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

Center For The Evaluation Of Risks To Human Reproduction

NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Propylene Glycol

March 2004 *NIH Publication No. 04-4482*

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Preface

The National Toxicology Program (NTP) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in 1998. The CERHR is a publicly accessible resource for information about adverse reproductive and/or developmental health effects associated with exposure to environmental and/or occupational chemicals. The CERHR is located at the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health and Dr. Michael Shelby is the director.[1]

The CERHR broadly solicits nominations of chemicals for evaluation from the public and private sectors. The CERHR follows a formal process for review and evaluation of nominated chemicals that includes multiple opportunities for public comment. Chemicals are selected for evaluation based upon several factors including the following:

- potential for human exposure from use and occurrence in the environment
- extent of public concern
- production volume
- availability of scientific evidence for reproductive and/or developmental toxicity.

The CERHR convenes a scientific expert panel that meets in a public forum to review, discuss, and evaluate the scientific literature on the selected chemical. Public comment is invited prior to and during the meeting. The expert panel produces a report on the chemical's reproductive and developmental toxicities and provides its opinion of the degree to which exposure to the chemical is hazardous to humans. The panel also identifies areas of uncertainty and where additional data are needed. The CERHR expert panels use explicit guidelines to evaluate the scientific literature and prepare the expert panel reports. Expert panel reports are made public and comments are solicited.

Next, the CERHR prepares the NTP-CERHR monograph. The NTP-CERHR monograph includes the NTP brief on the chemical evaluated, the expert panel report, and all public comments. The goal of the NTP brief is to provide the public, as well as government health, regulatory, and research agencies, with the NTP's interpretation of the potential for the chemical to adversely affect human reproductive health or children's health. The NTP-CERHR monograph is made publicly available electronically on the CERHR web site and in hard copy or CD-ROM from the CERHR.

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[1]Information about the CERHR is available on the web at <*http://cerhr.niehs.nih.gov*> or by contacting the director:

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Information about the NTP is available on the web at <*http://ntp-server.niehs.nih.gov*> or by contacting the NTP Office of Liaison and Scientific Review at the NIEHS:

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Introduction

In 1999, the CERHR Core Committee, an advisory committee composed of representatives from NTP member agencies, recommended propylene glycol and ethylene glycol for expert panel review. Propylene glycol was selected because it is a high production volume chemical and there is widespread human exposure. Ethylene glycol is the subject of a separate monograph.

Propylene glycol is used as a chemical intermediate in the manufacture of unsaturated polyester resins, and is found in cosmetics, personal care products, pharmaceuticals, food, liquid detergents, deicing fluids, antifreeze/engine coolant, paints, coatings, and tobacco products. Propylene glycol also is used in the production of plasticizers, 2-methylpiperazine, 1,2-propylene diamine, hydroxylated polyester, polyester-type fluorescent resin matrix, and polyether polyols.

As part of the evaluation of propylene glycol, the CERHR convened a panel of scientific experts (Appendix I) to review, discuss, and evaluate the scientific evidence on the potential reproductive and developmental toxicities of the chemical. There was a public meeting of the CERHR Ethylene Glycol/Propylene Glycol (EG/PG) Expert Panel on February 11-13, 2003. The CERHR received public comments throughout the evaluation process.

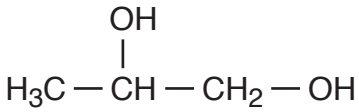
The NTP has prepared an NTP-CERHR monograph for propylene glycol. This monograph includes the NTP brief on propylene glycol, a list of the expert panel members (Appendix I), the expert panel's report on propylene glycol (Appendix II), and all public comments received on the expert panel's report on propylene glycol (Appendix III). The NTP-CERHR monograph is intended to serve as a single, collective source of information on the potential for propylene glycol to adversely affect human reproduction or development. Those interested in reading this monograph may include individuals, members of public interest groups, and staff of health and regulatory agencies.

The NTP brief included within this monograph presents the NTP's interpretation of the potential for exposure to propylene glycol to cause adverse reproductive or developmental effects in people. It is based on information about propylene glycol provided in the expert panel report, the public comments, and additional scientific information available since the expert panel meetings. The NTP brief is intended to provide clear, balanced, scientifically sound information on the potential for propylene glycol exposures to result in adverse health effects on development and reproduction.

NTP Brief on Propylene Glycol

What is Propylene Glycol?
Propylene Glycol (PG) is a small, hydroxy-substituted hydrocarbon with the chemical formula $C_3H_8O_2$ and the structure shown in Figure 1.

Figure 1. *Chemical structure of PG*



PG is used as a chemical intermediate in the production of unsaturated polyester resins. PG is used in liquid detergents, deicing fluids, antifreeze/engine coolant, paints and coatings. PG is one of the chemicals 'generally recognized as safe' (GRAS) by the U.S. Food and Drug Administration and is used in foods, cosmetics, pharmaceuticals and tobacco products.

Commercial PG is manufactured by direct hydrolysis of propylene oxide by water. In 1999, 1,083 million pounds of PG were produced in the U.S. with apparent consumption of 854 million pounds.

PG can be released into the environment from industrial disposal and PG-containing consumer products. PG is water-soluble and has the potential to leach into groundwater, but is rapidly degraded. The half-life of PG in water is estimated to be 1 to 4 days under aerobic conditions and 3 to 5 days under anaerobic conditions.

Are People Exposed to PG?*
Yes. The general public is exposed to PG by dermal contact with or ingestion of PG-containing products. Inhalation of PG vapors from such products may also occur. Dermal exposure can result from contact with PG-containing products such as cosmetics, antifreeze solutions, coolants, windshield deicers, or pharmaceutical creams. Oral exposure to PG can occur through its use in food and tobacco products and in prescription and over-the-counter medicines. PG is rapidly degraded in water; no information was located on PG levels in drinking water.

There is limited information on average U.S. exposure levels and no information on exposure levels due to dermal contact was noted. The average U.S. daily intake of PG from food products is estimated at 34 mg/kg bw/day for a 70 kg person. *[NOTE: mg/kg bw/day=milligrams per kilogram of body weight per day.]* Since PG has GRAS status and may not be listed as a specific ingredient in some foods, dietary intake based upon product labeling could result in an underestimation of intake. PG is also an ingredient in both over-the-counter and prescription pharmaceuticals. In adult humans, the mean serum half-life of PG is approximately 2 to 4 hours.

Occupational exposure to PG may occur through dermal contact or inhalation. Exposure studies indicate that exposure levels vary depending on protective gear worn, route of exposure, and length of exposure. A threshold limit value has not been defined for PG. However, the American Industrial Hygiene Association, Workplace Environmental Exposure Level recommended guide is "50 ppm as an eight-hour time-weighted average (TWA8) for total vapor and aerosol, and 10 mg/m^3 as a TWA8 for aerosol alone."

Can PG Affect Human Development or Reproduction?
Probably Not. There are no studies available on the effect of PG on human reproduction or development. Laboratory animal studies reviewed by the expert panel showed no effect on develop-

* Answers to this and subsequent questions may be: *Yes, Probably, Possibly, Probably Not, No or Unknown*

ment and/or reproduction at the highest doses tested (Figure 2).

Scientific decisions concerning health risks are generally based on what is known as a "weight-of-evidence" approach. In this case, recognizing the lack of human data and lack of adverse effects in laboratory animals after exposure to high doses (Figure 2), the NTP judges the scientific evidence sufficient to conclude that PG probably does not adversely affect human development or reproduction.

