 22 and 63. Treatment had no adverse effects on developmental landmarks such as incisor eruption, vaginal opening, testes descent, or wire-grasping skills. In addition, no adverse treatment-related effects were noted for exploratory behavior during the preweaning period and performance on a visual discrimination test at 12–14 weeks of age. The study authors concluded that no toxicity was observed at 250 mg/kg bw/day, while maternal and offspring effects were noted at 1,250 and 2,250 mg/kg bw/day, respectively. **[In agreement with study authors, the Expert Panel concluded that these data indicate a maternal NOAEL of 250 mg/kg bw/day and a developmental toxicity NOAEL of 1,250 mg/kg bw/day.]**

Strengths/Weaknesses: The Price et al. *(112*) study is GLP-compliant with adequate numbers of animals per group and a design that permits evaluation of dose-response relationships. This study was primarily designed to examine postnatal effects following prenatal exposure, so the design was different than that used by Price et al. *(98*). The highest dose chosen was similar to the middle dose used by Price et al. *(98*) and demonstrated similar effects on neonatal body weight with the caveat that the study designs were different.

Utility (Adequacy) for CERHR Evaluation Process: The Price et al. *(112*) study is not directly applicable to risk extrapolation. The exposures were delivered as bolus doses by means of gavage administration, which are unlikely to mirror expected human exposure. However, it does provide evidence of the transient nature of some of the adverse effects of high-dose ethylene glycol exposure during gestation on body weight (decreased at pnd 1, 4, and 22, but not different from control at pnd 63), as well as the lack of effect on several developmental landmarks and behavioral tests.

Table 3-7. Postnatal Toxicity Study of Ethylene Glycol in CD Rats by Price et al. *(112)*.

Effect	Doses (mg/kg bw/day)			
	0	**250**	**1,250**	**2,250**
Maternal body weight gain on gd 6–20 (g)	106.2	104.6	102.5	84.3**
Gestational length (days)	21.26	21.29	21.58**	21.84**
Dams with renal pathology/no. examined	0/18	0/14	4/15	5/15
Maternal kidney weight (g)	2.20	2.24	2.28	2.42*
Maternal relative kidney weight (% body weight)	0.79	0.80	0.81	0.87**
Maternal uterine weight (g)	4.62	4.58	4.73	4.08*
Maternal relative uterine weight (% body weight)	1.66	1.62	1.68	1.46*
Live litter size, pnd 1	13.67	12.63	13.58	11.87**
Live litter size, pnd 4	13.21	12.92	13.26	11.42**
% Cumulative pup mortality/litter - pnd 1	6.7	9.1	9.0	17.1**
% Cumulative pup mortality/litter - pnd 4	9.1	8.4	11.3	22.3**
Pup body weight/litter – pnd 1 (g), Male:	6.33	6.47	6.49	5.88**
Female:	5.97	6.07	6.06	5.60**
Pup kidney weight/litter, pnd 22 (g), Male: [a]	0.612	0.616	0.626	0.543*
Female: [a]	0.638	0.630	0.637	0.549**
Pup brain weight/litter, pnd 22 (g), Male: [a]	1.417	1.433	1.409	1.325*
Female: [a]	1.400	1.358	1.320	1.242**
Average pup kidney weight/litter, male – pnd 63 (g) [a]	3.260	3.208	2.981*	2.909*
Relative pup kidney weight/litter, female – pnd 63 (% body weight) [a]	1.01	0.96	0.91**	0.92*
No. litters with skeletal malformations/no. examined, pnd 22 (%), Male: [a]	0/23	0/22	0/24	6/20**
Female: [a]	0/24	2/23	0/24	12/20***
NOAELs		Maternal	Fetal	

Protocol: Dams received ethylene glycol by gavage on gd 6–20. Litters were delivered and fostered with untreated dams. Postnatal growth and development were evaluated up to pnd 63 in pups from 33–42 litters/group.
Notes:
[a] Data shown are from only those pups sacrificed on pnd 22 or 63.
*= p<0.05, **=p<0.01, ***= p<0.001
There were no effects on implantation sites, offspring kidney or liver lesions, developmental landmarks, or offspring performance on neurotoxicity testing.

Marr et al. *(113)* investigated the effects of maternal ethylene glycol exposure on pre- and postnatal skeletal development. Pregnant Crl:CD BR VAF/Plus outbred Sprague-Dawley rats

were treated with 0 or 2,500 mg/kg bw/day ethylene glycol (99% pure) in water via oral gavage (5 mL/kg) from gd 6 to 15. Based on previous studies, a dose sufficient to cause reduced fetal/pup weight was selected. Each dose group was subdivided and 4–7 dams and litters/group were sacrificed on gd 18 or 20 or pnd 1, 4, 14, 21, or 63. Endpoints examined were fetal and pup weights and skeletal malformations and degree of ossification (by staining with Alcian Blue and Alizarin Red S). Data were analyzed using Student's t-test for normally distributed continuous data and Mann-Whitney U test for comparisons of non-normal continuous data ($p<0.05$).

Results are summarized in Table 3-8. Maternal weight gain during the treatment period (gd 6–15) was significantly decreased (by 27%) in the ethylene glycol group, and gestational weight gain was significantly decreased (by 13%) in the treatment group. Fetal body weight was significantly decreased (by ~25%) in the group treated with ethylene glycol and examined on gd 18 and 20. The mean pup body weight per litter was significantly reduced (by 10%) in the treated group examined on pnd 1 only; other treated groups examined later had reduced weights that were not significant. The percentages total ossification, sternebrae ossification, and vertebral centra ossification were significantly reduced in the treatment groups on gd 20 and all pnd except 63. When these data were co-varied with fetal/pup weights, the delayed ossification during gestation was no longer significant, but this effect remained significant for the postnatal data. This suggests that the ossification effects observed postnatally were not influenced by body weight changes, while those seen on gd 20 may have resulted from body weight effects. Percent of malformed pups per litter (predominantly axial skeletal defects) were significantly increased in treated groups examined on all days except pnd 63. The authors conclude that offspring effects observed early in development as a result of ethylene glycol treatment may not be permanent since they were not observed at pnd 63.

Strengths/Weaknesses: The purpose of the Marr et al. *(113)* study was to evaluate the potential reversibility of ethylene glycol-related effects seen in previous studies. The strength of the study is that it provides a level of confidence about the long-term impact of the skeletal findings observed in near-term fetuses of high-dose litters. Its weakness is the absence of historical control data for some of the endpoints observed at the time points used.

Utility (Adequacy) for CERHR Evaluation Process: The Marr et al. *(113)* study is not directly applicable to risk extrapolation. It does, however, provide evidence that many of the skeletal changes (most of which are variations) appear to be transient.

Table 3-8. Effects of Ethylene Glycol in CD Rat Prenatal/Postnatal Study by Marr et al. *(113)*.

Effect	Significant Offspring Effects on Each Day of Examination in Treated Versus Control Animals						
	gd 18	gd 20	pnd 1	pnd 4	pnd 14	pnd 21	pnd 63
Pup body weight (g)	0.93* vs 1.26	2.75* vs 3.63	6.21* vs 6.90	9.22 vs 10.07	33.20 vs 34.78	51.18 vs 53.28	320.6 vs 338.7
No. malformations per litter/no. examined (%)	10.3/7 vs 0/7 (76* vs 0)	13.1/7 vs 0.2/6 (88* vs 1)	7.7/6 vs 0/6 (95* vs 0)	11.3/4 vs 1.0/6 (83* vs 6)	5.0/5 vs 0/5 (77* vs 0)	7.0/4 vs 0/7 (87* vs 0)	2.2/5 vs 0.6/5 (28 vs 8)
No. litters with skeletal malformations /no. examined (%)	7/7 vs 0/7 (100* vs 0)	7/7 vs 1/6 (100* vs 17)	6/6 vs 0/6 (100* vs 0)	4/4 vs 3/6 (100 vs 50%)	5/5 vs 0/5 (100* vs 0)	4/4 vs 0/7 (100* vs 0)	4/5 vs 1/5 (80 vs 20)
Reduced Ossification	[a]	[a]	[a]	[a]	[a]	[a]	[a]

Protocol: Rats gavaged with 0 or 2,500 mg/kg bw/day ethylene glycol on gd 6–15. Pups in 4–7 litters/group were examined during late gestation or during the postnatal period.
Notes: *=p<0.05
[a]See text for details.
gd = gestation day, pnd = postnatal day

3.2.2 Inhalation Exposure

3.2.2.1 Mouse

Tyl et al. *(114)* [also *(115)*] examined mouse prenatal developmental toxicity in a study conducted according to GLP. Crl:CD-1(1CR)BR mice, 25/dose group, were exposed to a respirable aerosol of ethylene glycol (100% purity; mass median aerodynamic diameter of 2.3 μm; whole-body exposure) for 6 hours/day at concentrations of 0, 150, 1,000, or 2,500 mg/m^3 on gd 6–15. Doses were based on results of range finding studies. Chamber concentrations were verified and it was found that chamber concentrations were below target concentrations (119, 888, and 2,090 mg/m^3). At scheduled sacrifice on gd 18, 22–25 dams/group were evaluated for body, liver, and kidney weight. The gravid uterus was weighed and examined for status of implantation sites. All live fetuses from 22–25 litters were counted, weighed, sexed, and examined for external malformations. In half of the fetuses, heads were fixed in Bouin's solution and examined and viscera were evaluated by the Staples method. The other half of fetuses were stained with Alizarin Red S and examined for skeletal malformations. Litters were considered the unit of comparison. Continuous variables were analyzed by the Levene's test, ANOVA, and t-tests with Bonferroni probabilities. Nonparametric data were analyzed with the Kruskal-Wallis Test followed by the Mann-Whitney U test. Incidence data were evaluated with the Fisher's Exact Test. For all statistical tests, a probability value of $p<0.05$ (two-tailed) was used as the critical level of significance.

Incidences of statistically significant effects in the Tyl et al. *(114)* study are summarized in Table 3-9. Maternal body weight was significantly decreased in the mice exposed to 1,000 and 2,500 mg/m^3, but corrected weight gain was not affected. The numbers of viable implants per litter were

significantly reduced at 2,500 mg/m^3. Significant embryo/fetal effects at 1,000 and 2,500 mg/m^3 consisted of reduced numbers of live fetuses/litter, reduced fetal body weight/litter, increased numbers of non-viable implants/litter, and increased litters with external, visceral, and skeletal malformations and variations. The types of malformations observed included cleft palate, exencephaly, and defects of the nasopharynx, tongue, brain, vertebra, ribs, and face. Authors identified a maternal and developmental toxicity NOEL **[NOAEL[5]]** of 150 mg/m^3. The authors speculated that a majority of the dose received could be from ingestion of ethylene glycol while grooming the fur. Authors estimated that inhalation and ingestion of ethylene glycol resulted in a total dose of 410–606 and 966–1,428 mg/kg bw/day in the 1,000 and 2,500 mg/m^3 groups, respectively. Doses were estimated by measuring ethylene glycol levels in fur to determine potential ingestion and then using inhalation rates with assumed 10 and 90% retention to determine potential inhalation exposure.

Strengths/Weaknesses: This GLP-compliant study by Tyl et al. *(114)* was designed to permit the evaluation of exposure-response relationships for whole-body inhalation exposures to mice. The author was alert to observe ingestion of test agent from the coats of animals during preening, after the animals had been removed from the exposure chambers. Satellite investigations demonstrated that most of the maternal exposure (~94%) was attributed to ingestion of ethylene glycol deposited on the fur during exposure to the aerosol.

Utility (Adequacy) for CERHR Evaluation Process: The Tyl *(114)* study is scientifically sound. The author identified potential confounding through a second exposure route and then verified and quantified exposure occurring through ingestion. Due to the confounding caused by exposure via two routes, the results of this study are not useful for evaluating effect levels for inhalation of ethylene glycol.

[5] Since the Expert Panel is considering only adverse effects in the selection of effect levels, the terminology of NOAEL will be used throughout this document.

Table 3-9. Major Effects of Ethylene Glycol in Prenatal Toxicity Study in CD-1 Mice by Tyl *(114)* [also *(115)*].

Effect	Doses (mg/m^3)			
	0	**150**	**1,000[a]**	**2,500[b]**
Maternal body weight gain on gd 6–15 (g)	13.63 ± 1.50	14.33 ± 1.93	11.28 ± 2.46**	9.42 ± 3.06***
Viable implants/litter (g)	10.7 ± 1.8	11.8 ± 2.2	9.3 ± 2.8	8.0 ± 2.9***
Non-viable implants/litter (g)	1.4 ± 1.0	1.1 ± 1.2	2.9 ± 2.0**	4.2 ± 2.9**
% Live fetuses/litter	88.5 ± 8.4	91.3 ± 10.1	76.2 ± 16.5**	65.2 ± 22.9***
Fetal body weight/litter (g)	1.33 ± 0.08	1.29 ± 0.10	1.07 ± 0.14***	0.94 ± 0.14***
No. litters with external malformations/ No. examined (%)	1/25 (4.0)	4/22 (18.2)	7/23* (30.4)	16/22* (72.7)
No. litters with visceral malformations/ No. examined (%)	2/25 (8.0)	3/22 (13.6)	8/23* (34.8)	16/22* (72.7)
No. litters with skeletal malformations/ No. examined (%)	18/25 (72.0)	18/22 (81.8)	23/23* (100.0)	22/22* (100.0)

Protocol: Mice inhaled ethylene glycol mists (whole-body exposure) from gd 6–15 and were sacrificed for evaluation of prenatal toxicity in fetuses from 22–25 litters/group on gd 18.
Notes: *= p<0.05, **=p<0.01, ***==p<0.001
[a]Authors estimated a total dose of 410–606 mg/kg bw/day from inhalation and ingestion.
[b]Authors estimated a total dose of 966–1,428 mg/kg bw/day from inhalation and ingestion.
There was no effect on corrected maternal weight gain.

Tyl et al. *(116)* [also *(117)*] next conducted a study to examine the role of ethylene glycol inhalation alone on prenatal developmental toxicity in Crl:CD-1(1CR)BR mice. In a study conducted according to GLP, timed-pregnant CD-1 mice, 30 per dose group, were exposed by nose-only to ethylene glycol (99% purity) aerosol target concentrations of 0, 500, 1,000, or 2,500 mg/m^3 for 6 hours/day, on gd 6–15. A positive control group received a whole-body exposure to an aerosol target concentration of 2,100 mg/m^3. The MMAD of the aerosols was 2.6 microns. Two groups of 30 negative control rats were exposed to water vapor. Dose selections were based on results observed in a previous experiment with whole-body inhalation exposure *(114)*. Ethylene glycol concentrations in chambers were measured, and it was found that actual concentrations were below target concentrations **[see table 1 of the study]**. Based on measurement of ethylene glycol levels in the fur of satellite animals, the amounts of ethylene glycol potentially available for oral ingestion were found to be 330 mg/kg bw for nose-only exposure to 2,500 mg/m^3 and 1,390 mg/kg bw for whole-body exposure to 2,100 mg/m^3. At scheduled sacrifice on gd 18, 22–29 adult mice/treatment group were weighed and kidneys were retained for subsequent microscopic examination. The gravid uterus was weighed and examined

and the status of uterine implants was recorded. Each live fetus from a total of 21–29 litters/group was examined for external, visceral, and skeletal malformations. Visceral defects were evaluated using the Staples method and the skeleton was stained with Alizarin Red S. The heads of half the fetuses were preserved in Bouin's solution and examined. Litters were considered the unit of comparison. Continuous variables were analyzed by the Levene's test, ANOVA, and t-tests with Bonferroni probabilities. Nonparametric data were analyzed with the Kruskal-Wallis test followed by the Mann-Whitney U test. Incidence data were evaluated with the Fisher's Exact Test. For all statistical tests, the fiducial limit of 0.05 (two-tailed) was used as the criterion for significance.

Statistically significant effects for the Tyl et al. *(116)* study are listed in Table 3-10. The only maternal effects in the nose-only exposure groups were significant increases in absolute kidney weight at 1,000 and 2,500 mg/m^3 and relative kidney weight at 2,500 mg/m^3. There were no kidney lesions or differences in body weight gain, water intake, or the number of total or viable implants/litter. Significant fetal effects in the 2,500 mg/m^3 nose-only group included reduced body weights/litter, an increase in one type of skeletal malformation (fused ribs), and increases in some individual types of skeletal variations. When statistical analysis was conducting by combining all malformations or variations, statistical significance was not achieved in any group. Ossification was significantly reduced in several bones from litters of the 2,500 mg/m^3 group. One type of variation (extra ossification sites in the sagittal suture) was significantly increased in all nose-only treatment groups. Significant effects in the whole-body exposure positive control group included increased resorptions, decreased body weights, and increased skeletal malformations and variations. The study authors concluded that the nose-only exposure data indicate a NOEL **[NOAEL[6]]** of 1,000 mg/m^3 for developmental toxicity and 500 mg/m^3 for maternal toxicity.