Supporting Evidence

As presented in the Expert Panel Report on PG (see report for details and literature citations), the panel concluded that PG does not produce developmental toxicity in offspring of laboratory animals treated with the highest oral doses tested, i.e., 1,230 mg/kg bw/day in rabbits; 10,400 mg/kg bw/day in mice; 1,600 mg/kg bw/day in rats; 1,550 mg/kg bw/day in hamsters.

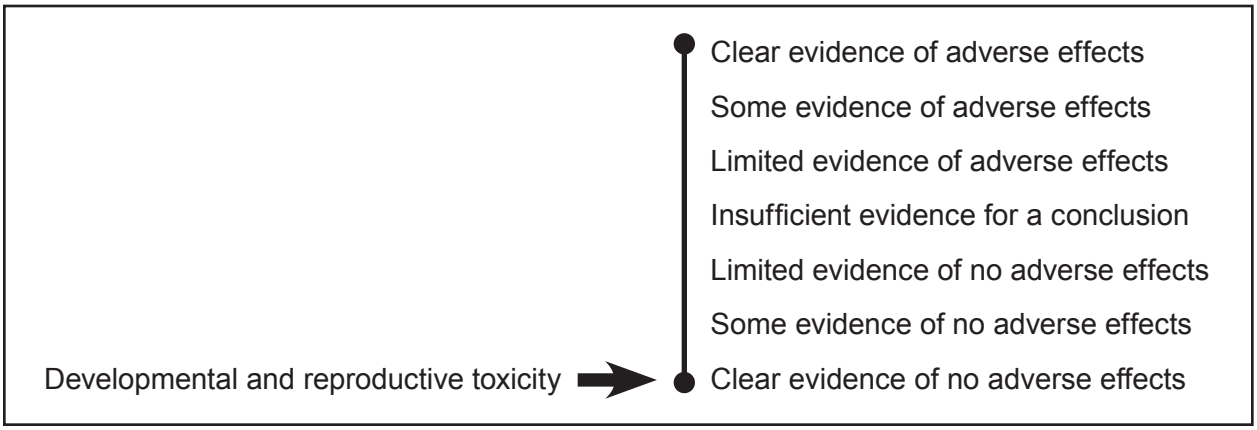
In an NTP continuous breeding study, no effects on fertility were observed in male or female mice that received PG in drinking water at doses up to 10,100 mg/kg bw/day. No effects on fertility were seen in either the first or second generation of treated mice.

The expert panel noted that the pharmacokinetics of PG are reasonably well understood in animals and humans. Information on the absorption, distribution, metabolism, and excretion of PG indicates that the absence of adverse effects in laboratory animals is likely to be relevant to humans. The rate-limiting step in PG metabolism is its conversion to lactaldehyde by alcohol dehydrogenase. Studies indicate that this reaction saturates in humans at doses that are 8-10 fold lower than needed to saturate the same step in laboratory animals. Saturation of this metabolic step is thought to be protective since PG has lower general toxicity than its metabolites.

Are Current Exposures to PG High Enough to Cause Concern?

Probably Not. Metabolism studies indicate that PG has a short half-life in humans. These data, combined with evidence that saturation of human metabolism occurs at doses 8-10 fold lower than observed in laboratory animals, suggest that human exposure levels are not high enough to cause concern. While there are no data on the PG exposures of the general U.S. population, it has been estimated that adults are exposed to approximately 34 mg/kg bw/day through food products. Limited data suggest that occupational exposures are not

Figure 2. *The weight of evidence that PG causes adverse developmental or reproductive effects in laboratory animals*



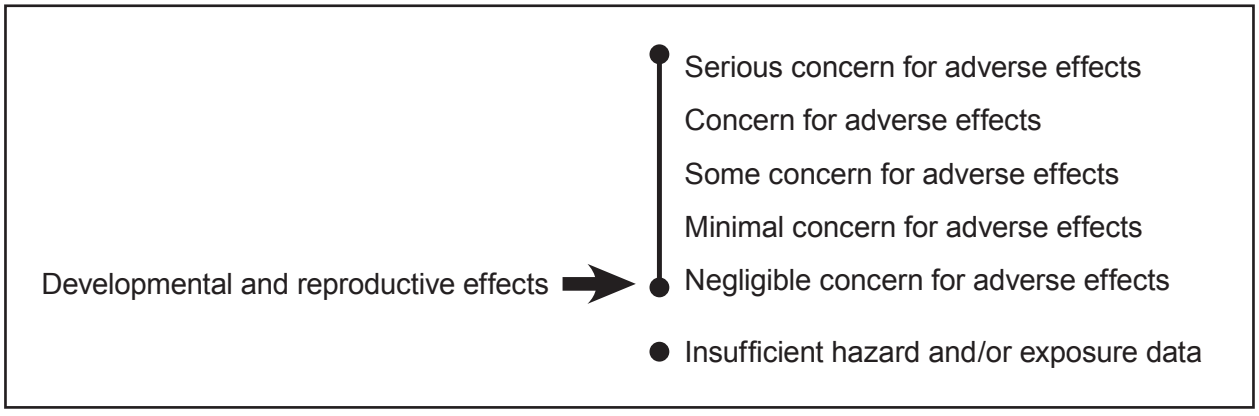
excessive. Based on the limited exposure data, pharmacokinetic studies, and laboratory animal studies the NTP offers the following conclusion (Figure 3):

The NTP concurs with the CERHR EG/PG Expert Panel that there is negligible concern for adverse developmental or reproductive toxicity from PG exposures in humans.

Studies evaluated by the expert panel indicate that high oral doses of PG produced no adverse developmental or reproductive effects in multiple laboratory animal species.

These conclusions are based on the information available at the time this brief was prepared. As new information on toxicity and exposure accumulate, it may form the basis for either lowering or raising the levels of concern expressed in the conclusions.

Figure 3. *NTP conclusions regarding the possibilities that human development or reproduction might be adversely affected by exposure to PG*



References

No additional references found.

Appendix I. NTP-CERHR Ethylene Glycol/Propylene Glycol Expert Panel

A 9-member panel of scientists covering disciplines such as toxicology, epidemiology, biostatistics and industrial hygeine was recommended by the Core Committee, a federal oversight committee for CERHR, and approved by the Director of the National Toxicology Program. The panel critically reviewed documents and identified key studies and issues for plenary discussions. At a public meeting held February 11-13, 2003, the expert panel discussed these studies, the adequacy of available data, and identified data needed to improve future assessments. The expert panel reached conclusions on whether estimated exposures may result in adverse effects on human reproduction or development. Panel assessments were based on the scientific evidence available at the time of the public meeting. The expert panel report was made available for public comment on May 15, 2003, and the deadline for public comments was July 14, 2003 (*Federal Register* 68:94 [15 May 2003] pp. 26325-26326). The Expert Panel Report on PG is provided in Appendix II and the public comments received on the report are in Appendix III. Input from the public and interested groups throughout the panel's deliberations was invaluable in helping to assure completeness and accuracy of the reports. The Expert Panel Report on PG is also available on the CERHR website <*http://cerhr.niehs.nih.gov*>.

Appendix I. NTP-CERHR Ethylene Glycol/Propylene Glycol Expert Panel

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

Center For The Evaluation Of Risks To Human Reproduction

Appendix II

NTP-CERHR EXPERT PANEL REPORT ON THE REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF PROPYLENE GLYCOL

May 2003 *NTP-CERHR-PG-03*

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Appendix II

ABBREVIATIONS

ACC	American Chemistry Council
ADH	alcohol dehydrogenase
ALDH	aldehyde dehydrogenase
ANOVA	analysis of variance
ATP	adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the concentration versus time curve
Avg	average
BIBRA	British Industrial Biological Research Association
bw	body weight
C	Celsius
cc	cubic centimeters
cm^2	centimeters squared
C_{max}	peak concentration
CAS RN	Chemical Abstracts Service Registry Number
CERHR	Center for the Evaluation of Risks to Human Reproduction
CNS	central nervous system
CSF	cerebral spinal fluid
d	day
dL	deciliter
DMBA	dimethylbenzanthracene
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
e.g.	*exempli gratia*; "for example"
EPA	Environmental Protection Agency
f	female
FAO/WHO	Food and Agriculture Organization/World Health Organization
FDA	Food and Drug Administration
fl oz	fluid ounces
g	gram
GC	gas chromatography
gd	gestation day
GRAS	generally recognized as safe
GSH	glutathione
hr	hour
HCG	human chorionic gonadotrophin
Hg	mercury
HPLC	high pressure liquid chromatography
HSDB	Hazardous Substances Data Bank
IM	intramuscular
IP	intraperitoneal
IPCS	International Programme on Chemical Safety
IU	international units

IV	intravenous
k_{abs}	absorption coefficient
kg	kilogram
K_m	Michaelis constant
K_{ow}	octanol-water partition coefficient
K_S	solubility constant
L	liter
lb	pound
LD_{50}	lethal dose, 50% mortality
LOAEL	lowest observed adverse effect level
m	male
M	molar
max	maximum
m^3	meters cubed
m^2	meters squared
meq	milliequivalents
mg	milligram
min	minute
mL	milliliter
mM	millimolar
mmol	millimole
MS	mass spectroscopy
mw	molecular weight
n, no., #	number
NAD	nicotinamide adenine dinucleotide
NZW	New Zealand White
ng	nanogram
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute of Occupational Safety and Health
nmol	nanomole
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
OSHA	Occupational Safety and Health Administration
Osm	osmolal
PBPK	physiologically based pharmacokinetic
PG	propylene glycol
PMSG	pregnant mare serum gonadotrophin
pnd	postnatal day
ppm	parts per million
RBC	red blood cell
RIA	radioimmunoassay
SCE	sister chromatid exchange
SD	standard deviation
SEM	standard error of the mean

SIDS	screening information data set
SPF	specific pathogen free
TLV	threshold limit value
T_{max}	maximum time
USDA	United States Department of Agriculture
USP	United States Pharmacopeia
v	volume
V_{max}	maximal velocity of metabolism
V_d	volume of distribution
VOC	volatile organic compound
wt	weight
wk	week
wt%	weight percentage
µg	microgram
µmol	micromole

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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.

Propylene glycol was selected for evaluation by the CERHR based on its high production and widespread public exposure due to its use as an antifreeze and deicing agent, as well as its use in paints, coatings, foods, drugs, and cosmetics.

This evaluation results from the efforts 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 propylene 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 exposures of 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 Propylene 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:

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A REPORT OF THE CERHR ETHYLENE GLYCOL AND PROPYLENE GLYCOL EXPERT PANEL:

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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, followed by 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 in the synopses of the study. **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.

1.0 CHEMISTRY, USAGE, AND EXPOSURE

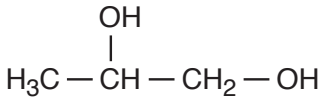
1.1 Chemistry

1.1.1 Nomenclature

The Chemical Abstracts Service Registry Number (CAS RN) for propylene glycol is 57-55-6. Synonyms or trade names for propylene glycol include: 1,2-propanediol; 1,2-dihydroxypropane; methylethylene glycol; trimethyl glycol; 1,2-propylene glycol; monopropylene glycol; propane-1,2-diol; alpha-propylene glycol; Dowfrost; PG 12; Sirlene; Solar Winter Ban; propanediol *(1)*; 2-dihydroxypropanol; methylethyl glycol; methyl glycol; 2,3 propanediol; and alpha propylene glycol *(2)*. The American Chemistry Council (ACC) *(3)* stated that the name Sirlene is no longer used.

1.1.2 Formula and Molecular Weight

Figure 1-1: *Chemical Structure of Propylene Glycol*



Chemical Formula: $C_3H_8O_2$
Molecular Weight: 76.095

1.1.3 Chemical and Physical Properties

Viscous, colorless, odorless hydroscopic liquid with a low vapor pressure. Physicochemical properties are listed in Table 1-1.

Table 1-1: Physicochemical Properties of Propylene Glycol[a]

Property	*Value*
Vapor Pressure	0.07 mm Hg at 20°C[b]
Melting Point	<-60°C
Boiling Point	188.2°C
Density	1.0361 g/cc at 20 °C[b]
Solubility in Water	Soluble
Log K_{ow}	-0.912[b]
Stability	Stable
Reactivity	Can react with oxidizing agents

[a]HSDB *(2)*, [b]ATSDR *(4)*

1.1.4 Technical Products and Impurities

According to the ACC *(3)*, impurities of propylene glycol include chlorides (1 ppm max), iron (1.0 ppm max), water (0.2 wt% max), and dipropylene glycol (<0.2%).

Manufacturers of propylene glycol are The Dow Chemical Company, Freeport, TX and Plaquemine, LA; Lyondell Chemical Company in Pasadena, TX; Huntsman Corporation in Port Neches, TX; and Arch Chemicals, Inc., in Brandenburg, KY *(3*).

1.2 Use and Human Exposure

1.2.1 Production

Commercial propylene glycol is manufactured by direct hydrolysis of propylene oxide by water *(5*). Propylene oxide is made using the chlorohydrin process where the propylene oxide is recovered as a pure product before conversion to the glycol. In 1999, 1,083 million pounds of propylene glycol were produced in the U.S. with apparent consumption of 854 million pounds *(5*).

1.2.2 Use

Of the 854 million pounds of propylene glycol consumed in the U.S., uses included (in million pounds and % wt) as a chemical intermediate in the manufacture of unsaturated polyester resins (228, 26.7%), cosmetics and personal care products, pharmaceuticals, and human food (170, 19.9%), liquid detergents (135, 15.8%), deicing fluids (85, 10%), antifreeze/engine coolant (55, 6.4%), paints and coatings (40, 4.7%), tobacco humectant (25, 2.9%), other fluids (32, 3.8%), and other applications (84, 9.8%) *(5*). Propylene glycol is also used in the production of plasticizers (e.g., polypropylene adipate), 2-methylpiperazine, 1,2-propylene diamine, hydroxylated polyester, polyester-type fluorescent resin matrix, and polyether polyols *(2*).

The following summary obtained from the Agency for Toxic Substances and Disease Registry (ATSDR) *(4*) and the Hazardous Substances Data Bank (HSDB) *(2*) provides information about propylene glycol uses and exposures:

> Propylene glycol is a colorless, odorless, water-soluble liquid considered safe for use in commercial formulations of foods, drugs, and cosmetics. Propylene glycol has been approved as safe in various food colors, flavorings, drugs, cosmetics, and as a direct additive to food. It is used as a humectant in tobacco, pet food, and in dentifrices; in veterinary medicine it is used as a glycogenic in ruminants. Propylene glycol is commonly used in the pharmaceutical industry as a solvent for drugs, as a stabilizer for vitamins, and in ointments for medicinal applications. It is used as a lubricant or heat transfer fluid in situations where leakage could lead to contact with food. It is used as an antifreeze, deicing solution, and as an additive to latex paints and coatings to improve freeze-thaw capability. Propylene glycol is also used in the generation of artificial mists and fogs used in fire safety training, and theatrical and stage productions. This widespread use of propylene glycol stems from its low level of toxicity.