Strengths/Weaknesses: The purpose of this GLP-compliant study by Tyl et al. *(116)* was to determine the effect of inhalation of ethylene glycol aerosols under conditions that obviated the confounding observed in Tyl et al. *(114)*. The authors recognized that this study also suffered from confounding. The dose received by each animal occurred by two routes because some material was still available for ingestion via preening of the face after removal from the aerosol, although not nearly as much as had been ingested after the whole-body exposure. Furthermore, the animals struggled a great deal during restraint, which was required for the nose-only exposure. A subsequent study *(118)* demonstrated that nose-only inhalation exposure of restrained pregnant mice to water aerosol resulted in variations and malformations that were qualitatively similar to those observed in the treated animals of the Tyl et al. *(116)* study, but occurring at a lower incidence. Therefore the Tyl et al. *(118)* study suggests that restraint of the mice could have contributed to the developmental effects observed in the litters of animals inhaling ethylene glycol mists.

Utility (Adequacy) for CERHR Evaluation Process: The Tyl et al. *(116)* study was well conducted. However, due to confounding caused by exposure via two routes and stress associated with restraint of the animals, the results of this study are not useful for evaluating effect levels for inhalation of ethylene glycol.

[6] Since the Expert Panel is considering only adverse effects in the selection of effect levels, the terminology of NOAEL will be used throughout this document.

Table 3-10. Prenatal Toxicity Study of Ethylene Glycol in CD Mice by Tyl *(116)* [also *(117)*].

Effect	Concentrations (mg/m^3)			
	0	**500**	**1,000**	**2,500**
Maternal kidney weight (g)	0.431	0.458	0.466*	0.472**
Maternal relative kidney weight (% body weight)	1.354	1.415	1.415	1.444*
Fetal body weight/litter (g)	1.289	1.281	1.310	1.184**
No. litters with fused ribs/ No. examined (%)	1/22 (4.5)	2/23 (8.7)	0/26 (0.0)	8/21* (38.1)
No. litters with reduced ossification	[a]	[a]	[a]	[a]
No. litters with extra ossification in sagittal suture/no. examined	12/22 (54.5)	23/23** (100)	24/26** (92.3)	21/21** (100)

Protocol: Mice inhaled ethylene glycol aerosols (nose-only exposure) from gd 6–15 and were sacrificed for evaluation of prenatal toxicity in fetuses from 21–26 litters/group on gd 18.

Notes:

*=p<0.05, **=p<0.01, ***=p<0.001

There were no effects on maternal body weight gain, water intake, kidney lesions, implantation sites or significant increases in total external, visceral, or skeletal malformations or variations.

[a]See text for details.

A positive control group exposed to 2100 mg/m^3 ethylene glycol (whole-body) had significant increases in resorptions and total skeletal malformations.

3.2.2.2 Rat

Tyl et al. *(114)* [also *(115)*] examined rat prenatal developmental toxicity in a study conducted according to GLP. Timed-pregnant Crl:COBS CD(SD)BR rats, 25/dose group, were exposed to a respirable aerosol of ethylene glycol (100% purity; MMAD of 2.3 μm; whole-body exposure) for 6 hours/day at daily doses of 0, 150, 1,000, or 2,500 mg/m^3 on gd 6–15. Concentrations were based on results of range-finding studies. Chamber concentrations were verified and it was found that chamber concentrations were below target concentrations (119, 888, and 2,090 mg/m^3). At scheduled sacrifice on gd 21, 20–25 dams/group were evaluated for body liver and kidney weight. The gravid uterus was weighed and examined for status of implantation sites. All live fetuses in 20–24 litters/group were counted, weighed, sexed, and examined for external malformations. In one-half of the fetuses, heads were fixed in Bouin's solution and examined and viscera were evaluated by the Staples method. The other half of fetuses were stained with Alizarin Red S and examined for skeletal malformations. Litters were considered the unit of comparison. Continuous variables were analyzed by the Levene's test, ANOVA, and t-tests with Bonferroni probabilities. Nonparametric data were analyzed with the Kruskal-Wallis test followed by the Mann-Whitney U test. Incidence data were evaluated with the Fisher's Exact test. For all statistical tests, a probability value of $p < 0.05$ (two-tailed) was used as the critical level of significance.

Incidences of statistically significant effects in the Tyl *(114)* [also *(115)*] study are summarized in Table 3-11. Maternal food and water consumption and body weights were unaffected by the chemical exposures. Absolute and relative liver weights of rats in the 2,500 mg/m^3 group were increased; organ histopathology was not evaluated. There were no effects on reproductive parameters including numbers of implantation sites and corpora lutea. Treatment had no effect on

prenatal mortality, fetal body weight, or the incidence of external, visceral, and skeletal malformations. **[Study tables, but not text, report a statistically significant increase in total visceral malformations for litters of the 2,500 mg/m^3 groups but this may be an error since only 1 fetus was observed to have a visceral malformation.]** In fetuses from the 1,000 and 2,500 mg/m^3 dose group, some fetal toxicity was expressed as reduced ossification (see Table 3-11). **[It does not appear that statistical analyses were conducted for total incidences of skeletal variations within dose groups.]** Authors identified a fetal NOEL of 150 mg/m^3. **[The Expert Panel disagreed with the authors selection of a NOEL [NOAEL[7]]. Reduced ossifications, deemed compound-related by authors, were seen at different sites in animals of the 1,000 and 2,500 mg/m^3 exposure groups. The lack of a dose-response relationship at ≥1,000 mg/m^3 suggests that the NOAEL exceeds 150 mg/m^3.]** The role of inhaled ethylene glycol in causing effects in this study was uncertain because the authors speculated that a majority of the dose received could have been from ingestion of ethylene glycol while grooming the fur. Authors estimated that inhalation and ingestion of ethylene glycol resulted in a total dose of 279–402 and 656–947 mg/kg bw/day in the 1,000 and 2,500 mg/m^3 groups, respectively. The dose estimates were formulated by measuring ethylene glycol levels in fur to determine potential ingestion and then using inhalation rates with assumed 10% and 90% retention to determine potential inhalation exposure.

Strengths/Weaknesses: This GLP-compliant study by Tyl et al. *(114)* was designed to permit the evaluation of exposure-response relationships for whole-body inhalation exposures to rats. As in the case with the study in mice, the exposed animals ingested test agent while preening after they had been removed from the exposure chambers. Confounding resulted from exposure occurring through two exposure routes.

Utility (Adequacy) for CERHR Evaluation Process: Due to the confounding caused by exposure via two routes, the results of the Tyl et al. *(114)* study are not useful for evaluating effect levels for inhalation of ethylene glycol.

[7] Since the Expert Panel is considering only adverse effects in the selection of effect levels, the terminology of NOAEL will be used throughout this document.

Table 3-11. Prenatal Toxicity Study in CD Rats by Tyl *(114)* [also *(115)*].

Effect	Doses (mg/m^3)			
	0	**150**	**1,000[a]**	**2,500[b]**
Maternal liver weight (g)	13.84 ± 1.72	14.01 ± 1.10	14.30 ± 1.39	15.00 ± 1.31*
Maternal Relative liver weight (% body weight)	4.76 ± 0.34	4.88 ± 0.34	4.86 ± 0.32	5.07 ± 0.34*
No. litters with some poorly ossified proximal phalanges/no. examined (%)	14/22 (63.6)	18/22 (81.8)	22/24* (91.7)	17/20 (85.0)
No. litters with some poorly ossified metatarsals/no. examined (%)	18/22 (81.8)	20/22 (90.9)	24/24* (100.0)	18/20 (90.0)
No. litters with poorly ossified humerus/no. examined (%)	0/22 (0.0)	0/22 (0.0)	1/24 (4.2)	4/20* (20.0)
No. litters with poorly ossified zygomatic arch/no. examined (%)	9/22 (40.9)	8/22 (36.4)	10/24 (41.7)	15/20* (75.0)

Protocol: Rats inhaled ethylene glycol mists (whole-body exposure) from gd 6–15 and were sacrificed for evaluation of prenatal toxicity in fetuses from 20–24 litters/group on gd 21.
Notes:
*=p<0.05, **=p<0.01, ***= p<0.001
[a]Authors estimated a total dose of 279–402 mg/kg bw/day from inhalation and ingestion.
[b] Authors estimated a total dose of 656–947 mg/kg bw/day from inhalation and ingestion.
There were no effects on maternal body weight, food or water intake, or implantation sites, prenatal mortality, fetal body weight, or total external, visceral, or skeletal malformations.

3.2.3 Dermal Exposure Studies

Tyl et al. *(119)* [also *(103)*] studied prenatal development toxicity resulting from dermal exposure to ethylene glycol in mice in order to determine if skin absorption contributed to developmental toxicity in a previous mouse whole-body inhalation exposure experiment *(114)*. The study was conducted according to GLP. Timed-pregnant Crl:CD-1 (ICR) BR mice (30/dose group) were exposed to ethylene glycol (100% purity) by occluded cutaneous application for 6 hours/day on gd 6–15 at doses of 0, 12.5, 50, or 100% ethylene glycol (w/v). Deionized water was used as the vehicle for the two lower doses. According to the study authors, those doses are equivalent to 0, 404, 1,677 and 3,549 mg/kg bw/day. A positive control group of 30 mice received a gavage dose of ethylene glycol in deionized water at 3,000 mg/kg bw/day on gd 6–15. The rationale for the dermal doses was not stated; but the oral dose was selected because it produced developmental toxicity in a study by Price et al. *(98)*. Concentrations of dosing solutions were verified. At scheduled sacrifice on gd 18, renal histopathology was examined in dams of the high-dose and control groups and implantation sites were examined in all dams (18–29 dams/group). A total of 16–23 litters/group were examined. In all fetuses, viscera were examined by the Staples method and skeletal effects were examined by staining with Alizarin Red S. Heads from half the fetuses were fixed in Bouin's solution and examined. The litter was the unit of comparison for analyses of data. Continuous variables were analyzed by Levene's test for equal variances, ANOVA, and

t-tests with Bonferroni probabilities. Nonparametric data were analyzed with the Kruskal-Wallis test followed by the Mann-Whitney U test. Fisher's Exact Test was used to evaluate incidence data. For all statistical tests, the fiducial limit of 0.05 (two-tailed) was used as the criterion for significance.

Statistically significant results of the Tyl et al. *(119*) study are outlined in Table 3-12. Dermally treated dams experienced no chemical-related clinical signs or adverse effects on body weight, water intake, liver or kidney weight, or renal histopathology. The number of resorptions or total implants/litter were not affected in the dermally treated group. The only fetal effect observed with dermal treatment was a statistically significant increase in the incidence of 2 skeletal variations (reduced ossification of skull bone and phalanges) in the 3,549 mg/kg bw/day group. Total external, visceral, or skeletal malformations were not significantly increased in any dose group. Fetuses in the positive control group experienced reduced body weights and increases in visceral malformations and skeletal malformations and variations. The authors concluded that the maternal and developmental toxicity NOEL **[NOAEL[8]]** for dermal exposure to ethylene glycol was at or near 3,549 mg/kg bw/day for undiluted (100%) ethylene glycol.

Strengths/Weaknesses: This GLP-compliant study by Tyl et al. *(119*) was designed to assess the potential developmental toxicity of cutaneously applied ethylene glycol on pregnant mice. The occlusion of the exposure sites precluded possible ingestion of ethylene glycol and thereby minimized potential confounding by multiple routes of exposure.

Utility (Adequacy) for CERHR Evaluation Process: The Tyl et al. *(119*) study is useful because it establishes the lack of effect (or very low impact) on development of dermally applied ethylene glycol in mice. Because blood levels were not measured, it is not known how much, if any, of the ethylene glycol was absorbed. This study is not useful for extrapolation of doses to humans.

[8] Since the Expert Panel is considering only adverse effects in the selection of effect levels, the terminology of NOAEL will be used throughout this document.

Table 3-12. Effects Associated with Dermally Applied Ethylene Glycol in a Prenatal Toxicity Study in CD-1 Mice by Tyl et al. *(119)* [also *(103)*].

Effect	Doses (mg/kg bw/day)			
	0	**404**	**1,677**	**3,549**
Maternal corrected body weight change[a] (g)	3.310	4.040	4.114	5.044*
No. litters with poorly ossified parietal/no. examined (%)	7/23 (30.4)	8/20 (40.0)	5/17 (29.4)	12/18* (66.7)
No. litters with unossified phalanges/no. examined (%)	8/23 (34.8)	12/20 (60.0)	8/17 (47.1)	13/18* (72.2)
NOAELs				Maternal and Fetal

Protocol: Mice were treated dermally with 0, 12.5, 50, or 100% ethylene glycol for 6 hours/day on gd 6–15. Sacrifice occurred on gd 18 for evaluation of prenatal developmental toxicity in fetuses from 17–23 litters/group.
Notes:
*=p<0.05, **=p<0.01, ***= p<0.001
[a]Body weight minus gravid uterine weight.
There were no effects on maternal body weight, water intake, liver or kidney weight, or renal histopathology or total visceral, skeletal, or external malformations or variations.
A positive control group treated with ethylene glycol by gavage had increases in visceral and skeletal malformations.

3.2.4 Mechanistic Studies

A series of studies were performed to determine if ethylene glycol-induced developmental toxicity is caused by ethylene glycol, its metabolites such as glycolic acid, or from metabolic acidosis or hyperosmolarity. In order to provide perspective, Table 3-13 outlines blood ethylene glycol and glycolic acid levels associated with human poisonings and developmental toxicity in rats. Additional studies have examined the developmental toxicity of other ethylene glycol metabolites, but there are no reports of those metabolites being detected in blood at significant levels in animal studies. For example, the blood level of oxalic acid was usually below the quantifiable limit of 4.9 μg/g **[0.054 mM]** in rats dosed with up to 2,500 mg/kg bw ethylene glycol *(44)*. In rats gavaged with 1,000 mg/kg bw ethylene glycol, blood levels of unresolved glyoxylate/glyoxal were about 2 orders of magnitude lower than ethylene glycol blood levels; glycolaldehyde was only detected once, at a level roughly 2 orders of magnitude lower than ethylene glycol, during a 12-hour period following dosing *(41, 43)*. The detection limit for glycolaldehyde was 33 ng/100 μL **[0.006 mM]**.

Table 3-13. Examples of Ethylene Glycol and Glycolic Acid Blood Levels.

Ethylene Glycol Exposure (Reference)	Estimated Peak Blood Ethylene Glycol Level (Reference)	Estimated Peak Blood Glycolic Acid Level (Reference)
1,000 mg/kg bw/day; LOAEL for developmental toxicity in rats *(107)*	14.3–21 mM *(41, 43, 44)*	4.8 mM *(44)*
2,500 mg/kg bw/day; teratogenic concentration in rats *(65)*	45–57 mM *(44)*	5.7–8.8 mM *(44, 65)*
Human poisoning cases *(56, 120)*	1–130 mM[a] *(56, 120)*	0–30 mM[a] *(56, 120)*

[a]May not be peak concentrations since they were measured from 1.5 to 30 hours following exposure.

In vivo and *in vitro* mechanistic studies are presented below in chronological order, according to publication dates.

Grafton and Hansen *(121)* investigated the direct effects of ethylene glycol on the *in vitro* development of whole CD rat embryos. Gd 10.5 embryos (11–18/group) were cultured in rat serum-containing medium with 0, 30, or 40 µL/mL **[0, 535, or 714 mM]** ethylene glycol (99.6% pure). The embryos were cultured for 8 hours (either hours 0–8 or hours 8–16 of a 48-hour culture period). Exposure duration was based on preliminary tests demonstrating that exposure to 30 µL/mL for 16 hours produced 100% embryo lethality. **[There was no discussion on rationale for dose selection.]** Following exposure, embryos were washed and transferred to fresh serum for either 40 or 32 hours. Embryos were evaluated for development, viability, and anomalies. Data were analyzed using one-tailed Dunnett's test for continuous data and Chi-Square for comparisons of discrete data ($p<0.05$). One embryo in each exposure group was dead after the culture period. Significant effects in the 40 µL/mL group included decreased crown-rump length and DNA content at hours 0–8 and 8–16 and decreased head length and somite numbers at hours 0–8. Significantly decreased protein content was seen in embryos exposed to both dose levels of ethylene glycol, with dose-related increases in hypoplastic telencephalon, lack of optic and otic development, absent hindlimb bud, and absent yolk sac circulation also observed. Most of the lesions were more numerous in the groups exposed during hours 8–16 (Table 3-14). Since the enzymes involved in ethylene glycol metabolism, alcohol and aldehyde dehydrogenases, were not present in the culture medium, the authors speculate that the observed effects were due to ethylene glycol itself.

Strengths/Weaknesses: The whole embryo culture technique is a good choice to examine the direct embryotoxic potential of ethylene glycol due to the embryo's very limited ability to metabolize the compound. Adequate numbers of embryos were evaluated in each group and statistical analysis appears appropriate. However, no rationale was presented for dose selection or for time of exposure. The levels of ethylene glycol that were used are much higher than blood levels observed with teratogenic exposures, and the levels producing abnormalities *in vitro* would probably never be achieved *in vivo*.

Utility (Adequacy) for CERHR Evaluation Process: Because of the high concentrations of ethylene glycol used, this work by Grafton and Hansen *(121*) is of no utility for the CERHR evaluation process.

Table 3-14. Embryotoxicity Observed Following *In Vitro* Exposure to Ethylene Glycol, Grafton and Hansen *(121*).