Propylene glycol is used as a softener for cellulose films in the United Kingdom *(2, 6*).

Propylene glycol is Food and Drug Administration (FDA) approved for use in food, tobacco, and pharmaceutical products as an inert ingredient *(7*). It is considered to be generally recognized as safe (GRAS) for direct addition to foods *(7*). GRAS substances, such as propylene glycol, are also permitted in packaging materials as long as the substances "are used in amounts not to exceed that required to accomplish their intended physical or technical effect" *(7*). Inert ingredients are required

to be listed in over-the-counter drugs *(8)*.

Propylene glycol is a humectant in pet food products, but not in cat foods. Because of the sensitivity of the cat erythrocyte to Heinz body formation (denatured proteins, primarily hemoglobin) by propylene glycol and the possibility of inducing anemia in cats, propylene glycol was removed from cat food products (semi-moist cat food) by the FDA in 1996 *(9)*.

1.2.3 Occurrence

Propylene glycol is released into the environment from industrial disposal and from consumer products containing this chemical. Airports are required by the Environmental Protection Agency (EPA) *(10)* to monitor storm water runoff and to recycle deicing solutions. Propylene glycol is water-soluble and has the potential to leach into groundwater, but is rapidly degraded. The half-life of propylene glycol in water is estimated to be 1−4 days under aerobic and 3−5 days under anaerobic conditions *(4)*. No information was found on this compound in any environmental medium. Propylene glycol was not listed as an organic wastewater contaminant in a recent report by Kolpin et al. *(11)*.

1.2.4 Human Exposure

1.2.4.1 General Population Exposure

The general population can be exposed to propylene glycol through dermal contact with consumer products such as cosmetic products, antifreeze solutions, coolants, windshield deicers, or pharmaceutical creams. Oral exposure to propylene glycol can occur through its use in food and tobacco products and as a solvent for pharmaceutical products *(2)*. In Japan, average daily intake of propylene glycol as a food additive has been reported to be 43.0 mg/person **[43 mg/60 kg = 0.71 mg/kg bw/day]** (Louekari et al. *(12)* [from Market Basket Study, Japan 1982]).

Data for per capita daily intake of propylene glycol in food products have been estimated for the United States in a recent report by the United Nations Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives *(13)*. In reviewing the annual volume of production of 31 flavoring agents, propylene glycol per capita consumption was estimated at 2,400,000 μg/day **[34.28 mg/kg bw/day for a 70 kg person]**. (This value was based upon the 1995 update of data collected since 1972 by the Flavor and Extract Manufacturers' Association.)

In a review by the Cosmetic Ingredient Review Expert Panel *(14)*, data on the percent concentration and use of propylene glycol in cosmetics was summarized; these data are presented in Table 1-2. These data were based upon information provided to the FDA in 1984 on propylene glycol use in cosmetic formulations and consisted of a total of 5,676 cosmetic products in 74 categories, with 2,597 product formulations containing between 1 and 10% propylene glycol.

Table 1-2. Product Formulation Data for Propylene Glycol (Adapted from Cosmetic Ingredient Review (14))

Product Category	*Number of product formulations within each concentration range*					
	>50%	*50–10%*	*10–1%*	*1–0.1%*	*Unknown*	*Total*
Baby shampoos			3	1	3	7
Baby lotions/oils/powders/creams			6		2	8
Other baby products			2	1		3
Bath oils/tablets/salts		6	2	18	3	29
Bubble baths		1	21	64	37	123
Other bath preparations		3	15	25	5	48
Eyebrow pencil			1			1
Eyeliner		2	49	4		55
Eye shadow		3	89	44	39	175
Eye lotion			3	1		4
Eye makeup remover		4	9	4		17
Mascara		4	36	6	14	60
Other eye makeup preparations			27	7	9	43
Colognes/toilet waters		5	35	56		96
Perfumes		1	7	20		28
Powders dusting/talcum, aftershave talc			1	8	1	10
Sachets		10	14	4		28
Other fragrance preparations	1	4	28	10		43
Hair conditioners			24	20	14	58
Hair sprays (aerosol fixatives)			1	8	1	10
Hair straighteners			22			22
Permanent waves		3	2	27	11	43
Rinses (noncoloring)			4	6	3	13
Shampoos (noncoloring)		4	70	83	54	211
Tonics/dressings/other hair grooming aids		2	18	8	3	31
Wave sets		1	6	11		18
Other hair preparations		2	6	7	1	16
Hair dyes/colors (requiring caution statement)		60	222	1	5	288
Hair rinses (coloring)			22	7		29
Hair shampoos (coloring)			1	2		3
Hair lighteners with color			1			1
Hair bleaches			6			6
Other hair coloring preparations		1	3		1	5
Blushers (all types)	1	3	45	19	17	85
Face powders			16	13		29
Foundations		45	150	11	56	262
Leg and body paints			3			3
Lipstick			4	633	544	1181
Makeup bases		12	261	9	52	334

Table 1-2 (continued)

Product Category	*Number of product formulations within each concentration range*					
	>50%	*50–10%*	*10–1%*	*1–0.1%*	*Unknown*	*Total*
Rouges		2	11	9	8	30
Makeup fixatives				1	3	4
Other makeup preparations		4	25	31	41	131
Cuticle softeners			9	3		12
Nail creams/lotions			6	1		7
Nail polish and enamel removers				2		2
Other manicuring preparations		3	3			6
Dentifrices (aerosol/liquid/paste/powder)		1	1			2
Mouthwashes/breath fresheners			3			3
Other oral hygiene products				1		1
Bath soaps/detergents			11	28		39
Deodorants (underarm)	19	13	71	12	9	124
Douches		5	1	1		7
Feminine hygiene products			1	1		2
Other personal cleanliness products		3	33	17		53
Aftershave lotions		1	54	36	6	97
Beard softeners		2			1	3
Preshave lotions			1	3	4	8
Shaving cream (aerosol brushless lather)		2	18	9	5	34
Other shaving preparations		1	5	5	2	13
Skin cleansing products (cold creams/lotions/liquids/pads)		17	195	35	29	276
Depilatories		2	2	2		6
Face/hand/body (excl. shaving preparations)		15	168	79	55	417
Foot powders/sprays			1			1
Hormone products		1	3	1		5
Moisturizing products		7	269	58	24	358
Night preparations		5	59	9	10	83
Paste masks (mud packs)		2	15		2	19
Skin lighteners		1	66	32	37	136
Skin fresheners		1	8	4	1	14
Wrinkle-smoothing products (removers)		1	8	4	1	14
Other skin care preparations		5	76	32	32	149
Suntan gels/creams/liquids		2	34	15	15	76
Indoor tanning preparations			10		2	12
Other suntan preparations		1	9	1	4	15
Ingredient Total	***21***	***279***	***2,597***	***1,579***	***1,200***	***5,676***

Propylene glycol is rapidly degraded in water and CERHR was unable to locate any information on propylene glycol in drinking water.

Propylene glycol may be released by some carpeting *(2)*. In a technical study by Hodgson et al. *(15)*, emissions of volatile organic compounds (VOC) from four different types of new carpets were measured. Exposure chamber air samples were collected onto multisorbent samplers packed with Tenax-TA, Ambersorb XE-340, and activated charcoal, in series. The chemicals were thermally desorbed from the sampler, concentrated, and injected into a capillary gas chromatograph with a mass spectrometer used as a detector. One carpet with a polyvinyl chloride backing emitted propylene glycol, vinyl acetate, formaldehyde, isooctane, and 2-ethyl-1-hexanol. Propylene glycol and vinyl acetate had the highest concentrations and emission rates for this carpet. The estimated emission rates ranged from 690 μg/m^2/hr 24 hours after installation to 193 μg/m^2/hr at 168 hours after installation. The other three carpet types did not emit propylene glycol.

The FDA estimated that the human daily dietary intake of propylene glycol to be a 'few mg per kg **[body weight]** per day' *(16)*. **[No details were given on how exposures were estimated.]** In a 2002 report by the United Nations Joint FAO/WHO Expert Committee on Food Additives *(13)*, per capita consumption of propylene glycol in the United States was estimated at 2,400,000 μg/day **[34.28 mg/kg bw/day for a 70 kg person]**. The average daily dietary intake of propylene glycol in Japan was estimated to be 43 mg/person **[0.7 mg/kg bw/day based on a 60 kg person]** *(12)*. The WHO food additive series *(17)* lists the acceptable human daily intake of propylene glycol at <25 mg/kg bw/day.

1.2.4.2 Medical Exposure

Propylene glycol is used in some pharmaceuticals that are administered intravenously (see Table 2-8). This represents a unique exposure route for certain subpopulations.

1.2.4.3 Occupational Exposure

Occupational exposure to propylene glycol may occur through direct dermal contact while handling products containing this compound or through inhalation of airborne propylene glycol resulting from heating or spraying processes *(2)*.

Neither the Occupational Safety and Health Administration (OSHA) nor the American Conference of Governmental Industrial Hygienists (ACGIH) has established exposure limits for propylene glycol vapors. No Threshold Limit Value (TLV) has been defined for propylene glycol, but an American Industrial Hygiene Association (AIHA) Workplace Environmental Exposure Level (WEEL) guide of 50 ppm (total exposure) and inhalation aerosol exposure of 10 mg/m^3 has been determined *(18)*.

A 1981−1983 National Occupational Exposure Survey (NOES) of U.S. workers led NIOSH to estimate that 1,748,454 people were potentially exposed to propylene glycol at the workplace *(2)*. Ninety-eight percent of exposures are with trade name products containing propylene glycol, rather than in the production of propylene glycol itself *(2)*.

Norbäck et al. *(19)* studied the exposure of Swedish painters to VOCs from indoor application of water-based paints. VOCs were sampled on different sorbents within the personal breathing zone of the painter and analyzed by gas chromatography (GC)/mass spectroscopy (MS). Propylene glycol was

one of the VOC constituents measured. Exposure measurements for propylene glycol were taken over a 1-hour period of water-based paint application for 20 batches of paint from 5 different manufacturers. Propylene glycol was detected in 12 of the 20 samples. Personal exposure to propylene glycol during application of water-based paints yielded a geometric mean of 350 μg/m^3 with a maximum value of 12,700 μg/m^3.

Laitinen et al. *(20)* examined exposure to ethylene and propylene glycol in Finnish motor servicing workers. 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 given as 1.9 cm^3/m^3 and 3.2 cm^3/m^3, respectively. Urine samples were collected after the work shift and analyzed for ethylene glycol, oxalic acid, and propylene glycol **[method of urine collection, storage, and extraction and quality control not reported]**. There were no differences found between controls and propylene glycol-exposed mechanics.

Deicing fluids are low viscosity glycols used to remove ice or snow that would increase drag on the aircraft. The antifreeze components in a deicing solution vary with the manufacturer, usage, and environmental conditions. Commercial Type I fluid is applied hot as a mixture of fluid and hot water to deice the exterior of aircraft. Type IV fluids are usually applied after the aircraft is deiced to keep ice from reforming. Approximately 90% of Type I fluids and 50% of Type IV fluids are propylene-glycol based *(3, 5)*. Performance criteria for deicing fluids are governed by specifications of the Aerospace Division of the Society of Automotive Engineers (SAE) *(21)*. Both inhalation and dermal exposures to workers using deicing solutions can occur.

The levels of propylene glycol in aircraft deicing workers (n=7, age 31−52 years, sex not given) using either undiluted or water-diluted propylene glycol heated to 60°C was measured in urine samples collected pre- and post-shift *(22)*. Workers were wearing coats, rubber gloves, and masks. The detection limit for the method used to measure propylene glycol in urine was 20 μg/L. Urine samples were also collected from a comparison group of non-exposed persons (n=16, sex and age not given). For the exposed workers, the median pre-shift urine level was 1.49 mg/L (range 0.72−13.44 mg/L) and 1.67 mg/g creatinine (range 0.41−10.58 mg/g creatinine) and the median post-shift urine level was 2.07 mg/L (range 0.77−9.04 mg/L) and 2.46 mg/g creatinine (range 1.22−10.27 mg/g creatinine). Propylene glycol concentrations in the post-shift worker urine samples were only slightly higher than those of the unexposed comparison group.

In a study simulating concentrations of propylene glycol mist used in aviation emergency training, Wieslander et al. *(23)* concluded that short (1 minute), high exposure (geometric mean concentration of 309 mg/m^3, range 176−851 mg/m^3) to propylene glycol mist may cause acute ocular and upper airway irritation. The duration of these effects was not measured, as measurements were taken within 15 minutes of exposure.

A Health Hazard Evaluation (HHE) on occupational exposure to propylene glycol during aircraft deicing operations was conducted by NIOSH *(24)*. Evaluation of deicing procedures was conducted at

the Denver International Airport (DIA) in March 1996. At DIA, United Airlines uses a 50% solution of propylene glycol in water, heated to 180° F for deicing aircraft. Trucks with dual 800-gallon tanks, spray hoses, and booms are used. The amount of fluid used for deicing each plane ranges from 50 to 200 gallons. Personal breathing-zone air samples were collected from six ground sprayers, one basket man, and one truck driver. Air samples were collected on XAD-7 OVS tubes at a flow rate of 0.5 L/min for 6 hours and analyzed by GC/MS for propylene glycol according to NIOSH Method 5523. Seven workers (Table 1-3) had a range of exposures from 10 to 21 mg/m^3 with a mean of 15 mg/m^3, based on a 6-hour collection.

Table 1-3. Exposure to Airborne Propylene Glycol HETA 95-0069 (24)

Job	*Concentration (mg/m^3)*
Ground Sprayer	14
Ground Sprayer	10
Ground Sprayer	16
Ground Sprayer	11
Ground Sprayer	17
Ground Sprayer	94*
Truck Driver	19
Basket Man	21

*Air sample was visibly contaminated with liquid propylene glycol. This was caused by a worker being accidentally sprayed with the deicing fluid during sampling.

The author concluded that "there was no hazard from overexposure to deicing fluid. ...Airborne exposure to propylene glycol was low and propylene glycol has low toxicity."

Propylene glycol does not bioaccumulate in organisms and rapidly biodegrades in the soil and in water *(25)*. However, this process is oxygen-demanding and can deplete dissolved oxygen levels in water *(26)*. The Clean Water Act requires airports to implement plans for deicer management to control storm water contamination. Therefore, airports must monitor propylene glycol storm water runoff and scavenge and recycle deicing solutions *(10)*.