Concentration (µL/mL)	Exposure Period (hr)	Number of Embryos With Defect/ Number of Embryos Evaluated				
		Yolk Sac Circulation Absent	Hypoplastic Telencephalon	Absent Hindlimb Bud	No Otic Development	No Optic Development
0	-	0/18	0/18	0/18	0/18	0/18
30	0–8	2/12	1/12	1/12	1/12	1/12
30	8–16	7/15	4/15	5/15	0/15	0/15
40	0–8	4/16	0/16	2/16	5/16	5/16
40	8–16	11/15	2/15	9/15	2/15	7/15

In a two-phase study, Khera *(122*) investigated the roles of maternal acid-base electrolyte imbalance and histological changes in maternal/extraembryonic tissues in ethylene glycol-induced developmental toxicity. Pregnant Crl:Sprague-Dawley rats were treated on gd 11 (plug day=gd 1) with ethylene glycol (water vehicle) using several different exposure routes and doses, depending on the type of endpoint to be examined. The ethylene glycol was of unspecified purity but was described as analytical grade. Data were analyzed using Student's t-test ($p<0.05$).

An acid-base electrolyte imbalance study conducted first demonstrated that rats experience increased osmolal gap, hyperosmolality, and metabolic acidosis following exposure to 1,250, 2,500, or 5,000 mg/kg bw ethylene glycol by gavage or 3,333 mg/kg bw ethylene glycol administered SC. Maternal toxicity (depressed reflexes, ataxia, lethargy) was seen in rats dosed orally with 5,000 mg/kg bw ethylene glycol or SC with 3,333 mg/kg bw ethylene glycol; these symptoms were less marked at the other doses. Combined ethylene glycol-sodium bicarbonate ($NaHCO_3$) treatment resulted in significantly, but not entirely reduced osmolal gap, acidosis, and osmolality.

In the teratology portion of the experiment, Khera *(122*) dosed 10–15 dams/group with 2,800 or 3,333 mg/kg bw ethylene glycol by SC injection on gd 11. In parallel with each ethylene glycol treatment scenario, animals were simultaneously given 530 mg/kg bw sodium bicarbonate by gavage and drinking water containing 2.65 mg/mL sodium bicarbonate. This parallel group allowed the researchers to observe any effects sodium bicarbonate treatment might have on acid-base-electrolyte imbalance or fetal anomalies associated with ethylene glycol exposure. Negative water control groups were also included in the experimental design. Three of 13 dams in the 3,333 mg/kg bw group died; no maternal deaths were seen when sodium bicarbonate was simultaneously administered. Two-thirds of the fetuses were examined for skeletal effects and the remaining fetuses were checked for visceral defects. Fetal body weights were reduced in the group treated with 3,333 mg/kg bw ethylene glycol and skeletal anomalies (ribs, vertebrae, and sternebrae) were increased in the 2,800 and 3,333 mg/kg bw ethylene glycol groups. As noted in Table 3-15, treatment with sodium bicarbonate mitigated the fetal body weight effect in the 3,333 mg/kg group, and significantly reduced incidences of total skeletal anomalies in the 2,800 and

3,333 mg/kg bw groups. **[Reporting of statistical significance was limited to comparisons between groups dosed with ethylene glycol and groups dosed with ethylene glycol and sodium bicarbonate.]**

Strengths/Weaknesses: Generally adequate numbers of animals were used in each experimental group and fetuses were evaluated for signs of developmental toxicity using appropriate methods. This study monitored various clinical chemistry parameters following a teratogenic dose of ethylene glycol (e.g., plasma pH, PCO_2, bicarbonate levels, electrolyte levels, hemoglobin, osmolality). Sodium bicarbonate was administered to reverse some of the physiological effects of ethylene glycol; teratogenicity as well as the clinical chemistry parameters were determined in animals with and without sodium bicarbonate. No rationale was presented for the doses or the treatment time selected. Malformation data were not presented on a per litter basis. Insufficient detail was presented to determine if the statistical analysis was appropriate.

Utility (Adequacy) for CERHR Evaluation Process: These data would appear to be of little use for the CERHR evaluation process. Khera *(122)* demonstrated that some of the teratogenic effects of the high dose of ethylene glycol could be due to the metabolic acidosis produced by the chemical.

Table 3-15. Fetal Effects of Ethylene Glycol Exposure and Sodium Bicarbonate Treatment in Rats, Khera *(122)*.

Treatment	Parameter	
	Fetal Weight (g)	No. Fetuses With Skeletal Anomalies/No. examined [%]
Water Control	5.2	4/110 [3.6]
$NaHCO_3$ Control	5.2	11/106 [10.4]
2,800 mg/kg bw Ethylene Glycol	4.8	55/136 [40.4]
2,800 mg/kg bw Ethylene Glycol + $NaHCO_3$	5.1	20/128 [15.6]*
3,333 mg/kg bw Ethylene Glycol	4.6	70/82 [85.4]
3,333 mg/kg bw Ethylene Glycol + $NaHCO_3$	4.9*	46/83 [55.4]*

*Statistically significantly difference ($p<0.05$) compared to group dosed with the same concentration of ethylene glycol but no $NaHCO_3$.

A histopathological examination of maternal and placental tissues was performed by Khera *(122)* on dams treated with ethylene glycol SC on gd 7–13 with 3,333 mg/kg bw or with 500 mg/kg bw by gavage **[this group is apparently mistakenly described as "5,000 mg/kg/day" in the Methods section, but is later described as "500 mg/kg/day" in the Results section and in Figure 8]**. Seven of 8 conceptuses examined at 24 hours postdosing in the 3,333 mg/kg bw ethylene glycol group exhibited lesions in the chorioallantoic labyrinth and/or allantois, while none of these lesions were observed in controls. Simultaneous administration of sodium bicarbonate reduced this number to 4/12. A significant increase in the ratio of maternal to fetal vascular area in the labyrinth were seen at 48 hours postdosing in the 3,333 mg/kg bw ethylene glycol group; the ratio was reduced, but still significantly increased compared to control, when sodium bicarbonate was simultaneously administered. Larger maternal vascular spaces and

proportionally smaller allantoic villi and placental basal zone were also seen in the 500 mg/kg bw/day oral gavage dose group.

Khera *(122*) postulated that maternal metabolic acidosis and hyperosmolality may have contributed to reduction in villigenesis and developmental effects. The ethylene glycol-induced homeostatic changes in the mother and histologic changes in placentae were postulated to affect embryonic nutrition and, consequently, development.

Strengths/Weaknesses: Khera *(122*) used a dose of ethylene glycol that had previously been shown to be developmentally toxic. Although the uteri of 3–8 dams/test group were fixed, it is not clear how many conceptuses from each litter were examined.

Utility (Adequacy) for CERHR Evaluation Process: Although interesting, the results from the Khera *(122*) study appear to be of little use for the CERHR evaluation process. The results are consistent with the author's hypothesis that the observed placental changes could have affected embryonic nutrition.

Carney et al. *(123*) conducted two *in vitro* studies in rat embryos in order to determine the role of ethylene glycol, glycolic acid, acidity, and hyperosmolarity in ethylene glycol-induced developmental toxicity. In each study conducted according to GLP, embryos were obtained from Crl:CD (Sprague-Dawley) rats on gd 10.5 and treated with ethylene glycol, glycolic acid, or sodium glycolate (all chemicals greater than 98% pure) for 46 hours. Gd 10.5 embryos were selected to maintain consistency with the studies conducted by Grafton and Hansen *(121*) and Khera *(122*). Following the exposure period, embryos were monitored for viability, growth, and morphology. Before and after the exposure period, concentrations of ethylene glycol and glycolic acid were monitored by GC/MS to verify target concentrations and to ensure that ethylene glycol was not metabolized to glycolic acid by embryos. The pH of the media was also monitored. Statistical analyses included the Fisher's exact test for percentage data and Bartlett's test, ANOVA, Dunnett's test, and/or the Wilcoxon Rank Sum test with Bonferroni's correction for continuous data.

In the first study, Carney et al. *(123*) exposed 10 embryos/group to 0.5, 2.5, 12.5, 25.0, or 50.0 mM ethylene glycol or glycolic acid for 46 hours. A positive control group of 10 embryos was exposed to 1.0 mM sodium valproate. **[A negative control group was also used, but treatment of that group was not specified.]** The only effect in embryos treated with ethylene glycol was a very slight but significant reduction in the Brown-Fabro morphology score *(124*) in the 50 mM group, which the authors interpreted as an insignificant delay in development. In contrast, numerous developmental effects were noted in the embryos treated with ≥12.5 mM glycolic acid, including significant reductions in crown-rump length, somite number, morphology score, and embryo protein content. Embryo viability and yolk sac protein content were significantly reduced with exposure to ≥25 mM glycolic acid. Dysmorphogenesis was noted in 70 and 60% of embryos in the 12.5 and 50 mM glycolic acid groups, respectively, while no dysmorphogenesis was noted in controls. The structures in which morphological abnormalities were most commonly observed included the maxillary process, mid-facial regions, and telencephalic hemispheres. In the positive control valproate group, 100% dysmorphogenesis was observed in addition to signs of decreased growth.

In the second study, Carney et al. *(123*) incubated 12 embryos/group in a control medium with a pH of 7.41, control medium with a pH of 6.74, medium with 12.5 mM glycolic acid (pH=6.74), or medium with 12.5 mM sodium glycolate (pH=7.42). Results of that study are listed in Table 3-16. Adverse effects on growth and dysmorphogenesis in the 12.5 mM glycolic acid group were

consistent to those observed in the 12.5 mM glycolic acid group in the first study. Effects in the 12.5 mM sodium glycolate group were virtually identical to those observed in the 12.5 mM glycolic acid groups, but the magnitude of effects was slightly less. For example, the percentage of dysmorphogenic embryos in the glycolic acid and sodium glycolate groups was 67 and 58%, respectively. Significant effects noted in the pH 6.74 control group were reductions in head length and embryo- and yolk sac-protein content.

Based on the results of these experiments, Carney et al. *(123*) concluded that glycolic acid is the proximate developmental toxicant following ethylene glycol exposure and that acidity of culture medium, is only a minor contributor to the effects observed *in vitro*. The authors further explain that acidification of culture medium does not simulate metabolic acidosis occurring *in vivo* since it is a dynamic process that involves other factors such as reductions in PCO_2 and bicarbonate along with increases in lactate and glucose. Lastly, the authors noted that development was not apparently affected by the osmolarity of culture medium since no major effects were noted with exposure to a very hyperosmolar solution of 50 mM ethylene glycol.

Strengths/Weaknesses: The whole embryo culture technique is a good choice to examine the direct embryotoxic potential of ethylene glycol and glycolic acid due to the embryo's very limited ability to metabolize these compounds. The concentrations of both compounds were determined at the beginning as well as at the end of the culture period to ensure the correct starting concentration as well as to determine if there was embryonic metabolism. The doses of chemicals used were chosen based on previous pharmacokinetic data. The Carney et al. *(123*) study was also conducted under GLP conditions, and a positive control group was added to the study. Additionally, the types of dysmorphogenesis observed are similar to malformations produced *in vivo*. In the second experiment, a group was added to control for decreased pH as well as adding a group to specifically test the effect of the glycolate anion. The number of embryos treated in each group in both studies is somewhat small (n=5–12 embryos) and the developmental stage at the beginning of the culture period was not controlled (although not specifically stated, the animals were shipped from the supplier after verification of pregnancy and this probably resulted from an overnight breed).

Utility (Adequacy) for CERHR Evaluation Process: These data by Carney et al. *(123*) are of high utility for defining the proximate developmental toxicant following ethylene glycol exposure in rats. Carney et al. *(123*) is a well-conducted study that demonstrated developmental toxicity of glycolic acid specifically that was not due to changes in pH or hyperosmotic conditions in the media. This study also demonstrated no developmental toxicity of ethylene glycol at concentrations similar to those observed in *in vivo* studies.

Table 3-16. Effects Observed in an *In Vitro* Study of Ethylene Glycol, Carney et al. *(123)*.

Effect	Treatment			
	Control (pH=7.41)	Control (pH=6.74)	12.5 mM Glycolic Acid (pH=6.74)	12.5 mM Sodium Glycolate (pH=7.42)
Crown-rump length (mm)	4.8	4.5	3.6*	4.3*
Head length (mm)	2.6	2.3*	2.1*	2.2*
Embryo protein [units not specified]	989	771*	399*	596*
No. somites	31.8	30.5	20.2*	25.8*
Morphology score	48.9	47.9	41.0*	43.9*
Visceral yolk sac (mm)	6.9	6.6	5.8*	6.1*
Visceral yolk sac protein (μg)	456	345*	301*	330*
No. dysmorphogenic/ no. evaluated (%)	0/12 (0)	1/12 (8%)	8/12 (67%)*	7/12 (58%)*

Notes: *=p<0.05

In a subsequent publication, Carney et al. *(65)* examined the roles of glycolic acid and metabolic acidosis in producing developmental toxicity *in vivo* using Crl:Sprague-Dawley rats in a GLP-compliant study. Twenty-five rats/group received one of the following treatments on gd 6–15: gavage with 2,500 mg/kg bw ethylene glycol (40.3 mmol/kg bw; 99.98% purity); gavage with 650 mg/kg bw glycolic acid (8.5 mmol/kg bw; 99.7% purity); SC injection with 833 mg/kg bw sodium glycolate (8.5 mmol/kg bw; ≥98% purity); or gavage with the deionized water vehicle. Concentrations of dosing solutions were verified. In the first phase of the study, it was verified that each treatment produced identical peak serum-glycolate levels (8.4–8.8 mM) and that metabolic acidosis was produced in the groups receiving ethylene glycol or glycolic acid by gavage but not in the group receiving sodium glycolate by SC injection. However, the AUC for glycolate was found to be 3-fold higher when ethylene glycol exposure data were compared to glycolic acid or sodium glycolate exposure data. Following sacrifice of dams on gd 21, at least half the fetuses were dissected under a stereomicroscope and examined for visceral malformations according to the Staples method; the heads of those fetuses were preserved in Bouin's solution and examined. The skeletons of the remaining fetuses were evaluated by staining with Alizarin Red S. Data were evaluated by Bartlett's test, ANOVA, 2-sided Dunnett's test, Wilcoxon Rank-Sum test with Bonferroni's corrections, and/or Fisher Exact Probability Test.

A total of 21–25 litters/group were examined. Treatment-related deaths were observed in four dams of the glycolic acid group. Resorptions were slightly elevated in all treatment groups and reached statistical significance in the ethylene glycol group. Fetal weights were significantly reduced in all three treatment groups, with the effect most pronounced with ethylene glycol

treatment. The primary effects in the ethylene glycol group included significantly increased incidences of axial skeleton defects, cranial neural tube defects, craniofacial defects, abdominal wall defects, and skeletal variations. The pattern of malformations in the glycolic acid group was similar to that of the ethylene glycol group except that there were no cranial neural tube and craniofacial defects observed. Incidences of malformations in each treatment group are outlined in Table 3-17. Several skeletal malformations were significantly increased in the glycolic acid group but occurred at a lower incidence than the ethylene glycol group. The only significant effects in the sodium glycolate group were increased incidence of skeletal variations that were also observed with ethylene glycol treatment. The severity of malformations in the sodium glycolate group was less than that of the ethylene glycol and glycolic acid groups. All malformations seen in the glycolic acid and sodium glycolate group also occurred in the ethylene glycol group. According to the study authors, the data indicate that glycolate ion alone can produce developmental toxicity, but that metabolic acidosis is a major exacerbating factor. The authors also speculated that the reason why cranial neural tube and craniofacial defects were observed only with exposure to ethylene glycol was because of the 3-fold higher glycolate AUC that occurred with ethylene glycol versus glycolic acid or sodium glycolate treatment.

Strengths/Weaknesses: Carney et al. *(65*) is a well-conducted GLP study performed in accordance with regulatory guidelines and standard practices using appropriate numbers of animals and statistical analyses. Fetuses were evaluated for signs of developmental toxicity using appropriate methods. Although only single doses of each compound were used, pharmacokinetic analyses were conducted on gd 10 to ensure that plasma levels of glycolic acid were similar in the treatment groups. The defects observed in this study after ethylene glycol treatment are similar to those observed by Price et al. *(98*).

Utility (Adequacy) for CERHR Evaluation Process: The Carney et al. *(65*) study provides data suggesting that both glycolic acid and the metabolic acidosis produced by ethylene glycol or glycolic acid are involved in the mechanism of teratogenicity. There was some evidence of maternal toxicity in the groups administered ethylene glycol and glycolic acid and this may have contributed to the observed developmental toxicity. There was no evidence of maternal toxicity in the sodium glycolate group (except for increased liver weight), and no malformations (only skeletal variations) were observed in this group. The increased AUC for glycolic acid after ethylene glycol administration (which would probably be further exacerbated by repeated dosing with ethylene glycol [not discussed by the authors]), provides a plausible explanation for the higher incidences and more severe defects produced by ethylene glycol.

Table 3-17. Incidence of Rat Malformations Following Exposure to Glycolic Acid, Sodium Glycolate, or Ethylene Glycol, Carney et al. *(65)*.