1.3 Utility of Data

Limited human exposure data for propylene glycol were available for Expert Panel review. The utility of the occupational exposure data available is limited by either the small sample size or a high proportion of non-detected values. Estimates of propylene glycol workplace exposures are based on a 1981−1983 NOES of U.S. workers and may not reflect current occupational exposure. These data are insufficient to evaluate occupational exposure to propylene glycol.

An estimate of U.S. consumer exposure was available from a 2002 report by the United Nations Joint FAO/WHO Expert Committee on Food Additives *(13)*. In reviewing the annual production volume of 31 flavoring agents, per capita consumption of propylene glycol was estimated at 2,400,000 µg/day **[34.28 mg/kg bw/day for a 70 kg person]**. This value exceeded the estimated per capita

consumption in Japan (1982) by approximately 50-fold **[43 mg/60 kg = 0.71 mg/kg bw/day]**. These estimates of human exposure are for food products and do not include exposure from pharmaceutical products or exposure through inhalation. Propylene glycol is found in many pharmaceuticals that are administered intravenously. There are limited data on the effects and exposure levels of chronic (intravenous) administration of propylene glycol in infants and children and no information was found on chronic exposure in pregnant women.

1.4 Summary of Human Exposure Data

In 1999, 1,083 million pounds of propylene glycol were produced in the U.S. with apparent consumption of 854 million pounds *(5)*. Of the apparent amount consumed, uses included, in million pounds and percentages, unsaturated polyester resins (228, 26.7%); cosmetics and personal care products, pharmaceuticals, and human food (170, 19.9%); liquid detergents (135, 15.8%); deicing fluids (85, 10%); antifreeze/engine coolant (55, 6.4%); paints and coatings (40, 4.7%); tobacco humectant (25, 2.9%); other fluids (32, 3.8%); and other applications (84, 9.8%) *(5)*. Propylene glycol is approved by the FDA for use in food, tobacco, and pharmaceutical products and has GRAS status for direct addition to foods.

The general population is exposed to propylene glycol by oral intake, dermal contact, and inhalation. The average daily intake of propylene glycol from food products in the United States has been estimated at 2,400 mg/day **[34 mg/kg bw/day for a 70 kg person]** *(13)*. In Japan, the estimated average daily intake of propylene glycol as a food additive was reported to be 43 mg per person **[43 mg/60 kg=0.71 mg/kg bw/day]** (Louekari et al. *(12)* [from Market Basket Study, Japan 1982]). The Joint FAO/WHO Expert Committee on Food Additives *(13)* concluded that "the safety of these substances [propylene glycol and propylene glycol stearate] would ... not be expected to be of concern." Since propylene glycol has GRAS status and may not be listed as a specific ingredient in some foods, dietary intake based upon product labeling would result in an underestimation of intake. Propylene glycol is an inert ingredient in some pharmaceutical preparations and is also found in many pharmaceuticals that are administered intravenously, which represents a unique exposure route for certain subpopulations.

Occupational exposure to propylene glycol may occur through dermal contact or through inhalation of airborne propylene glycol from heating or spraying processes. No TLV has been defined for propylene glycol, but an AIHA WEEL guide of 50 ppm (total exposure) and an inhalation aerosol exposure of 10 mg/m^3 have been determined. NIOSH estimated that 1,748,454 people (1981−1983 NOES survey as cited in NIOSH report, 1983 *(2)*) are potentially exposed to propylene glycol in the workplace, primarily through contact with trade name products containing propylene glycol.

Several small occupational exposure studies measuring propylene glycol were located. In a study by Laitinen et al. *(20)*, motor-servicing worker exposure to propylene glycol and ethylene glycol was measured. Propylene glycol was below the detection level in air and levels in the urine of exposed workers did not differ from urinary levels in unexposed controls. As dermal exposure to workers was not measured, it was not possible to determine whether urinary levels of propylene glycol found in the workers were due to low exposure or to low dermal absorption.

Norbäck et al. *(19)* measured airborne propylene glycol exposure of Swedish painters during indoor application of water-based paints. Propylene glycol was detected in 12 of 20 samples with a geometric

mean of 350 μg/m^3 and a maximum value of 12,700 μg/m^3.

The levels of propylene glycol in aircraft deicing workers (n=7, age 31−52 years, sex not given) using either undiluted or water-diluted propylene glycol heated to 60ºC was measured in urine samples collected pre- and post-shift *(22)*. Urine samples were also collected from a comparison group of non-exposed persons (n=16, sex and age not given). For the exposed workers, the median pre-shift urine level was 1.49 mg/L (range 0.72−13.44 mg/L) and 1.67 mg/g creatinine (range 0.41−10.58 mg/g creatinine). For the exposed workers, the median post-shift urine level was 2.07 mg/L (range 0.77−9.04 mg/L) and 2.46 mg/g creatinine (range 1.22−10.27 mg/g creatinine). For the unexposed comparison group, the median urine level was 1.35 mg/L (range 0.29−10.7 mg/L) and 1.18 mg/g creatinine (range 0.46−18.77 mg/g creatinine).

In a Health Hazard Evaluation (HHE) conducted by NIOSH on workers (n=8) using propylene glycol during aircraft deicing operations *(24)*, personal breathing-zone air samples over a 6-hour period were collected. Seven workers had exposures ranging from 10 to 21 mg/m^3 with a mean of 15 mg/m^3 (1 worker sample excluded due to a suspect high value).

2.0 GENERAL TOXICOLOGICAL AND BIOLOGICAL PARAMETERS

2.1 Toxicokinetics and Metabolism

The toxicokinetics and metabolism data for propylene glycol were initially examined by consulting authoritative reviews *(4, 27*) and an independent review *(28*). The toxicokinetics sections in those reviews were somewhat brief, and a decision was made by CERHR to review relevant original studies in humans and studies in animals pertinent to reproductive and developmental toxicity.

2.1.1 Absorption

2.1.1.1 Human

Studies of the pharmacokinetics of propylene glycol in humans have been conducted primarily in conjunction with on-going patient therapy where propylene glycol was administered as a vehicle for medications.

Oral

Yu et al. *(29*) examined the pharmacokinetic profile of propylene glycol during multiple oral-dosing regimens. The 22 subjects were outpatients who participated in a phenytoin bioavailability study where propylene glycol was used as a solvent. In one study, 16 adults received a 20.7 g/dose 3 times daily for a minimum of 3 days. In another study, 6 individuals received a 41.4 g/dose twice daily for a period of 3 days. These oral doses were given in conjunction with 100 mg phenytoin in 7.25 mL of alcohol USP, 6 µL of Peach Flavor, 5 mL of glycerin USP, and 8 mL of 70% (w/w) fructose. Propylene glycol was rapidly absorbed from the gastrointestinal tract with maximum plasma concentrations obtained within 1 hour of dosing. The average serum half-life of propylene glycol for the study with 16 and 6 individuals was determined by the authors to be 3.8 and 4.1 hours, respectively. The average total body clearance was determined by the authors to be approximately 0.1 L/kg/hr, although there was significant variability in clearance rate among individuals. The apparent volume of distribution was determined by the authors to be approximately 0.5 L/kg, which approximates the volume of distribution of total body water *(29*).

Strength/Weaknesses: This study by Yu et al. *(29*) provides data on the oral absorption of propylene glycol as well as on serum half-life, and apparent volume of distribution and total body clearance after repeated oral doses of either 20.7 g 3 times daily or 41.4 g 2 times daily, for a minimum of 3 days. The results are in agreement with expectations for a highly water-soluble, small molecule: rapid absorption, distribution into total body water, relatively short half-life, and rapid total body clearance. One study limitation is the study subjects' concomitant exposure to ethanol; propylene glycol and ethanol are substrates that compete for alcohol dehydrogenase in the initial step of metabolism. While the doses of propylene glycol were high, the data do indicate ready bioavailability of the chemical. The half-life estimates are generally consistent with the results of Speth et al. *(30*) to be discussed later.

Utility (Adequacy) for CERHR Evaluation Process: Data in the Yu et al. *(29*) study are generally adequate to estimate kinetic parameters, but inadequate for quantitative determination of bioavailability.

Rectal

In a study using human volunteers, Kollöffel et al. *(31*) studied rectal absorption and other kinetic

parameters in children and adults. Propylene glycol and water (1:1) were used as solvents in the formulation of a rectal solution of paracetamol. Absorption of propylene glycol through the rectum was rapid with peak concentrations obtained at 1 ± 0.6 hour (average ± SD) in children (5−12 years old) and 1.5 ± 0.3 hours in adults. Peak plasma concentrations were measured at 171 mg/L **[2.2 mM]** in 4 children dosed with 0.173 g/kg bw propylene glycol and 119 mg/L **[1.6 mM]** in 10 adults dosed with 8.64 g propylene glycol **[123 mg/kg bw assuming a 70 kg bw]**. The serum half-life was determined to be 2.8 ± 0.7 hours in adults and 2.6 ± 0.3 hours in children. The apparent volume of distribution was 0.79 ± 0.30 L/kg in adults and 0.77 ± 0.17 L/kg in children *(31)*.

Strength/Weaknesses: Kolloffel et al. *(31)* determined C_{max} and T_{max} and then used a linear curve-fitting program to recalculate C_{max} and T_{max}, values as well as half-life, apparent volume of distribution, and clearance after different doses of propylene glycol were administered per rectum to adults and children. The small number of children (n = 4) and the age range (5−12 years) does not permit a judgment as to whether bioavailability may differ as a function of age within childhood or between children and adults. The values reported are in the expected range providing confirmatory evidence for the reliability of kinetic parameters determined by Speth et al. *(30)*. Plasma levels in children (age 5−12 years) were only slightly higher than in adults. The half-life was virtually the same in children as in adults, which is in agreement with alcohol dehydrogenase activity reaching adult levels by the age of 5 years *(32)*. The extent of oral absorption cannot be judged from these data but a visual inspection of plasma concentrations after intravenous (IV) infusion *(30)* and rectal administration *(31)* indicate very high bioavailability. Thus, oral bioavailability will also be very high. Although it appears that children absorb propylene glycol significantly faster and attain higher peak plasma concentration than adults, the differences are modest and of doubtful toxicological significance.

Utility (Adequacy) for CERHR Evaluation Process: The study by Kolloffel et al. *(31)* is useful to indirectly assess bioavailability.

Dermal

There is limited information on the absorption of propylene glycol through intact human skin. In a study of human skin biopsy specimens from adults 19−50 years of age, MacKee *(33)* found no penetration of radioactive tracer materials after up to 1 hour permeation time using propylene glycol alone as a vehicle **[visual evidence of tracer uptake into biopsied skin, but no analytical confirmation provided]**. Enhancers, such as surfactants, increased absorption.

Three studies are described briefly below that involved patients with significant medical complications. In 45 patients (0.5−87 years old) with second- and third-degree burns on 21−95% of their body, propylene glycol was absorbed through skin following dermal treatment with sulfadiazine in a propylene glycol vehicle; serum levels of propylene glycol in those patients ranged from 0 to 0.98 g/dL **[0 to 129 mM]** *(4, 34)*. In an 8-month-old infant with second- and third-degree burns and complicating toxic epidermal necrolysis over 78% of his body, dermal treatment with silver sulfadiazine in propylene glycol resulted in a peak propylene glycol blood level of 1.059 g/dL **[139 mM]** *(35)*. A blood propylene glycol level of 0.070 g/dL **[9.2 mM]** in an infant was attributed to Mycostatin cream usage for diaper rash *(36)*.

Strengths/Weaknesses: The MacKee study *(33)* showed what is expected of a highly water-soluble substance: that dermal absorption of propylene glycol through the intact skin is very limited.

Weaknesses of this study are the insensitive, non-quantitative method for assessing chemical uptake and the extensive manipulation of the skin following the permeation period (excision which apparently produced bleeding), which may have lead to losses of both skin and permeated chemical from handling the tissue. The three clinical studies *(34-36*) present evidence of propylene glycol bioavailability in circumstances that preclude confident extrapolation to a healthy general population. They do indicate that once the stratum corneum is impaired (removed such as in burns or irritated), dermal absorption may become a significant source of exposure.

Utility (Adequacy) for CERHR Evaluation Process: The MacKee *(33*) study has minimal utility for drawing conclusions regarding propylene glycol penetration across healthy human skin. However, when combined with the rat dermal penetration *in vitro* study *(37*) also showing no uptake, and given the difficulty water soluble molecules generally have penetrating the stratum corneum, the Panel concluded that the dermal absorption rate across intact skin is likely to be slow. Therefore, it can also be expected that any dermal exposure to propylene glycol will result in systemic levels far below saturation of metabolic clearance.

Inhalation

Bau et al. *(38*) **[as reported in HSDB *(2*)]** reported that less than 5% of a technetium-labeled aerosol containing 10% propylene glycol **[propylene glycol not directly measured]** in deionized water was taken up by humans after inhalation for 1 hour in a mist tent. The authors measured the aerosol mass median diameter to be 4.8−5.4 microns, a size small enough to have enabled penetration to the deep lung. Ninety percent of the dose was found in the nasopharynx and it rapidly entered the stomach with very little entering the lungs. Propylene glycol was not measured. The low vapor pressure (0.07 mmHg, approx equal to ~90 ppm or ~270 mg/m^3) of propylene glycol in combination with the short half-life before saturation of metabolism does not allow the build up of toxicologically relevant doses.

Strength/Weaknesses: Since propylene glycol was not directly measured by Bau et al. *(38*), absorption through the nasal mucosa cannot be determined. However, the low dose rate from inhalation exposure and the small surface area would not lead to significant absorption of propylene glycol.

Utility (Adequacy) for CERHR Evaluation Process: Since inhalation of chemicals is kinetically related to IV infusion, it is of interest to know if propylene glycol is efficiently absorbed from the lungs. As a small, water soluble molecule, it is reasonable to predict that propylene glycol would be absorbed by the lungs. However, with a low vapor pressure (0.07 mm Hg), inhalation of toxicologically relevant doses of propylene glycol is not possible unless heated to higher temperatures. Therefore, the remaining question is whether propylene glycol in a carrier medium could lead to significant exposure by inhalation. Bau et al. *(38*) provides a quantitative answer. Of an average of 263 mL of nebulized aerosol, 8.1 mL containing 10% propylene glycol was retained per hour, corresponding to about 0.8 g of compound, which in turn amounts to 0.09 g/kg per 8 hours. Therefore, it can be concluded that under normal conditions of exposure, propylene glycol via inhalation is of limited toxicological relevance.