Malformation	% of Affected Litters in Each Treatment Group			
	Control	Glycolic acid	Sodium Glycolate	Ethylene Glycol
Meningoencephalocele	4.2	0	0	25.0*
Exencephaly	0	0	0	25.0*
Cleft lip	0	0	0	29.2*
Cleft palate	0	0	0	29.2*
Omphalocele	0	0	0	54.2*
Dilated cerebral ventricles	0	19.0	0	33.3*
Hemivertebra	0	71.4*	4.0	95.8*
Extra vertebrae	0	4.8	0	29.2*
Missing vertebrae	0	28.6*	0	62.5*
Fused vertebrae	0	19.0	0	75.0*
Fused centra	0	4.8	0	33.3*
Fused ribs	0	42.9*	0	95.8*
Missing ribs	0	71.4*	4.0	91.7*

*Significantly different from control values by censored Wilcoxon test (α=0.05).

Munley et al. *(125)* examined the developmental toxicity of glycolic acid in Crl:CD®BR rats. Twenty-five dams/group were randomly assigned to groups and dosed with 0 (water control), 75, 150, 300, or 600 mg/kg bw glycolic acid (99.6% purity) in water by gavage on gd 7–21. Dose levels were based on a screening study that demonstrated maternal and developmental toxicity at 350 mg/kg bw and greater. **[Blood levels of glycolic acid were not measured. However, based on data by Carney et al. *(65)*, it is expected that the glycolic acid blood level in the 600 mg/kg bw group would be 8 mM or lower and that glycolic acid blood level was obtained following gavage treatment with 2,500 mg/kg bw ethylene glycol *(44, 65)*.]** Concentrations of dosing solutions were verified through an acid/base titration method. Maternal toxicity was evaluated by assessing body weight and food intake. On gd 22, dams were sacrificed for an evaluation of implantation sites and fetal toxicity. Uteri of apparently non-pregnant dams were stained with ammonium sulfide to check for resorptions. A total of 23–25 litters/group were evaluated. Fetuses were sexed, weighed, and examined for external malformations. Skeletal effects were examined in all fetuses by fixing them in 70% ethanol, macerating with 1% potassium hydroxide, and staining with Alizarin Red S. Visceral effects were examined according to the Staples method in every other fetus and in fetuses with skeletal malformations. Heads of half the fetuses were fixed in Bouin's solution and examined. Statistical analyses for maternal effects included ANOVA or the Cochran-Armitage test. The litter was considered the statistical unit for evaluation of developmental effects and statistical analyses included Jonckheere's test and analysis of covariance (ANCOVA).

Maternal body weight gain during treatment and final body weight adjusted for gravid uterine weight were significantly reduced in the 600 mg/kg bw/day group. A slight but significant

reduction in food intake was noted at this dose only from gd 21 to 22. Clinical signs observed in dams of the 600 mg/kg bw/day group included abnormal gait, lung noises (wheezing and/or rattling), and irregular respiration. Lung noises (wheezing and/or rattling) were also heard in 2 dams of the 300 mg/kg bw/day group. There were no effects on reproductive parameters including resorptions, numbers of corpora lutea and implantation sites, litter size, or sex ratio at any dose. Statistically significant effects in fetuses are outlined in Table 3-18. Developmental toxicity was observed in the 600 mg/kg bw/day group and included significantly reduced fetal weight and increased numbers of litters containing fetuses with skeletal malformations and variations. Malformations included missing ribs and fused ribs, vertebrae, and sternebrae. In the 300 mg/kg bw/day dose group, fused ribs and sternebra were noted in 2 fetuses from 2 litters (p=0.055); the authors considered the effect to be relevant to treatment since it was consistent with effects noted at 600 mg/kg bw/day. Fetal mortality was not affected at any dose. The study authors identified 150 mg/kg bw/day as a maternal and developmental NOEL **[NOAEL[9]]**.

Strengths/Weaknesses: Munley et al. *(125)* is a well-conducted study done according to regulatory guidelines and standard practices using appropriate numbers of animals. The length of the dosing period was slightly different than that used by Carney et al. *(65)*. Plasma glycolic acid levels were not determined in this study, but the high dose of glycolic acid administered was nearly the same as that administered by Carney et al. *(65)* and should have produced similar blood levels. Although many of the skeletal malformations observed in this study are the same as those observed by Carney et al. *(65)*, the visceral and external defects observed by Carney et al. were not observed. Malformations were only observed at the highest dose used, and that dose produced maternal toxicity (decreased body weight and weight gain and increased clinical signs). Some of the clinical signs observed in this study were similar to those observed by Carney et al. *(65)*.

Utility (Adequacy) for CERHR Evaluation Process: These data are useful for the CERHR evaluation process. Data from the Munley et al. *(125)* study suggests that glycolic acid may be teratogenic, but only at doses that also produce maternal toxicity.

Table 3-18. Incidence of Skeletal Malformations in Rats Administered Glycolic Acid, Munley et al. *(125)*.

Malformation	No. Affected Litters/No. Examined (%) in Control and Two Highest Dose Groups		
	Control	300 mg/kg bw	600 mg/kg bw
Missing rib	0	0	3/23 (13.0)*
Fused ribs	0	2/23 (8.7)**	9/23 (39.1)*
Fused sternebrae	0	0	3/23 (13.0)*
Non-fused sternebrae	4.0	1/23 (4.3)	5/23 (21.7)*
Fused vertebrae	0	2/23 (8.7)**	6/23 (26.1)*
Hemivertebrae	0	1/23 (4.3)	8/23 (34.8)*

*Statistically significant (p≤0.05)
**p=0.0555

[9] Since the Expert Panel is considering only adverse effects in the selection of effect levels, the terminology of NOAEL will be used throughout this document.

Klug et al. *(126*) conducted an *in vitro* study to examine the developmental toxicity of ethylene glycol and its metabolites in rat embryos. Embryos were obtained from Wistar rats (Bor: Wisw/SPF, TNO) on gd 9.5 and incubated for 48 hours in media containing 50–200 mM ethylene glycol, 0.03–0.3 mM glycolaldehyde, 1–10 mM glycolic acid, 3–6 mM glyoxal, 0.3–1 mM glyoxylic acid, or 1–2 mM oxalic acid **[purity of chemicals not specified]**. Untreated control embryos were also examined. A total of 38 embryos in control groups and 5–19 embryos/treatment group were evaluated for viability, growth, differentiation, and dysmorphogenesis. Statistical analyses included the Mann-Whitney test and t-test. Effects and their statistical significance are outlined in Table 3-19. In embryos treated with 200 mM ethylene glycol, significant effects included reduced yolk sac diameter, crown-rump length, protein content, and differentiation score. A 47% rate of dysmorphogenesis was noted, and effects most commonly observed included defects in the head region, incomplete flexion, and unclearly shaped somites. Growth and differentiation were adversely affected in embryos treated with ≥3 mM glycolic acid. Rates of dysmorphogenesis were 25 and 88% in the 6 mM and 10 mM glycolic acid groups, respectively. Defects were most often noted in the head and shape (rotation) of the embryo; somites could not be counted in the 10 mM group. Similar effects were noted with the other metabolites that were about 3- to 10-fold more potent than glycolic acid (Table 3-19). As noted by study authors, embryotoxic levels of glycolic acid *in vitro* were consistent with glycolic acid blood levels observed following administration of developmentally toxic doses of ethylene glycol to rats. However, the embryotoxic concentrations of ethylene glycol and the other metabolites *in vitro* most likely exceeded blood levels that would be obtained with exposure to toxic doses of ethylene glycol.

Strengths/Weaknesses: The whole embryo culture technique is a good choice to examine the direct embryotoxic potential of ethylene glycol and its various metabolites due to the embryo's very limited ability to further metabolize these compounds. Six different compounds were tested *in vitro* with multiple doses of each compound used. However, dose-response data were limited with glyoxal, glyoxylic acid, and oxalic acid since only two doses of each of these compounds were used. For most of the treatments, adequate numbers of embryos (15–19) were evaluated; however, in some groups only a few embryos (5–7) were examined. All data from control embryos were pooled, and the same control data are presented for each compound. Although it was not stated that the developmental stage of the embryos was controlled at the beginning of the experiment, a shortened breeding period (2 hours) was used, so the developmental stage of all embryos should have been similar at the beginning of culture. Insufficient detail was presented to determine if the statistical analysis was appropriate.

Utility (Adequacy) for CERHR Evaluation Process: The data in the Klug et al. *(126*) study are somewhat useful for the CERHR evaluative process in that the data on ethylene glycol and glycolic acid basically confirm the data presented by Carney et al. *(123*). Neither study found developmental toxicity at doses of ethylene glycol that could be reasonably achieved *in vivo* and glycolic acid at 3 or 12.5 mM was embryotoxic. These concentrations of glycolic acid in the Klug et al. *(126*) study are within the range achieved after a teratogenic dose of ethylene glycol *in vivo* [~8 mM; Carney et al. *(65*)]. The concentrations of the other metabolites are higher than would be anticipated *in vivo* after a toxic exposure to ethylene glycol. The utility of the rest of the data to the CERHR evaluative process is limited by the pooling of control data and questionable statistical analysis.

Table 3-19. *In Vitro* Experiment with Ethylene Glycol and Metabolites, Klug et al. *(126)*.

Chemical: Concentration in mM (number embryos evaluated)	Effects				
	Yolk Sac (mm)	Crown-Rump Length (mm)	Somites (n)	Protein (μg/embryo)	Score
Control (38)	**4.47**	**3.57**	**26**	**188**	**36**
Ethylene glycol:					
50 (7)	4.20	3.36	25	180	35
100 (15)	4.20*	3.48	25	175	35
200 (15)	4.08**	3.36*	26	142**	33**
Glycolic acid:					
1 (5)	3.90**	3.66	26	219	37
3 (17)	4.44	3.42**	26	149*	35
6 (16)	4.20*	2.91**	24** (3)[a]	106**	32**
10 (16)	3.21**	2.40*	ND	68**	25**
Oxalic acid:					
1 (16)	4.68	3.42*	26	193	36
2 (16)	3.90**	3.00**	23** (3)[a]	136**	25**
Glycolaldehyde:					
0.03 (19)	4.56	3.66	26	163	36
0.1 (16)	4.50	3.72	26	205	37*
0.2 (18)	4.05**	3.39*	25	161*	36
0.3 (5)	3.00**	2.70**	21 (1)[a]	114**	29**
Glyoxal:					
3 (16)	4.5	3.66	27*	188	37*
6 (18)	3.60**	2.79**	26	113**	30**
Glyoxylic acid:					
0.3 (19)	4.32*	3.60	26	190	36
1 (17)	2.88**	2.52**	24** (8)[a]	77**	29**

Notes: *=0.01≤p≤0.05 [sic], **=p≤0.01
[a]Number in parentheses indicates the numbers of embryos in which somites could be counted.
ND=Could not be determined.

Carney et al. *(61)* conducted a study to determine the dose-rate effects of ethylene glycol on developmental toxicity. Pregnant CD (CRL: CD (SD) IGS BR) rats were randomly assigned to groups that received 0, 1,000, or 2,000 mg/kg bw/day ethylene glycol (99.91% purity) on gd 6–15. Eighteen rats/group received bolus doses by SC injection, while 16–20 rats/group were slowly and continuously dosed through SC pumps. Distilled water was the vehicle control. The lower dose was approximately equal to the LOAEL observed in the Neeper-Bradley et al. *(107)* study in rats, and the high dose was the maximum amount that could be delivered by SC pump. The SC route was previously demonstrated to be effective in producing ethylene glycol-induced developmental toxicity by Khera *(122)*. Concentrations of dosing solutions were confirmed to be within 100–102% of target values. Blood levels of ethylene glycol and glycolic acid were measured by GC/MS in rats treated by bolus injection or continuous infusion (3 rats/group) on gd 7, 9, 12, and 15. Blood samples were taken from the bolus exposure group at 3 hours following treatment, but it is not clear when blood was taken from animals treated by continuous infusion.

Maternal toxicity was assessed by observing clinical signs and measuring body weight gain, food consumption, and urinalysis parameters on gd 7 and 15, and liver and kidney weight on gd 21. On gd 21, dams were sacrificed and necropsied. Implantation sites were examined, corpora lutea were counted, and uteri of apparently non-pregnant rats were stained with 10% sodium sulfide to determine if early resorptions occurred. All fetuses were sexed, weighed, and examined for viability and external malformations. About half of the fetuses were stained with Alizarin Red S and examined for skeletal malformations. Heads from the remaining fetuses were preserved in Bouin's solution and sectioned to evaluate craniofacial defects. Statistical analyses included Bartlett's test for equality of variances, ANOVA, Dunnett's test, the Wilcoxon Rank-Sum test with Bonferroni's correction, the Fisher exact probability test with Bonferroni's correction, and binomial distribution test.

In the 1,000 and 2,000 mg/kg bw/day bolus groups, the mean blood levels of ethylene glycol (9.5 mM and 21.9 mM, respectively) and glycolic acid (3.3 mM and 6.3 mM, respectively) were higher compared to mean ethylene glycol (2.3 mM and 4.3 mM, respectively) and glycolic acid (0.1 mM and 1.0 mM, respectively) blood levels in rats treated by SC pump. Maternal and fetal toxicity were evident in the groups treated with bolus doses. Dams in both ethylene glycol bolus groups had reddish-colored urine, changes in urinalysis parameters (i.e., non-significant increases in protein, bilirubin, and blood), and increased relative and absolute liver weight. Additional effects noted in dams of the 2,000 mg/kg bw/day bolus group included uncoordinated gait, reduced activity, decreased body weight gain on gd 6–9 and 6–21, reduced food intake on gd 6–9, and increased relative kidney weight (no histopathology conducted). In litters from the 1,000 mg/kg bw/day bolus group, a non-significant increase in extra vertebrae and ribs and significantly reduced ossification of several axial skeleton structures were considered to be treatment-related by authors. Also noted in litters of the 2,000 mg/kg bw/day group were significant increases in axial skeleton malformations and significantly reduced fetal body weight. No dose-related evidence of maternal or fetal toxicity was noted in rats treated with infusion pumps. According to the study authors, increased body weight gain in dams of the 2,000 mg/kg bw/day infusion pump group was likely due to increased litter size. When terminal body weights were corrected for gravid uterine weight, the 2,000 mg/kg bw/day infusion group did not differ from controls. Carney et al. *(61*) concluded that "these data support the hypothesis that dose-rate is a critical determinant of ethylene glycol-induced developmental toxicity and demonstrate how bolus dosing studies can greatly overestimate the risk to humans of ethylene glycol exposures, which typically involve low doses and/or slow dose-rates." **[Bolus dosing may, however, provide useful data for predicting effects resulting from poisonings.]**

Strengths/Weaknesses: Carney et al. *(61*) is a well-conducted GLP study using appropriate numbers of animals and statistical analyses. Fetuses were evaluated for signs of developmental toxicity using appropriate methods. The purity of the test chemical was known, and the doses administered to animals were analyzed and found to be 100–102% of their target.

Utility (Adequacy) for CERHR Evaluation Process: The data presented in the Carney et al. *(61*) study are useful for the CERHR evaluative process and demonstrate that developmental toxicity is more closely related to blood concentrations of glycolic acid than to administered dose of ethylene glycol. The data presented in this study are consistent with previous reports. The malformations observed in this study after bolus administration of 2,000 mg/kg bw (primarily skeletal defects – fused or missing ribs, decreased ossification of vertebral centra) were similar to defects reported by Khera *(122*) after SC administration of 2,800 mg/kg; glycolic acid levels in these animals were approximately 6.3 mM. No malformations were observed when the same dose of ethylene glycol was administered by SC pump; blood levels of glycolic acid in these animals were 1.0 mM. Previous *in vitro* studies had shown embryotoxicity of glycolic acid at 3–6 mM

(126) or 12.5 mM *(123)*. Therefore, these data support the hypothesis that the proximate teratogen is glycolic acid. A detailed evaluation regarding toxicokinetic parameters of this study is included in Section 2.1.3.2.

Carney et al. *(45)* conducted preliminary and probe studies to determine if differences in susceptibility to ethylene glycol-induced toxicity in rabbits and rats is due to differences in toxicokinetics or in the amount of toxic metabolites reaching the embryo. In both GLP-compliant experiments, isotopically normal and/or $^{13}C_2$-ethylene glycol (99.7% purity and 96.7% purity, respectively) were administered in aqueous solution by gavage to pregnant 5–6-month-old New Zealand White rabbits and 8–9-week-old Sprague-Dawley rats. Doses were based on effects observed in teratogenicity studies *(35, 97)* as described in Section 3.2.1. Dosing solutions were analyzed to verify target concentrations, stability, and homogeneity. Levels of ethylene glycol and its metabolites in body fluids were analyzed by GC/MS. Because low numbers of animals were used, results were only evaluated by descriptive statistics such as mean±SD.

In the preliminary study, 3 pregnant rabbits were gavage dosed with 2,500 mg/kg bw ethylene glycol on gd 9, and blood samples were collected at time intervals between 0.5 and 24 hours after dosing for an analysis of acid-base balance and ethylene glycol, glycolic acid, and oxalic acid levels. None of the rabbits experienced metabolic acidosis as determined by blood pH, PCO_2, or bicarbonate levels. Absorption of ethylene glycol was rapid with peak blood levels of parent compound occurring at 1 hour post-dose. Glycolic acid levels increased slowly with no clear C_{max} in most animals. Authors stated that blood glycolic acid levels remained constant and within peak values at 4–12 hours post-dose. Blood oxalic acid levels were not consistently increased over baseline levels. The study authors noted that in contrast to rats, rabbits do not develop metabolic acidosis with exposure to 2,500 mg/kg bw ethylene glycol and experience a slower increase in blood glycolic acid levels with peak blood levels occurring at approximately 1 and 4–12 hours, respectively.

A probe study was next conducted by Carney et al. *(45)* to compare levels of ethylene glycol, glycolic acid, and oxalic acid in maternal blood and extraembryonic fluids in rats and rabbits. Gavage doses of 500 and 2,500 mg/kg bw were administered to 6 pregnant rats/dose group on gd 10 and 6 pregnant rabbits/dose group on gd 9. Authors stated that gd 10 in rats and gd 9 in rabbits represent equivalent periods (early somite stages) of development. Blood and extraembryonic fluid (pooled by litter) were collected at 1 hour post-dose in 3 rats and rabbits/dose group, at 3 hours post-dose in 3 rats/dose group, and at 6 hours post-dose in 3 rabbits/dose group. The first collection period was based on the estimated time of peak ethylene glycol concentrations and the second collection period was based on estimated time of peak glycolic acid levels in each species. Concentrations of ethylene glycol and glycolic acid in maternal blood and extraembryonic fluids are listed in Table 3-20. Maternal blood levels of ethylene glycol were very similar in rats and rabbits at both doses and time periods, leading study authors to conclude that oral absorption does not account for the different sensitivity in ethylene glycol toxicity in these two species. Levels of ethylene glycol in extraembryonic fluid were about twice as high in rats at 1 hour post-dose, but were approximately equal at 3 and 6 hours post-dose in rats and rabbits, respectively. In rabbits, glycolic acid concentrations were 3–18 fold lower in blood and 4–38 fold lower in extraembryonic fluid than in rats. The authors suggested this may represent a quantitative difference in metabolism or elimination between the two species. Levels of glycolic acid in extraembryonic fluid exceeded maternal blood levels in rats, but in rabbits levels of glycolic acid were usually lower in extraembryonic fluid than in maternal blood. According to authors, glycolate ion "trapping" appears to occur in the rat but not rabbit conceptus. "Trapping" is a term used to describe passage of the non-ionized form of a weak acid (present at low blood pH) across cell membranes to the extraembryonic fluid; the higher pH of the extraembryonic fluid causes the

weak acid to become ionized and thus unable to diffuse back across cell membranes. Levels of oxalic acid were near or lower than background levels, but were slightly elevated in EEF from rats dosed with 2,500 mg/kg bw. In cases where oxalic acid was detected, levels in rabbits were about half of those in rats. Study authors postulated that oxalic acid may be an additive factor in rat developmental toxicity at very high doses.

It was noted by Carney et al. *(45*) that absolute blood values of ethylene glycol and its metabolites were about 3-fold higher in the preliminary toxicokinetic study compared to values obtained at comparable time periods in rabbits from the probe study. They determined that differences were most likely due to variability between animals and slight differences in analytical conditions.

Strengths/Weaknesses: The Carney et al. *(45*) study was conducted under GLP conditions with test material of known purity. Doses of ethylene glycol that had previously been examined *in vivo* were used. Although the study was well conducted, very small numbers of animals were used, and only descriptive statistics were presented.

Utility (Adequacy) for CERHR Evaluation Process: These data by Carney et al. *(45*) are very interesting, but due to their preliminary nature, they are not very useful for the CERHR evaluative process. They do present a plausible explanation for differences in developmental toxicity between rats and rabbits. These results should be followed up by a more thorough experimentation.

Table 3-20. Comparison of Maternal Blood (MB) and Extraembryonic Fluid (EEF) Levels of Ethylene Glycol and Glycolic Acid in Rats and Rabbits Dosed with Ethylene Glycol, Carney et al. *(45*).

Ethylene Glycol mg/kg bw	Hours Post Dose	Ethylene Glycol Level (mM)[a]		EEF/MB for Ethylene Glycol	Glycolic Acid Level (mM)[a]		EEF/MB for Glycolic Acid
		MB	EEF		MB	EEF	
Rat:							
500	1	7.48	7.56	1.01	1.50	2.60	1.74
500	3	3.97	3.55	0.89	2.20	3.96	1.80
2,500	1	27.48	30.66	1.12	4.87	6.47	1.33
2,500	3	18.22	26.04	1.43	8.89	15.33	1.72
Rabbit:							
500	1	7.01	4.08	0.58	<0.07	<0.07	No data
500	6	2.87	4.66	1.62	0.73	1.04	1.42
2,500	1	26.93	16.72	0.62	0.56	0.18	0.32
2,500	6	18.58	28.79	1.55	3.62	2.67	0.74

[a]Study authors stated that this data should not be considered definitive.

The Panel noted some differences between placentas of rodents versus other species. Mice and rats, both of which exhibit developmental toxicity after exposure to ethylene glycol, have brief gestation periods (16–22 days). In order to establish a channel of physiologic exchange between the maternal system and the developing embryos, these quickly developing species establish an early placenta (the inverted visceral yolk sac placenta) that is eventually replaced by the definitive chorioallantoic placenta. Establishment of the rodent yolk sac placenta results in formation of a vesicle that contains the embryo and is filled with exocoelomic fluid. Relative to maternal

plasma, the yolk sac fluid of rats and mice concentrates weak acids, including glycolic acid. The rabbit, which has an incomplete yolk sac vesicle, does not concentrate glycolate or other weak acids in the fluid that surrounds the embryo *(45*) and does not exhibit developmental toxicity after exposure to ethylene glycol. Humans never have a yolk sac placenta nor are human embryos contained in a fluid filled yolk sac cavity. In contrast to rats and mice, but similar to rabbits, the fluid that surrounds human embryos does not concentrate weak acids relative to maternal blood levels. These species differences in placentation may play a key role in the developmental toxicity of ethylene glycol, although other aspects of pharmacokinetics and pharmacodynamics may also contribute.

3.2.5 Screening Studies

A series of screening studies demonstrated developmental toxicity that was generally consistent with effects noted in standard teratogenicity studies. Three studies reported effects on prenatal and postnatal growth and mortality in the offspring of CD-1 mice treated by gavage with ethylene glycol on gd 6–, 7–, or 8–14 and examined until pnd 4 *(99, 127, 128*). Pup weights on pnd 1 and 4 were significantly reduced in mice treated with 2,500 mg/kg bw/day *(128*). Significant reductions in the numbers of viable litters and live pups per litter and increases in numbers of dead pups at birth were observed in mice treated with 11,090 mg/kg bw/day ethylene glycol *(99, 127*). These studies were conducted to validate screening systems but provide little useful information for the CERHR evaluation process.

The Panel is aware of a Frog Embryo Teratogenesis Assay (FETAX) conducted with ethylene glycol *(129*). The Panel questioned the usefulness of the assay for evaluating human developmental toxicity since the assay is conducted in tetraploid amphibians, which do not share characteristics of mammals, and does not produce specific, reproducible malformations.

3.3 Utility of Data

There are no human data to evaluate developmental toxicity of ethylene glycol.

A series of GLP studies used appropriate methods to examine prenatal developmental toxicity of ethylene glycol in rats and mice exposed by oral gavage, rats exposed through diet, rabbits exposed by oral gavage, and mice exposed dermally during periods of organogenesis (gd 6–15 in mice and rats, gd 6–19 in rabbits). Appropriate endpoints such as teratogenicity, fetal growth, and embryo/fetal mortality were examined in those studies. Doses were appropriate since they exceeded concentrations relevant to expected human environmental and occupational exposures.

Scientifically sound studies that examined prenatal developmental toxicity in rats and mice exposed by inhalation were of little utility due to unavoidable confounding by oral exposure during whole-body and nose-only exposure and stress induced by restraint during nose-only exposure.

Postnatal growth and development were examined in a GLP study that exposed rats to ethylene glycol by gavage on gd 6–20; appropriate parameters were evaluated including a limited number of neurobehavioral tests (i.e., exploratory behavior and visual discrimination) and sexual development landmarks such as testes descent and vaginal opening.

Mechanistic studies in rats and toxicokinetic studies in rats and mice are useful to the evaluation process since they provide information on the likely proximate teratogen, effects of dose rate, and possible reasons for interspecies sensitivity in developmental toxicity.

In summary, the animal developmental toxicity data is adequate and assumed relevant for assessing hazards in humans exposed to ethylene glycol through the oral and dermal routes. However, the Panel acknowledges that sensitivities among laboratory animal species and humans may vary due to factors such as differences in placental development, toxicokinetics and metabolism, and ontogeny of metabolic enzymes.

3.4 Summary

3.4.1 Human Data

No human data were identified.

3.4.2 Experimental Animal Data

There are sufficient data to conclude that prenatal oral exposure to high doses of ethylene glycol causes developmental toxicity in rats and mice. The results of the studies are summarized below and outlined in Table 3-21. The Expert Panel noted that ethylene glycol was delivered by gavage in the majority of oral exposure studies, but that bolus doses do not represent expected human environmental exposures. Developmental toxicity was not observed in rabbits orally exposed to ethylene glycol at doses associated with severe maternal toxicity. Aerosol studies are inconclusive in determining if developmental toxicity occurs by this exposure route due to oral exposure associated with grooming. Dermal exposure was not associated with developmental toxicity in studies with mice.

3.4.2.1 Oral Exposure

3.4.2.1.1 Mice

Studies conducted by Price et al. *(98)* and Neeper-Bradley et al. *(107)* demonstrate that ethylene glycol is a developmental toxicant in CD-1 mice following gavage exposure to ≥500 mg/kg bw/day on gd 6–15. Exposure to 500 mg/kg bw/day increased the numbers of litters containing pups with malformations with no specific malformation elevated to a level of statistical significance. Malformations that primarily affected the axial skeleton were increased in litters exposed to ≥750 mg/kg bw/day. At a dose of 3,000 mg/kg bw/day, neural tube closure and craniofacial defects were observed. Additional evidence of developmental toxicity included reduced fetal body weight (≥750 mg/kg bw/day) and reduced numbers of live fetuses (3,000 mg/kg bw/day). The developmental NOAEL for mice was identified as 150 mg/kg bw/day. Maternal toxicity was limited to decreased absolute liver weight at doses of ≥1,500 mg/kg bw/day in the Price et al. *(98)* study, while no evidence of toxicity was noted in dams exposed to 1,500 mg/kg bw/day in the Neeper-Bradley et al. *(107)* study.

Developmental toxicity observed in mouse continuous-reproductive breeding studies *(130, 131)* was consistent with that observed in prenatal studies, although differences in study design and method of dosing preclude a direct comparison. These data are discussed in Section 4.2.

The Panel concluded there is sufficient evidence in mice that gavage exposure to ≥500 mg/kg bw/day ethylene glycol on gd 6–15 causes developmental toxicity in the form of malformations. Saturation of glycolic acid metabolism appears to correlate with the threshold above which developmental toxicity was observed (see also Sections 2.1.3.2 and 2.6.1.3).

3.4.2.1.2 Rats

Studies conducted by Price et al. *(98*) and Neeper-Bradley et al. *(107, 109*) demonstrate that high doses of ethylene glycol administered by gavage on gd 6–15 cause developmental toxicity in Sprague-Dawley rats. Skeletal malformations were found to be the most sensitive endpoint. In the Neeper-Bradley et al. *(107, 109*) study, an increase in axial skeleton malformations was noted in groups dosed with ≥1,000 mg/kg bw/day. In contrast, no skeletal malformations were noted in offspring of rats dosed with 1,250 mg/kg bw/day in the Price et al. *(98*) study; an increase in skeletal malformations was noted in groups dosed with ≥2,500 mg/kg bw/day. The Expert Panel noted that sacrifice of animals on different days (gd 20 for the Price et al. study and gd 21 for the Neeper-Bradley et al. study) complicates direct comparison of the two studies. Additional evidence of developmental toxicity in the Price et al. *(98*) and Neeper-Bradley et al. *(107, 109*) studies included reduced fetal body weights (1,000–5,000 mg/kg bw/day), increased soft tissue and external malformations consisting primarily of neural tube closure and craniofacial defects (2,500–5,000 mg/kg bw/day), reductions in live fetuses (2,500–5,000 mg/kg bw/day), and increased postimplantation loss (5,000 mg/kg bw/day). The developmental toxicity NOEL **[NOAEL[10]]** was identified as 500 mg/kg bw/day *(107, 109*). Effects noted in dams by Price et al. *(98*) and Neeper-Bradley et al. *(107, 109*) included reduced body weight gain with no effect on body weight corrected for gravid uterine weight (≥1,250 mg/kg bw/day), increased kidney weight and water intake (≥2,500 mg/kg bw/day), and decreased liver weight (5,000 mg/kg bw/day). There were no kidney lesions observed in dams at doses up to 2,500 mg/kg bw/day. The maternal NOEL **[NOAEL]** was identified as 1,000 mg/kg bw/day *(107, 109*).

In a dietary study where Fischer 344 rats received 40–1,000 mg/kg bw/day ethylene glycol on gd 6–15, there was no increase in malformed fetuses *(110*). A significantly increased fetal, but not litter, incidence of poorly or unossified vertebrae was considered by the study author to be evidence of delayed fetal maturation and suggestive of minimal toxicity. However, noting the absence of body weight effects or other consistent changes in skeletal integrity, the Expert Panel concluded that the 1,000 mg/kg bw/day dose should be classified as a NOAEL, not a LOAEL.

Price et al. *(112*) evaluated postnatal development in a study where Sprague-Dawley rats were gavage dosed with ethylene glycol on gd 6–20 and allowed to litter. Gestational length was increased at doses ≥1,250 mg/kg bw/day. An increase in pup mortality on pnd 1–4 and reduced pup weight gain on pnd 1–22 was noted at the 2,250 mg/kg bw/day dose level. There were no effects on neurobehavioral tests (i.e., exploratory behavior and visual discrimination tests) or developmental landmarks (i.e., incisor eruption, vaginal opening, testes descent, or wire grasping skills) at doses up to 2,250 mg/kg bw/day. Malformations in pups were consistent with those observed in prenatal studies.

A study examining fetuses and pups of rats gavage dosed with 2,500 mg/kg bw/day ethylene glycol on gd 6–15 demonstrated fewer skeletal variations and malformations in pups examined on pnd 63 versus fetuses and pups examined at various time points up to pnd 21 *(113*). The study

[10] Since the Expert Panel is considering only adverse effects in the selection of effect levels, the terminology of NOAEL will be used throughout this document.

suggests that many ethylene glycol-induced skeletal changes, most which are variations, are reversible.

The Expert Panel concluded there is sufficient evidence in rats that gavage exposure to ≥1,000 mg/kg bw/day ethylene glycol on gd 6–15 causes developmental toxicity in the form of skeletal malformations. Saturation of glycolic acid metabolism appears to correlate with the threshold above which developmental toxicity was observed (see also Section 2.1.3.2 and 2.6.1.3).

3.4.2.1.3 Rabbits

A study conducted by Tyl et al. *(97)* demonstrated no developmental toxicity in rabbits following gavage exposure with up to 2,000 mg/kg bw/day on gd 6–19, as noted by a lack of teratogenicity, prenatal mortality, and effects on fetal growth. Severe maternal toxicity was observed at 2,000 mg/kg bw/day and included a 42% death rate, increased early delivery, renal lesions, and renal oxalate crystals. The maternal and fetal NOAELs were identified as 1,000 and 2,000 mg/kg bw/day respectively.

The Expert Panel concluded that data are sufficient to demonstrate a lack of developmental toxicity in rabbits following gavage of does with up to 2,000 mg/kg bw/day ethylene glycol on gd 6–19.

3.4.2.2 Inhalation Exposure

3.4.2.2.1 Mice

Tyl et al. *(114, 115)* exposed CD-1 mice to ethylene glycol aerosols by whole-body inhalation on gd 6–15 and found increased malformations at ≥1,000 mg/m^3. However, it was noted that ethylene glycol was deposited on the fur of animals and that oral ingestion through grooming could account for a large percentage of the total dose. In order to examine the role of ethylene glycol exposure alone, Tyl et al. *(116, 117)* repeated the study, exposing the mice to ethylene glycol by nose-only. The only developmental effects were skeletal variations at ≥500 mg/m^3 and increased fused ribs at 2,500 mg/m^3. The Expert Panel agreed with study author observations that the studies were confounded by exposure through ingestion in both the whole-body and nose-only study and stress induced by restraint in the nose-only study.

The Expert Panel concluded that the data are insufficient to determine if inhalation of ethylene glycol causes developmental toxicity in mice.

3.4.2.2.2 Rats

A whole-body inhalation study was conducted by Tyl et al. *(114)* in Sprague-Dawley rats. However as discussed above for mice, the findings of the study were confounded by oral intake from grooming of fur containing ethylene glycol. The Expert Panel concluded that the data are insufficient to determine if inhalation of ethylene glycol causes developmental toxicity in rats.

3.4.2.3 Dermal Exposure

Tyl et al. *(119)* exposed CD-1 mice to ethylene glycol by the dermal route for 6 hours/day on gd 6–15 and found no evidence of malformations, increased prenatal mortality, or delayed growth at

doses up to 1,677 mg/kg bw/day. The only fetal effects observed at the highest dose of 3,549 mg/kg bw/day were reduced ossification of skull bones and phalanges. The maternal and fetal NOELs **[NOAELs[11]]** were identified as 3,549 mg/kg bw/day.

The Expert Panel concluded that data are sufficient to demonstrate a lack of developmental toxicity in mice following dermal exposure with up to 3,549 mg/kg bw/day ethylene glycol for 6 hours/day on gd 6–15.

3.4.2.4 Mechanistic Issues

3.4.2.4.1 Variability Across Dose Routes and Dose-Rate Effects

Ethylene glycol was more toxic to the conceptus when administered to the pregnant dam by the oral versus the dermal route. Toxicokinetic studies reviewed in Section 2 demonstrated that in contrast to rapid and complete absorption through the oral route, dermal exposure in rats and mice is slow and incomplete *(41-43*).

Carney et al. *(61*) compared blood levels of ethylene glycol and glycolic acid and developmental toxicity in Sprague-Dawley rats administered 1,000–2,000 mg/kg bw/day ethylene glycol on gd 6–15 by bolus SC injection or slow and continuous infusion by an SC pump. Compared to bolus injection, continuous infusion resulted in lower blood levels of ethylene glycol and glycolic acid. There was no evidence of developmental toxicity with continuous infusion. The study suggested that a peak glycolic acid concentration of at least 3 mM is needed to produce developmental toxicity.

3.4.2.4.2 Proximate Teratogen

A series of *in vivo* and *in vitro* studies sought to identify the proximate developmental teratogen associated with ethylene glycol exposure. *In vitro* studies *(121, 126*) demonstrated that ethylene glycol produced dysmorphogenesis in 9.5–10.5-day-old rat embryos only at concentrations (≥200 mM) greatly exceeding blood levels (14–57 mM) observed with *in vivo* exposures of rats to teratogenic concentrations of ethylene glycol *(41, 43, 44*). All of these studies suggested that either a metabolite or metabolic acidosis may be responsible for developmental toxicity. In rats, coadministration of sodium bicarbonate and 2,800 or 3,333 mg/kg bw ethylene glycol by SC injection on gd 11 reduced metabolic acidosis effects in dams and decreased the incidence of skeletal defects in fetuses compared with administration of ethylene glycol alone *(122*). In Sprague-Dawley rats gavage dosed with ≥300 mg/kg bw/day glycolic acid on gd 7–21 *(125*), fetal malformations of the axial skeleton were consistent with those observed in rats dosed with ethylene glycol at ≥1,000 mg/kg bw/day ethylene glycol *(107*). *In vitro* exposure of 9.5–10.5-day-old rat embryos to glycolic acid *(123, 126*) resulted in dysmorphogenesis at media concentrations (3–12.5 mM) within the ranges of glycolic acid blood levels (4.8–8.8 mM) observed in rats administered teratogenic doses of ethylene glycol *(44, 65*). Other metabolites (i.e., oxalic acid, glycolaldehyde, glyoxal, and glyoxylic acid) caused effects at lower concentrations than ethylene glycol, but their concentrations greatly exceeded those levels observed in *in vivo* studies *(126*). In an *in vitro* study, dysmorphogenesis in gd 10.5 rat embryos treated with 12.5 mM sodium glycolate (pH=7.42) was similar but lower in magnitude than in embryos treated with 12.5 mM glycolic acid (pH=6.74) *(123*). In a subsequent study, Sprague-Dawley rats were treated with

[11] Since the Expert Panel is considering only adverse effects in the selection of effect levels, the terminology of NOAEL will be used throughout this document.

ethylene glycol, glycolic acid, or sodium glycolate at levels that produced identical peak levels of glycolate (8.4–8.8 mM), but a 3-fold higher AUC with ethylene glycol versus glycolic acid or sodium glycolate administration *(65*); metabolic acidosis occurred only in groups treated with ethylene glycol or glycolic acid. Skeletal defect patterns were similar in the ethylene glycol and glycolic acid groups, with a higher incidence in the ethylene glycol group; increased glycolic acid AUC with ethylene glycol dosing is a possible reason for the higher incidence and greater severity of defects. Only a few skeletal variations were observed in the sodium glycolate group. It is important to keep in mind that all of these studies were performed using rat embryos.

The Expert Panel concluded that the mechanistic data suggest that unmetabolized ethylene glycol is not likely to be the proximate teratogen in rodents. Glycolic acid and/or other down stream metabolites in combination with resulting metabolic acidosis is the most likely cause of developmental toxicity following exposure of rodents to ethylene glycol.

3.4.2.4.3 Interspecies Variability

A preliminary study suggested metabolic differences between rats and rabbits following exposure to a high dose of ethylene glycol *(45*). However, the preliminary nature of the data did not allow definite conclusions to be made about interspecies differences. The Panel does note interspecies differences in placental development that may provide insight on differing sensitivity between rats and rabbits. In early pregnancy, mice and rats develop an inverted yolk sac placenta that is eventually replaced by the chorioallantoic placenta. The yolk sac placenta, which never develops in rabbits or humans, tends to concentrate weak acids, including glycolic acid, in the fluid surrounding the embryo. The preliminary studies by Carney et al. *(45*) suggest that the rabbit does not concentrate weak acids in fluids surrounding the embryo.

Because a metabolite, glycolic acid or a subsequent metabolite, appears to be the proximate teratogen, the documented species differences in the ontogeny of enzymes such as alcohol dehydrogenase, aldehyde dehydrogenase, CYP2E1, and/or glycolate oxidase involved in the metabolic disposition of ethylene glycol may play a role in species-specific susceptibility to ethylene glycol developmental toxicity. These issues are discussed more fully in Section 2.1.3.4.

Table 3-21. Summary of Key Developmental Toxicity Studies.

Doses (mg/kg bw/day)	Exposure Regimen	Species/ Strain	Dose (mg/kg bw/day): Effect[b]	Reference
750 1,500 3,000	gd 6–15, gavage	CD-1 Mouse	**Dams:** **Maternal NOAEL=750** **Maternal LOAEL = 1,500:** ↓bw gain and ↓liver weight **3,000:** ↓bw gain and ↓liver weight **Fetuses:** **Fetal LOAEL = 750:** ↓bw/litter, ↑malformed fetuses/litter, ↑litters with malformed fetuses, ↑litters with skeletal malformations **1,500:** ↓bw/litter, ↑malformed fetuses/litter, ↑litters with malformed fetuses, ↑litters with skeletal malformations **3,000:** ↓Live fetuses/litter, ↓bw/litter, ↑malformed fetuses/litter, ↑litters with malformed fetuses, ↑litters with external, visceral, and skeletal malformations	Price et al. *(98)*
50 150 500 1,500	gd 6–15, gavage	CD-1 Mouse	**Dams:** **Maternal NOAEL=1,500 (HDT)** **Fetuses:** **Fetal NOAEL=150** **Fetal LOAEL = 500:** ↑Litters with malformations **1,500:** ↓bw/litter, ↑litters with malformations, ↑litters with skeletal malformations	Tyl and Frank *(108)* Neeper-Bradley et al. *(107)*
1,250 2,500 5,000	gd 6–15, gavage	CD Rat	**Dams:** **Maternal LOAEL = 1,250:** ↓bw gain **2,500:** ↓bw gain, ↑relative (to bw) kidney weight, ↑water intake **5,000:** ↓bw gain, ↓ liver weight, ↑relative (to bw) kidney weight, ↑water intake **Fetuses:** **Fetal NOAEL=1,250[a]** **2,500:** ↓Live fetuses/litter, ↓bw/litter, ↑malformed fetuses/litter, ↑ litters with malformed fetuses, ↑litters with skeletal malformations **5,000:** ↑Postimplantation loss/litter, ↓live fetuses/litter, ↓bw/litter, ↑malformed fetuses/litter, ↑litters with malformed fetuses, ↑ litters with external malformations, ↑litters with visceral malformations, ↑litters with skeletal malformations	Price et al. *(98)*

Doses (mg/kg bw/day)	Exposure Regimen	Species/ Strain	Dose (mg/kg bw/day): Effect[b]	Reference
150 500 1,000 2,500	gd 6–15, gavage	CD Rat	**Dams:** **Maternal NOAEL=1,000** **Maternal LOAEL = 2,500:** ↓bw gain, ↑water intake, ↑absolute and relative (to bw) kidney weights, ↑relative (to bw) liver weight **Fetuses:** **Fetal NOAEL=500 mg/kg bw/day** **Fetal LOAEL = 1,000:** ↓bw/litter, ↑litters with skeletal malformations **2,500:** ↓bw/litter, ↑litters with external, visceral, skeletal, and total malformations	Neeper-Bradley et al. *(107, 109*)
40 200 1,000	gd 6–15, diet	Fischer 344 Rat	**Dams:** **Maternal NOAEL=1,000 (HDT)** **Fetuses:** **Fetal NOAEL=1,000[a] (HDT)**	Maronpot et al. *(110*)
250 1,250 2,250	gd 6–20, gavage	CD Rat	**Dams:** **Maternal NOAEL=250** **Maternal LOAEL = 1,250:** ↑Gestational length, ↑renal lesions **2,250:** ↓bw gain; ↑gestational length, ↑renal lesions, ↑absolute and relative (to bw) kidney weights, ↓ absolute and relative (to bw) uterine weight **Pups:** **Pup NOAEL=1,250** **Pup LOAEL = 2,250:** ↓Live litter size and ↑ pup mortality on pnd 1 and 4, ↓postnatal weight gain, ↓kidney weight, ↓brain weight, ↑skeletal malformations	Price et al. *(112*)
100 500 1,000 2,000	gd 6–19, gavage	New Zealand White Rabbit	**Dams:** **Maternal NOAEL=1,000** **Maternal LOAEL = 2,000:** ↑Renal crystals and lesions and death **Fetuses:** **Fetal NOAEL=2,000 (HDT)**	Tyl et al. *(97*)
404 1,677 3,549	6 hours/day on gd 6–15, dermal	CD-1 mouse	**NOAEL=3,549 for both dams and fetuses (HDT)**	Tyl *(103*) Tyl et al. *(119*)

[a]The Expert Panel's selection of a NOAEL is higher than the study authors selection.
HDT Highest Dose Tested
[b]The Panel is using terms of NOAEL and LOAEL, whereas authors sometimes used terms of NOEL and LOEL. Please refer to text in Section 3 for further explanation.

4.0 REPRODUCTIVE TOXICITY DATA

4.1 Human Data

No human reproductive toxicity data were identified.

4.2 Experimental Animal Data

Using a continuous breeding protocol, Lamb et al. *(131)* [also cited in Morrissey et al. *(132)*] investigated the reproductive function of mice exposed to ethylene glycol. Ethylene glycol (99.6% purity) was administered in drinking water to male and female COBS Crl:CD-1 (ICR) BR outbred albino mice, 20/dose/sex, at concentrations of 0.25, 0.5, or 1.0% w/v. Forty control mice/sex were exposed to the vehicle. The approximate doses were stated by the authors to be 410, 840, and 1,640 mg/kg bw/day. Dose selection was based upon results of a range-finding study and the goal was to achieve no toxicity at the lowest dose and a 10% reduction in body weight at the highest dose. Concentrations of ethylene glycol in dosing solutions were verified. At 11 weeks of age, mice were continuously treated with the chemical during a 1-week pre-mating period, a 14-week cohabitation period, a 3-week segregation period, and at least until weaning of the offspring born during the 3-week segregation period. Newborn litters were examined, sexed, and weighed. With the exception of the last litter, the pups were immediately sacrificed, allowing the pairs to breed again. F_1 litters born after separation of males and females were reared and weaned; the treated F_1 offspring continued to receive ethylene glycol throughout their lifetime. Twenty F_1 pairs/group from the control and high-dose group were retained for subsequent mating within groups at 70 days of age. The F_1 mating pairs were cohabited until a copulatory plug was found or 7 days had elapsed, whichever came first. F_2 pups from 1 litter/pair were examined, sexed, weighed, and then discarded. F_1 mice were necropsied after the mating trial and weights were measured for liver, brain, pituitary, and male and female reproductive organs. **[Histopathology findings in reproductive organs were not reported.]** Each pair of mice was considered the experimental unit. Reproductive data were evaluated by the Chi-Square test for homogeneity and/or the Fisher's Exact test. Pup and litter data were evaluated by Chi-Square approximation to the Kruskal-Wallis test, Fisher's Exact Test, and the Mann-Whitney U test.

Results of the testing conducted in F_0 mice are listed in Table 4-1. Thirty-eight pairs of F_0 mice in the control group and 18–20 pairs in treatment groups survived. No treatment-related effects were observed on body weight, clinical signs, or water consumption at any dose level. The authors reported a slight but statistically significant decrease in number of litters/fertile pair ($p<0.01$), mean number of live pups/litter ($p<0.05$), and mean live pup weight ($p<0.01$) in the 1.0% ethylene glycol group. Neither the 0.25 or the 0.50% dose groups were significantly affected.

In the F_1 generation, 16 control pairs and 11 high-dose pairs produced litters. There were no significant differences in fertility, live litter size, or live pup weight between the control and 1.0% ethylene glycol groups. A number of F_1 animals in the 1,640 mg/kg bw/day group were noted to have unusual facial features. Further examination of the skeleton by staining with Alizarin Red in 4 mice/sex/group in the control and high-dose group revealed a pattern of skeletal defects affecting the skull, sternebrae, ribs, and vertebrae in both sexes of the high-dose group.

Strengths/Weakness: Lamb et al. *(131)* is a well designed study that used adequate numbers of animals and examined reproductive function in two generations. Limitations of the study include examination of reproductive function only in control and high-dose F_1 animals, no histopathology in reproductive organs, and no sperm measurements.

Utility (Adequacy) for CERHR Evaluation Process: Lamb et al. *(131)* is useful for demonstrating no effects on reproductive function in mice at doses up to 0.5% (840 mg/kg bw/day). Exposure to 1.0% (1,640 mg/kg bw/day) resulted in a minor effect on fertility (a slight decrease in the number of F_1 litters/pair of F_0 mice) and findings that were most likely developmental effects (reduced numbers of live F_1 pups/litter, decreased F_1 pup weight, and facial and skeletal malformations). No reproductive effects were observed in the high-dose F_1 mice.

Table 4-1. Effects Observed in a Continuous Breeding Study in CD-1 Mice, Lamb et al. *(131)*.

Effect	Dose in % (mg/kg bw/day)			
	0	**0.25 (410)**	**0.5 (840)**	**1.0 (1,640)**
No. F_1 Litters/Pair of F_0	4.9	4.7	4.9	4.5**
No. F_1 Live Pups/Litter	10.8	10.4	10.5	10.2*
Live F_1 Pup weight (g)	1.63	1.64	1.58	1.53**
Protocol: Reproductive function studied in 20–40 F_0 mice given water with 0, 0.25, 0.5, or 1.0% ethylene glycol and 19–20 F_1 mice given water with 0 or 1.0% ethylene glycol. **Notes:** *= $p<0.05$, **=$p<0.01$, ***= $p<0.001$ No effects were seen on F_0 and F_1 body weight or clinical signs and F_1 fertility, live litter size, or F_2 live pup weight.				

Gulati et al. *(130, 132)* continued and extended the continuous breeding study reported by Lamb et al. *(131)* by testing a higher dose level and looking at additional endpoints. This study was conducted according to GLP using ethylene glycol (99.6% purity) administered in drinking water at concentrations of 0, 0.5, 1.0, and 1.5% (w/v) to COBS Crl: CD-1 (ICR) BR outbred albino mice. Concentrations of dosing solutions were verified. The authors estimated doses at 0, 897, 1,798, and 2,826 mg/kg bw/day, respectively. At 11 weeks of age, 40 control mice/sex and 20 treated mice/sex were exposed during a 1-week premating period. Males and females were then paired 1:1 within dose groups and exposures continued through a 14-week cohabitation period, a 21-day separation period, and until weaning of the last litter. Pups born during the cohabitation period were examined, sexed, and weighed. With the exception of the last litter born, pups were discarded after examination so the parental animals could continue mating. F_1 litters born after separation of males and females (at the end of 14 weeks) were saved and nursed through weaning. When F_1 litters were weaned, a crossover mating trial was conducted in F_0 mice by breeding 20 male and female mice from the high-dose group to 20 control mice/sex. The purpose of the trial was to determine whether one or both sexes were affected. F_1 mice from all dose groups continued to receive treatment and at 74 days of age, 20 males and females/dose were mated within treatment groups. In both the crossover and F_1 mating studies, animals mated until a vaginal plug was detected or 7 days passed; litters were examined, sexed, weighed, and discarded. Fertility data were analyzed using the Cochran-Armitage test, Fisher's exact test or Chi-Square test for homogeneity. Pup and litter data were evaluated with the Kruskall-Wallis test, Jonckheere's test, the Wilcoxon's rank-sum test, F-test, t-test, and/or Williams test. Table 4-2 lists the major findings of this study.

Thirty-eight pairs of F_0 mice from the control group and 14–20 pairs of mice from treatment groups were fertile. Fertility index at all doses did not differ from controls. However, female pup weight and pup weight adjusted for litter size were significantly reduced at all doses; live pups/litter were significantly reduced in the 1.5% group. The crossover mating study confirmed that there was no reduction in fertility in high-dose males or females; the fertility rate was 50% in all groups. The only significant effect in the crossover study was reduced adjusted pup body weight in litters born to high-dose females mated with control males. At the end of the study, estrous cycles were monitored for 1 week and sperm analyses, necropsies, and histopathological examination were performed in control and high-dose F_0 mice. Organ sections were stained with hematoxylin-eosin for histopathological evaluation, but there was no mention of the fixation method. No treatment-related effect on estrous cyclicity was noted and histologic studies revealed no treatment-related effects on ovary, uterus, or vagina. In high-dose F_0 males, sperm number in a sample from the cauda epididymis was similar to controls but the incidence of abnormal sperm increased and motility decreased significantly in the 1.5% group. Table 4-3 outlines the main histopathological findings for male reproductive organs.

Testicular lesions that occurred at a higher frequency and severity in males treated with 1.5% ethylene glycol included degeneration of seminiferous tubules, loss of spermatozoa, spermatids, spermatogonia and spermatocytes, vacuolization of epithelial cells, and interstitial cell hyperplasia. Epididymal lesions were also observed. Kidney lesions and oxalate crystals were observed in the treated group. Body and absolute liver weight were significantly lower in males of the 1.5% dose group. There were no effects on male reproductive organ weights or female body, liver, and kidney weights. Blood calcium levels were not affected in any treatment group. **[The Expert Panel concluded that because effects on male reproductive organs and sperm parameters were only examined in controls and the 1.5% dose group, the data are inadequate to characterize these endpoints over this dose range.]**

A total of 13–18 pairs of F_1 mice/group were fertile. The treated F_1 mice showed no effect on mating or fertility index, number of live pups/litter, or sex ratio within litters. There was a significant decrease in adjusted live pup weight in all treatment groups with no evidence of a dose-response relationship. At the end of the study, estrous cycles were monitored for 1 week and sperm analyses and necropsies were performed for all dose levels in F_1 mice but histopathology was only conducted in control and high-dose groups. Significant decreases were observed for absolute seminal vesicle and right testis weight at all dose levels and absolute epididymis weight in the 1.0 and 1.5% groups. Relative right testis and epididymis weights were significantly decreased in mice from the 1.0 and 1.5% dose groups. Sperm motility was significantly reduced at the 1.0 and 1.5% groups. Sperm count was decreased by about 20% at all doses; though there was no dose response, statistical significance was achieved at the 2 lower doses. A dose-related increase in abnormal sperm was not significant. The major histological findings for male reproductive organs are listed in Table 4-3. Histological examination in high-dose mice revealed a higher incidence and severity of seminiferous tubule degeneration, epididymal lesions, and interstitial cell hyperplasia in mice of the 1.5% dose group. No chemical-related histopathological lesions were observed in the reproductive tissues from high-dose female mice and estrous cycles were not affected at any dose. Absolute liver weights were reduced in males and females of the 1.5% dose group. There were no lesions in livers or kidneys. An 18% mortality rate was observed in male mice from the 1.5% dose group prior to mating. Blood calcium levels were not affected in any treatment group. Facial abnormalities similar to those reported by Lamb et al. *(131*) were observed in F_1 mice from the 1.0 and 1.5% dose groups.

[The Expert Panel stated that at 1.5% ethylene glycol in drinking water there was evidence of some degenerative changes in the testes and altered sperm parameters. While 85% of the

treated animals revealed some degeneration of the seminiferous tubules, the control groups also exhibited a 57% incidence. There were no treatment-related microscopic lesions in the prostate gland or the seminal vesicles. There is no evidence of female reproductive toxicity at doses up to 1.5% in drinking water as noted by no effects on fertility, estrous cyclicity, or histopathology of female reproductive organs (i.e., ovary, uterus, or vagina).]

Strengths/Weaknesses: Dose-response limitations within the study protocol preclude establishing a NOAEL. In addition, reproductive parameters in the control group (e.g., degenerative changes in seminiferous tubules) were over 50% and hence render any scientific opinion inconclusive.

Utility (Adequacy) for CERHR Evaluation Process: The results of the Gulati et al. *(130)* study were essentially negative with respect to the effects of ethylene glycol on multigenerational findings in mice. According to Gulati et al. *(130)*, ethylene glycol does not exhibit any significant toxicological effects upon mouse reproductive processes.

Table 4-2. Major Effects Produced by Ethylene Glycol in a Continuous Breeding Study in CD-1 mice, Gulati et al. *(130)*.

Effect[c]	Dose in % (mg/kg bw/day)			
	0	**0.5 (897)**	**1.0 (1,798)**	**1.5 (2,826)**
F_0 Parents and F_1 Offspring:				
% Abnormal sperm in F_0	5.05	ND	ND	8.28*
% Motile sperm in F_0	94.3	ND	ND	80.6*
F_0 male body weight (g)	46.449	ND	ND	42.287*
No. F_1 litters/Pair of F_0	4.68	5.00*	4.85	4.43
F_0 male liver weight (g)	2.113	ND	ND	1.933*
No. live pups/litter	11.81	11.64	11.99	9.99*
Adjusted (per litter size) live pup weight (g)	1.58	1.53*	1.48**	1.43**
Adjusted live pup weight (g) in crossover study	1.68	ND	ND	1.54* /1.69[a]
F_1 Parents and F_2 Offspring:				
% Abnormal sperm in F_1	4.24	4.67	5.25	5.77
F_1 sperm count $x10^9$	1036	801*	855*	861
% Motile sperm in F_1	94.6	94.4	92.1*	84.1*
F_1 right testis weight (g)	0.140	0.124[b]*	0.119[b]**	0.120[b]*
F_1 seminal vesicle weight (g)	0.465	0.408*	0.409*	0.401*
F_1 epididymis weight (mg)	49.565	45.845	44.300[b]*	42.850[b]**
F_1 relative (to body weight) right testis weight (g)	0.137	0.125	0.120*	0.121*
F_1 relative (to body weight) epididymis weight (mg)	48.653	46.127	44.568*	43.212**
F_1 liver weight (g), male:	1.869	1.720*	1.739	1.692*
females:	1.662	1.730	1.630	1.518*
Adjusted (per litter size) live pup weight (g)	1.54	1.46*	1.46*	1.45*

Protocol: Reproductive function studied in 16–38 F_0 pairs/group, 16–20 F_0 treated x control pairs/group, and 20 F_1 pairs/group administered ethylene glycol through drinking water at 0, 0.5, 1.0, and 1.5%.
Notes: *=p<0.05, **=p<0.01, ***=p<0.001
ND=Not determined.
[a]Values for treated females x control male / control females x treated males.
[b]These values were listed in both Table 14 and 15 of the study but different levels of statistical significance were listed in the 2 tables.
[c]See text for description of effects on non-reproductive endpoints.
No effects were noted for fertility index, estrous cycles, and histopathology or weights of female reproductive organs in either generation.

Table 4-3. Summary of Histopathological Effects in Male Mouse Reproductive Organs Caused by 1.5% Ethylene Glycol in Drinking Water, Gulati et al. *(130)*.

Effect: Severity[a]	Dose in % (mg/kg bw/day)	
	0[b]	1.5 (2,826)[c]
F_0 Mice		
Seminiferous tubule degeneration:		
Minimal	11	11
Mild	1	3
Moderate	0	2
Severe	0	1
Interstitial cell hyperplasia:		
Minimal-to-moderate	0	3
Epididymal lesions:		
Minimal-to-moderate	1	9
Epididymal sperm reduction:		
Moderate	0	4
F_1 Mice		
Seminiferous tubule degeneration:		
Minimal	7	10
Mild	1	0
Moderate	0	2
Interstitial cell hyperplasia:		
Minimal-to-mild	0	2
Epididymal lesions		
Minimal-to-mild	0	4

Notes:
[a]Reported as total number of mice affected.
[b]21 F_0 and 20 F_1 mice were examined.
[c]20 F_0 and F_1 mice/generation were examined.
Statistical significance of effects was not reported.
There were no treatment-related effects on female reproductive organs.
See text for a description of kidney histopathology in F_0 males.

Bolon et al. *(133*) assessed differential follicle counts in ovaries from ten mice/group exposed to ethylene glycol using the NTP continuous breeding protocol *(132*) and reported ethylene glycol had no effect on follicular counts.

In a multi-generation and dominant lethal mutation study by DePass et al. *(104*) [also Woodside et al. *(134*)], ethylene glycol (99.82% purity) was added to the diet of male and female Crl:Fischer 344 rats to provide dosages of 0, 40, 200, or 1,000 mg/kg bw/day. Ethylene glycol levels in diet were adjusted every 2 weeks to maintain constant dose levels. During the second and third week of lactation, ethylene glycol levels were reduced 2- and 3-fold to adjust for large increases in food consumption that occur during this time. Authors verified that calculated doses based on nominal concentrations of ethylene glycol in diet were close to target doses. Preliminary studies indicated that the highest dose caused mild renal toxicity, and subsequently the dose selection was based upon the effects seen in the male since they appeared to be more susceptible

than the female. Two groups of control rats were fed diets without ethylene glycol. Exposure of the F_0 males and females to ethylene glycol began at about 49 days of age (~7 weeks before mating) and was continued for 3 generations. At about 100 days of age, 10 males in each dose group were mated to 20 females in the same dose group. The date of parturition and number of live and dead newborns were recorded for each litter. The litters were weighed at pnd 4 and 14 and individual pups were weighed at weaning (pnd 21). F_1 and F_2 rats were randomly selected for mating, ensuring that each litter was represented. **[The exact number of F_1 and F_2 rats mated was not specified.]** Necropsies and histopathological examination were performed on five males and five females at each dosage level in the F_2 parents and F_3 weanlings. **[Methods for histological evaluation were not discussed.]** For the dominant lethal mutation study, 155-day-old male F_2 rats (15/dose and control groups) were bred to 3 sets of untreated females at weekly intervals. Females were sacrificed on gd 12 for an examination of ovaries and numbers of live and dead fetuses. Fifteen F_2 males in the dietary control group were injected IP with 0.50 g/kg of triethylenemelamine to serve as positive controls. Continuous data were evaluated by ANOVA, Bartlett's test for homogeneity of variance, Duncan's multiple range test, and t-tests. Discontinuous data were analyzed by a multiple sum of ranks test, and frequency data by the x^2 test and Fisher's exact test. No effects on body weight gain or diet consumption were observed at any dose. Ethylene glycol treatment had no effect on fertility index, gestation index, gestation survival index, or days from first mating to litter in the F_0–F_1, F_1–F_2, or F_2–F_3 generation. There was also no effect on postnatal pup weight gain. No histopathologic effects were observed in accessory sex glands, epididymis, testes, uterus, ovaries, or kidneys of F_2 parents and/or F_3 weanlings. Ethylene glycol treatment resulted in no statistically significant or dose-related increase in dominant lethal mutations, while such effects were observed in the triethylenemelamine-positive control group. **[The Expert Panel concluded that these data provide adequate evidence that continuous dietary exposure of rats to 40–1,000 mg ethylene glycol/kg bw/day does not suppress the fertility index in 3 generations of rats mated once as mature adults.]**

Strengths/Weaknesses: This study by DePass et al. *(104)* examined reproductive function in three generations of rats and included a histopathological evaluation of reproductive organs in a limited number of F_2 and F_3 animals (five sex/dose). Limitations of the study included no information about the numbers of F_1 and F_2 animals mated, no information about histological procedures, inadequate reporting of histopathological findings, and no sperm measurements.

Utility (Adequacy) for CERHR Evaluation Process: The DePass et al. *(104)* study is useful for demonstrating no effects on reproductive function at doses up to 1,000 mg/kg bw/day in rats.

In an evaluation of a reproductive and developmental toxicity screen, Harris et al. *(128)* tested ethylene glycol in 14–16-week-old Swiss Crl:CD-1 mice. Ten male and female mice/group were gavaged with ethylene glycol in water at 0, 250, 700, and 2,500 mg/kg bw/day. Aliquots of ethylene glycol were analyzed by GC after dosing and found to be 93–100% of the target dose concentration. The rational for dose selection was to achieve a high dose that was about 1/3 the level of the reported LD_{50}. There were two separate groups of females in these studies. Group A females were dosed daily on study days 0–21. On study days 8–12 they were cohabited with treated males. Percentage pregnant and number of live implants were the only endpoints examined in Group A. In Group B, time-mated pregnant mice were dosed daily with ethylene glycol on gd 8–14 and followed until pnd 4. Fertility rate, body weight, litter size, and implantation sites were recorded. Males were treated daily from study day 3 to 20 and upon necropsy, liver, kidney, and testis weight were recorded as were epididymal sperm counts and motility. Data were analyzed by the Cochran-Armitage test for linear trend, Fisher's exact test, Kruskal-Wallis ANOVA, and/or Jonckheere's test for dose-response trends. Treatment of Group

A females with 2,500 mg/kg bw/day resulted in significant reductions in live implants/female and increases in the number of dead implants/female. Reduced litter weight on pnd 1 and 4 was the only significant effect noted in Group B females treated with 2,500 mg/kg bw/day. Evaluation of males on study day 20 revealed no effect on sperm count or motility. Testis and epididymis weights were unaffected. There were no treatment-related lesions in the testis and epididymis (preserved in Bouin's solution) or liver and kidney of males. Authors noted that the screening assay was less sensitive in detecting reproductive toxicity than continuous breeding studies such as the one conducted by Gulati et al. *(130*).

Strengths/Weaknesses: The Harris et al. *(128*) study was clear in its design and the data are well reported. Effects, presented as reduced number of live implants and reduced total litter weight, were seen only at the 2,500 mg/kg bw dose. The study also identified a lack of histopathologic effect in the kidney and testis at doses up to 2,500 mg/kg bw.

Utility (Adequacy) for CERHR Evaluation Process: Results support findings of other studies.

The Panel is aware of a study demonstrating that ethylene glycol induced vitellogenin gene expression in rainbow trout *(135*). An author of that study later released a letter stating that it is inappropriate to include ethylene glycol on lists of endocrine disrupters based on results of that study *(136*). According to Lech *(136*), the increase in vitellogenin mRNA induction could be unrelated to endocrine activity and occurred only at very high doses of ethylene glycol (1–20 g/kg). Lech *(136*) stated that ethylene glycol did not competitively bind to the estrogen receptor in other studies.

The Commission to the Council and the European Parliament *(137*) reviewed data regarding endocrine activity of numerous chemicals and concluded that ethylene glycol is not an endocrine disrupter based on the available information.

4.3 Utility of Data

There are no reproductive toxicity data in humans exposed to ethylene glycol.

Reproductive toxicity associated with ethylene glycol exposure was examined in two continuous breeding studies conducted in mice exposed through drinking water and in one multigeneration study in rats exposed through diet. The studies examined fertility in at least two generations of male and female animals, including animals exposed *in utero*. One mouse study examined estrous cycling and sperm parameters. Histopathology of reproductive organs was examined in control and high-dose mice of one continuous breeding study and a limited number of animals from all dose groups in the rat multigeneration study. In addition, histopathological evaluations of reproductive organs were conducted in chronic and subchronic studies reported in Section 2.

The reproductive toxicity studies examined appropriate endpoints in animals dosed with ethylene glycol at concentrations exceeding expected human environmental and occupational exposure levels. Therefore, the data are sufficient for assessing reproductive toxicity. Based on similarities in systemic toxicity observed between human poisoning case studies and high-dose animal studies, the Panel assumes that the animal data are relevant for assessing human hazard.

4.4 Summary

4.4.1 Human Data

No human data were identified.

4.4.2 Experimental Animal Data

Results of the key animal reproductive toxicity studies are outlined in Table 4-4.

4.4.2.1 Mice

In two continuous breeding studies, CD-1 mice were exposed to 410–2,826 mg/kg bw/day ethylene glycol in drinking water for 1 week prior to mating, 14 weeks of cohabitation, and the entire gestation and lactation period *(130, 131*). No adverse effect on fertility was noted in two generations of mice in either study. A slight reduction in the number of F_1 pups/litter following exposure to 1,640 mg/kg bw/day in 1 study *(131*) was not repeated in the second study *(130*). One study *(130*) included an evaluation of estrous cycles, reproductive organ histopathology in control and high-dose animals, and sperm parameters in control and high-dose F_0 males and F_1 males from all dose groups. No effects were noted for estrous cycles and histopathology of ovary, uterus, or vagina. A non-dose-related reduction in sperm count was observed in F_1 animals exposed to ≥897 mg/kg bw/day, with statistical significance obtained only in the 2 lower dose groups. Percent motile sperm were significantly reduced in F_1 mice exposed to ≥1,798 mg/kg bw/day and abnormal sperm were increased in F_0 mice of the 2,826 mg/kg bw/day group. Interstitial cell hyperplasia and epididymal lesions were increased in both generations of mice exposed to 2,826 mg/kg bw/day compared to control mice. The Expert Panel determined that the toxicological significance of seminiferous tubule degeneration was inconclusive due to the high incidence observed in control mice.

Histopathological results in a reproductive toxicity screen *(128*) support the conclusion that ethylene glycol produces no major reproductive toxicity effects in males. The study found no effects on sperm count and sperm motility and no treatment-related lesions in the testis or epididymis of males gavage dosed with up to 2,500 mg/kg bw/day ethylene glycol for 18 days.

Reproductive organ histopathology examined in subchronic and chronic toxicity studies (described in Section 2.2.2.1 and 2.4.2) found no treatment-related histopathology of reproductive organs including ovary, uterus, prostate, testis, seminal vesicles, or epididymis *(34, 91*). The results in female mice are consistent to those observed in the continuous breeding study *(130*). The lack of testicular lesions in subchronic and chronic studies *(34, 91*) suggests that the inconclusive finding of increased testicular lesions in the Gulati et al. *(130*) study was not treatment related. Interstitial cell hyperplasia and epididymal lesions observed in the breeding study were not observed in the chronic study despite higher concentrations and longer duration of dosing.

The Expert Panel concluded that data in mice are sufficient to demonstrate no effect on fertility of male or female mice following oral exposure of up to 2,826 mg/kg bw/day ethylene glycol for approximately 22 weeks.

4.4.2.2 Rats

A multigeneration study examined reproductive toxicity in 3 generations of male and female Fischer 344 rats exposed to 40–1,000 mg/kg bw/day ethylene glycol *(104)* and found no effect on fertility, gestation, offspring survival, or postnatal pup weight gain. No histopathological lesions were observed in reproductive organs (epididymis, testes, uterus, ovaries) of F_2 parents and F_3 offspring, although it was noted that histopathological procedures and findings were not adequately reported in the study. Estrous cycles and sperm parameters were not evaluated.

Results of histopathology evaluations (Section 2.2.2.1 and 2.4.2) conducted primarily in control and high-dose animals in chronic and subchronic studies are consistent to findings observed in the multigeneration study.

The data are sufficient to demonstrate that ethylene glycol is not a reproductive toxicant in male and female rats following dietary exposure to up to 1,000 mg/kg bw/day for 7 weeks prior to mating in parental rats or from the time of conception through mating in offspring.

Table 4-4. Summary of Key Reproductive Toxicity Studies

Dose (mg/kg bw/day	Exposure Regimen	Species/ Strain	Dose (mg/kg bw/day): Effects	Reference
410 840 1,640	In drinking water from 1 week prior to mating, 14 weeks of cohabitation, and throughout gestation and lactation	CD-1 Mouse	**NOAEL=840 mg/kg bw/day** **1,640:** ↓No. F_1 litters/F_0 pairs ↓No. live F_1 pups/litter ↓F_1 pup weight No reproductive effects in F_1 adults	Lamb et al. *(131)*
897 1,798 2,826	In drinking water from 1 week prior to mating, 14 weeks of cohabitation, and throughout gestation and lactation	CD-1 Mouse	**897:** ↑No. F_1 litters/F_0 pairs, ↓F_1 sperm count, ↓F_1 testis and seminal vesicle weight ↓F_1 and F_2 pup weight **1,789:** ↓F_1 sperm count and motility, ↓F_1 testis, seminal vesicle weight, and epididymis weight, ↓ F_1 relative (to bw) testis and epididymis weight, ↓F_1 and F_2 pup weight **2,826:** ↑Abnormal sperm in F_0, ↓F_0 and F_1 sperm motility, ↓F_1 testis, seminal vesicle weight, and epididymis weight, ↓F_1 relative (to bw) testis and epididymis weight, ↑F_0 and F_1 testicular and epididymal lesions, ↓ F_0 body weight (m), ↑ F_0 kidney lesions and oxalate crystals (m), ↑F_1 death (m), ↓F_1 and F_2 pup weight, ↓ F_1 pups/litter No effects were noted for fertility index, estrous cycles, or histopathology of female reproductive organs in either generation.	Gulati et al. *(130)*
250 700 2,500	Females were gavage dosed on study days 0–21 and males on study days 3–20. Mating occurred on study days 8–21	CD-1 Mouse	**2,500:** ↓ Live implants and ↑ dead implants/female No effect on sperm count and sperm motility, and no treatment-related lesions in the testis, epididymis, liver, or kidney of males.	Harris et al. *(128)*
40 200 1,000	In diet continuously for three generations	Fischer 344 Rat	**NOAEL=1,000** No effects on fertility, gestation index, gestation survival, histopathology of reproductive organs, or pup weight gain.	DePass et al. *(104)*

5.0 SUMMARIES, CONCLUSIONS, AND CRITICAL DATA NEEDS

5.1 Summary and Conclusions of Reproductive and Developmental Hazards

5.1.1 Developmental Toxicity

There were no data identified that permit the evaluation of developmental toxicity in humans. Ethylene glycol was tested for developmental toxicity in mice, rats, and rabbits. There were insufficient data to determine whether ethylene glycol causes developmental toxicity by inhalation exposure in mice and rats.

There were sufficient data to determine that CD-1 mice exposed to ethylene glycol by the dermal route for 6 hours/day on gd 6–15 showed no evidence of malformations, increased prenatal death, or delayed growth at doses up to 3,549 mg/kg bw/day. Maternal and fetal NOAELs were identified as 3,549 mg/kg bw/day. These findings are consistent with toxicokinetic data that indicates low absorption of ethylene glycol by the dermal route of exposure.

There were sufficient data to conclude that oral gavage exposure to high doses of ethylene glycol (CD-1 mice, ≥ 500 mg/kg bw/day on gd 6–15; Sprague-Dawley rats, ≥1,000 mg/kg bw/day on gd 6–15) causes developmental toxicity in mice and rats, including axial skeletal malformations, reduced body weights, external malformations, and increased post-implantation loss. Mechanistic data suggest that unmetabolized ethylene glycol is not likely to be the proximate teratogen in rodents. Glycolic acid or one of its metabolites or metabolic acidosis is the most likely cause of developmental toxicity following exposure of rodents to ethylene glycol.

The Expert Panel believes that knowledge of toxicokinetics can substantially inform the interpretation of the observed developmental toxicity of ethylene glycol in rodents and the assessment of its relevance to humans. Relevant toxicokinetic points are summarized below; more complete discussions are found in Sections 2.1 and 2.6.1.

The toxicokinetics of ethylene glycol have been extensively studied in rats, with more limited data in mice, dogs, monkeys, and humans. The data indicate extensive absorption via the oral and inhalation routes. Once absorbed, ethylene glycol is distributed into total body water and can be excreted unchanged in urine. However, the majority of the dose is metabolized via alcohol dehydrogenase (ADH)-mediated (and possibly also CYP2E1-mediated) oxidation. Ethylene glycol metabolism yields a variety of products with glycolic acid and oxalic acid of principle toxicological interest. There are several studies that have attempted to characterize the oxidation of glycolic acid to glyoxylate, a saturable process that leads to accumulation of glycolic acid. This is reflected most clearly in the non-linear increase in glycolic acid levels in blood and urine as the dose of ethylene glycol is increased. This saturation occurs beginning at doses as low as 150 mg/kg bw in rodents with the mouse enzyme system appearing to be somewhat more readily saturable than the rat system. It is evident that glycolic acid metabolism is saturated at the bolus doses in rats required to produce developmental toxicity (1,000 mg/kg bw). The role of saturation kinetics in developmental toxicity is also suggested by the lack of glycolic acid buildup and the lack of toxicity after ethylene glycol dermal or continuous infusion dosing. This lack of toxicity occurred at applied doses that, by bolus dosing, would produce both saturation and toxicity. The lower dose rate in these studies leads to a glycolic acid formation rate that apparently does not exceed saturation. It appears that in rats, maternal blood glycolic acid concentrations must be at

least 3 mM for developmental effects to ensue *(61, 126*). The threshold level has not been defined in other species.

Developmental toxicity was not observed in rabbits orally exposed to ethylene glycol at doses associated with severe maternal toxicity. Rabbits demonstrated no developmental toxicity following gavage exposure to doses as high as 2,000 mg/kg bw/day on gd 6–19, as noted by a lack of malformations, prenatal deaths, or decrease in fetal weights. Severe maternal toxicity was observed at 2,000 mg/kg bw/day as evidenced by maternal deaths, increased early delivery, and lesions as well as oxalate crystals in the kidneys. Maternal and fetal NOAELs were identified as 1,000 and 2,000 mg/kg bw/day, respectively. Thus, the data were sufficient to demonstrate a lack of developmental toxicity in rabbits following oral gavage throughout organogenesis at doses ≤2,000 mg/kg bw/day.

The Panel notes that renal elimination of ethylene glycol and glycolic acid is important to clearance of these compounds, especially under conditions of metabolic saturation. However, the role of renal clearance may be impaired due to oxalate-induced crystal formation and renal toxicity when high doses of ethylene glycol are administered. This, along with metabolic saturation, likely contributes to the very slow elimination rate of glycolic acid in high-dose human poisoning cases involving renal damage, which is in contrast to the much more rapid clearance of ethylene glycol and glycolic acid under lower dose conditions (inhalation exposures in two human volunteers).

In animal studies, the role of renal toxicity in contributing to glycolic acid buildup and developmental toxicity is unclear; developmental toxicity and subchronic studies reveal that mice are more resistant to ethylene glycol-induced renal toxicity than rats, but are somewhat more sensitive to ethylene glycol-induced developmental toxicity. Developmental toxicity studies demonstrate the opposite pattern in rabbits (more sensitive to renal toxicity but less sensitive to developmental toxicity than rats). The database of studies in rats suggests that renal toxicity from subchronic ethylene glycol exposure in male rats occurs at lower doses (LOAEL 500 mg/kg bw/day) *(60*) than those reported to produce developmental toxicity in the rat (LOAEL 1,000 mg/kg bw/day) *(107*).

The Expert Panel is confident that these developmental toxicity data are useful in judging the hazard to humans because the doses tested far exceeded the doses relevant to humans based on knowledge of absorption, distribution, metabolism, and excretion in rats, mice, and humans. However, it is recognized that the rat and mouse models are possibly more sensitive than humans because of the dependence of these species on the inverted yolk sac placenta, which is not present in humans (See discussion in Section 3.2.4). The Panel recognizes that other pharmacodynamic factors may impact such interspecies differences. It is further noted that the pattern of general toxicity is similar in experimental animal studies and instances of human poisoning.

Much of the toxicokinetic data, especially that which demonstrates a metabolic threshold, were developed in rats. The Panel finds that these data are relevant to humans based upon the likelihood that humans will also exhibit saturation of ethylene glycol and glycolic acid metabolism. In fact, a single *in vitro* study *(68*) provides kinetic constants for the saturable metabolism of glycolic acid in humans. This evidence suggests that human metabolism of glycolic acid saturates at a 4-fold lower concentration than in the rat.

5.1.2 Reproductive Toxicity

There were no data identified that permit the evaluation of reproductive toxicity in humans. Ethylene glycol was tested for reproductive toxicity in rats and mice. There are sufficient data to conclude that ethylene glycol is not a reproductive toxicant in rats exposed orally to 1,000 mg/kg bw/day via diet. The study in mice was essentially negative at doses up to 2,826 mg/kg bw/day via drinking water. The studies available for review included a continuous breeding study in mice, a two-generation study in rats, and sub-chronic toxicity studies in rats.

The Expert Panel concluded that data in mice are sufficient to demonstrate no effect on fertility of male or female mice following oral exposure to up to 2,826 mg/kg bw/day ethylene glycol for approximately 22 weeks.

The Expert Panel concluded that the data are sufficient to demonstrate that ethylene glycol is not a reproductive toxicant in male and female rats following dietary exposure with up to 1,000 mg/kg bw/day for 7 weeks prior to mating in parental rats or from the time of conception through mating in their offspring.

The Expert Panel is confident that these data are useful in judging hazard to humans because the doses tested far exceeded the doses relevant to humans based on knowledge of absorption, distribution, metabolism, and excretion in rats, mice, and humans. It was further noted that the pattern of general toxicity is similar in experimental animal studies and instances of human poisoning.

5.2 Summary of Human Exposure

Ethylene glycol is used as an engine coolant, in the manufacture of polyester and PET resins, and is found in deicing solutions, industrial coolants, hydraulic fluids, and surface coatings. In 1999, U.S. consumption of ethylene glycol was 5,497 million pounds *(8)*.

Limited data are available on general public exposures to ethylene glycol. Exposures to ethylene glycol by the general public can possibly occur from dermal contact with products such as antifreeze solutions, ingestion of food or beverages containing trace amounts of ethylene glycol that has leached from packaging materials, and inhalation of air and ingestion of soil near point source emissions. Very little ethylene glycol is expected to be in outdoor air, with the possible exception of point source emissions. Therefore, significant exposure through outdoor air is not expected for the majority of the population. Health Canada estimated human exposure to ethylene glycol as a worst-case-scenario for persons living next to an industrial point source in the range of 22–88 μg/kg bw/day *(19)*. [The Expert Panel acknowledges the limitations in these estimates as stated by Health Canada.]

Occupational exposures to ethylene glycol are not well characterized. Workers can be exposed to ethylene glycol during its use as a chemical intermediate and as an ingredient of automotive antifreeze, deicing solutions, and surface coatings. Occupational exposure most likely occurs from dermal contact with ethylene glycol-containing solutions and inhalation of airborne vapors and mists generated through heating and spraying processes. Abdelghani et al. *(10)* obtained 16 short-term (15-minute) ethylene glycol exposure measurements of <0.05–2.33 mg/m^3 aerosol and <0.05–3.37 mg/m^3 vapor. A study of airport personnel found personal ethylene glycol air exposures ranging from <17 to 190 mg/m^3 mists and <2.5–22 mg/m^3 vapors *(11)*; urinary concentrations of ethylene glycol were increased in some workers as compared to levels in a non-occupational comparison group. A study of automotive mechanics found increased urinary ethylene glycol levels compared to unexposed workers *(30)*. Limitations in the occupational studies *(11, 30)* do not allow a determination of whether the dermal or inhalation route predominates in occupational settings.

5.3 Overall Conclusions

Available data from rat studies suggest that oral doses associated with developmental toxicity (1,000 mg/kg bw) are greater than doses associated with renal toxicity (500 mg/kg bw).

Developmental toxicity, and evidence of some renal toxicity, are observed in rodents at doses that exceed saturation of glycolic acid metabolism, which clearly occurs at 500 mg/kg bw in rats. Limited human *in vitro* data suggest that saturation of glycolic acid metabolism occurs at ~125 mg/kg bw, but saturation is expected to require much higher doses for slower dose-rate (non-bolus) exposure or for routes characterized by poor absorption (e.g., dermal). The Panel believes that ethylene glycol exposures resulting in blood levels below the level of saturation should not result in hazard associated with developmental toxicity in humans.

There are no data that are viewed as reliable estimates of human exposure in the general human population. It was noted that Health Canada had estimated a worst-case-scenario for persons living in the immediate vicinity of an ethylene glycol point source in the range of 0.022–0.088 mg/kg bw/day. The Panel also constructed two occupational exposure scenarios based on data presented in Section 1.2.4.2:

- Occupational inhalation exposure to 188 mg/m^3 (irritation limit) for 15 minutes resulting in a burden of 0.8 mg/kg bw for 15 minutes (21 L/minute, 70 kg bw).

- Occupational inhalation exposure of 10 mg/m^3 (the Expert Panel-estimated median of deicing data) for 480 minutes resulting in a total exposure burden of 1.4 mg/kg bw/8 hours (21 L/minute, 70 kg bw).

A comparison of the exposures associated with these scenarios to the dose where saturation of human metabolism is estimated to occur (125 mg/kg bw) shows that all of these expected exposures in the human are at least 100- to 1,000-fold lower than those expected to result in metabolic saturation.

Scenarios involving continuous rather than acute exposures would have even a larger margin of safety due to dose rate phenomena. This comparison does not take into account the potential impact of human interindividual variability.

The Expert Panel judges the likelihood of adverse developmental toxicity in the humans from such levels of exposure to be of negligible concern.

The Panel concludes that the lack of reproductive toxicity in experimental animal studies indicates there is negligible concern for reproductive effects in humans.

5.4 Critical Data Needs

Although the following data needs are less critical for assessing toxicity for the general population, additional knowledge will facilitate more precise inter-species extrapolation and a better characterization of inter-individual variability that would be helpful in identifying susceptible sub-populations.

1. Better and up-to-date human exposure estimates including various subpopulations would be helpful.

2. Fetal toxicity corresponds with exposure to both glycolic acid and oxalic acid, both *in vivo* and *in vitro*; the key dose metric for toxicokinetic analysis is somewhat uncertain. Thus, mechanistic data are needed on this metabolic process.

3. There is uncertainty regarding which specific human and rodent ADH isozymes are capable of oxidizing ethylene glycol and also what role CYP2E1 might play. This uncertainty also extends to knowledge about the temporal- and tissue-specific expression of these enzymes (e.g., placenta, fetus) that may promote the *in situ* formation or removal of ethylene glycol or key metabolites. Thus, mechanistic data are needed that define which ADH isoenzymes and what role CYP2E1 plays in ethylene glycol metabolism.

4. Polymorphisms in ADH, ALDH, and CYP2E1, as well as glycolate oxidase functional differences across individuals, may cause a substantial degree of inter-individual variability that may decrease or enhance toxicity and is not possible to quantify at this time. Thus, a better definition of mechanisms leading to inter-individual metabolic variability is needed.

5. While the dose level required for metabolic saturation in rodents has been delineated, there is only limited data on where this saturation occurs in humans, suggesting a saturation concentration of glycolic acid that is 4-fold lower than that observed in rats. A critical data need is confirmatory studies regarding the saturation level of ethylene glycol and glycolic acid metabolism in humans. This information, as well as the information from the points above, needs to be incorporated into PBPK models that are predictive of human internal dose.

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