2.1.1.2 Animals

Oral

Animal studies demonstrate that propylene glycol is rapidly absorbed following oral exposure. ATSDR *(4)* reports the findings of a study by Christopher et al. *(39)* in which plasma levels of propylene glycol were measured at 19.1 and 8.4 mM in 2 cats fed a diet with 12% propylene glycol [1.60 g/kg bw/day] for 5 weeks. Morshed et al. *(40)* found that propylene glycol blood concentration (41.04 mM) reached its maximum level 1 hour after 4 New Zealand White (NZW) rabbits were administered 38.66 mmol/kg bw [2.942 g/kg bw] as a 28.4% aqueous solution by gavage. Morshed et al. *(41)* orally administered an aqueous solution of propylene glycol at 4.83−77.28 mmol/kg bw [0.368−5.881 g/kg bw] to 6 male Wistar rats/group and found that absorption occurred by a first order process; time to peak absorption was related to dose and ranged from roughly 10 minutes at the low dose to 2 hours at the high dose. An older study by Lehman and Newman *(42)* demonstrated peak blood levels of propylene glycol approximately 2−3 hours after oral dosing in dogs.

Strength/Weaknesses: The Christopher et al. *(39)* study provides very limited data (one time point only) on plasma concentration of propylene glycol after repeated administration of one of two dose rates administered in the diet. It is impossible to derive any kinetic information from such a study other than the qualitative statement that propylene glycol is absorbed to some extent by the cat from the diet.

In contrast, Morshed et al. *(41)* provided a more complete set of data indicating dose-dependent T_{max} for propylene glycol in the dose range of 0.4−5.9 g/kg. The authors did not calculate absorption half-lives or determine the extent of absorption. They concluded that gastrointestinal absorption occurred by a first order process because of the linear rise of plasma concentration at each of the five doses. **[This is an improper conclusion. Data are plotted on an arithmetic scale from which calculation of kinetic rate constants is not possible. There is no indication of curve stripping to calculate k_{abs}. The fact that elimination appears linear on an arithmetic scale indicates a zero order process. If absorption were first order, the absorption rate should increase with increasing concentration in the gastrointestinal tract. The fact that absorption rate did not increase in this manner suggests some limitation with higher bolus doses – e.g., possible delayed gastric emptying. In any case, more complete information is needed to assess bioavailability from the oral route (e.g., Vd, AUC, total body clearance rate, or a comparison IV study in rats).]** The other Morshed et al. *(40, 43)* papers and the Lehman and Newman *(42)* paper also do not provide data suitable for quantitative evaluation. There are reliable quantitative data for the gastrointestinal absorption of diethylene glycol in the rat *(44)* with absorption half-lives ranging from 5 to 40 min (average 16 min) amounting to 80−100% of the dose. Since diethylene glycol has a higher molecular weight but comparable hydrophilicity, it is likely that very rapid gastrointestinal absorption occurs also for propylene glycol. This is also the case for ethylene glycol as indicated by rapid urinary excretion *(45)*.

Utility (Adequacy) for CERHR Evaluation Process: Available animal data are not well suited for quantitative estimation of gastrointestinal absorption of propylene glycol. Nevertheless, all data including structure-activity relationships point toward very rapid and complete absorption. This is plausible for a highly water-soluble small molecule which will cross membranes with bulk flow of water across aqueous pores.

Dermal

Information on *in vivo* dermal absorption of propylene glycol in animals was not located. ATSDR notes that "*In vitro* studies of the penetration of propylene glycol through the rat abdominal stratum corneum have been conducted" *(4)*. Fresh abdominal skin from male Wistar rats was used in experiments in which propylene glycol, or a mixture of propylene glycol and oleic acid, were evaluated for absorption properties *(46)*. When propylene glycol was applied for up to 2 hours, no compound was detected in the dermis. However, when 0.15 M oleic acid was added to the propylene glycol, it was detected in the dermis after 30 minutes of exposure, but not after 5 or 15 minutes *(46)*. ATSDR *(4)* reported that hairless mouse skin overestimates absorption of propylene glycol by human skin while shed snake skin underestimates absorption. Therefore, the authors concluded that human skin should be used for absorption studies if possible.

2.1.2 Distribution

Speth et al. *(30)* reported on the pharmacokinetics of IV administration of propylene glycol involving six cancer patients who were sufficiently healthy to care for themselves and had normal liver and kidney function. They reported that clearance decreased as dose increased over a dose-range of 3−15 mg/m^2. There was a first order elimination with an average terminal half-life of 2.3 ± 0.7 hours. Considerable interpatient variation was noted. The apparent volume of distribution ranged from ~0.55−0.94 L/kg. In other studies with oral or rectal exposure, apparent volumes of distribution ranged from ~0.52−0.79 L/kg *(29, 31)*.

Strength/Weaknesses: This study *(30)* provides sound pharmacokinetic data from a limited number of individuals who were exposed intravenously to propylene glycol. However, the Speth et al. conclusion that clearance of propylene glycol in humans occurs by a first order process is questionable, as is the calculation of an average half-life of 2.3 ± 0.7 hours.

Utility (Adequacy) for CERHR Evaluation Process: This human study with IV exposure and those with oral and rectal exposure indicate that propylene glycol is uniformly distributed in total body water without a significant distribution to specific tissues. It can be predicted with certainty that propylene glycol will distribute into the water compartment of the placenta and fetus.

2.1.3 Metabolism

In what is considered to be the main pathway of propylene glycol metabolism in mammals *(4, 39)*, propylene glycol is oxidized by alcohol dehydrogenase to lactaldehyde, then to lactate by aldehyde dehydrogenase. The lactate is further metabolized to pyruvate, carbon dioxide, and water. Lactate also contributes to glucose formation through gluconeogenic pathways *(39)*. Lactate, via phosphoenol pyruvate, can be detoxified into glucose and stored as glycogen, as has been demonstrated by Wittman et al. *(47)* for propylene glycol in rats. Excess production of lactic acid resulting from very large exposures to propylene glycol can produce a metabolic anion gap **[anion gap = $(Na^+) - (Cl^- + \text{total } CO_2)$]** and metabolic acidosis *(4)*. Serum levels of >180 mg/L **[2.37mM]** can result in toxicity *(48)*.

In most mammals, part of the absorbed propylene glycol is eliminated unchanged by the kidney, while another portion is excreted by the kidneys as a glucuronic acid conjugate *(2, 28)*. The amount of propylene glycol eliminated by the kidneys has been estimated for humans at 45% *(48)*, for dogs at 55−88% *(49)*, and for rabbits at 24−14.2% *(50)*. Cats do not have the ability to produce the

glucuronidated metabolite *(28*). Alternate stereo-specific reaction pathways have been described for the metabolism of propylene glycol and are described below.

In adult humans, the mean serum half-life of propylene glycol is approximately 2−4 hours *(30*). Kelner and Bailey *(51*) studied the pharmacokinetics of propylene glycol in humans in conjunction with the IV administration of medications. Propylene glycol concentrations were measured in sera and cerebral spinal fluids (CSFs) from five patients receiving medication containing propylene glycol as a vehicle; lactate and pyruvate concentrations were also measured. The authors stated that all patients had normal hepatic and renal function based upon laboratory tests. The authors found a significant ($p < 0.01$) correlation of lactate concentrations in the serum and CSF to the corresponding propylene glycol concentrations in these fluids. The authors concluded that although the increase in serum lactate could be due to the patients' clinical conditions, it was unlikely in light of the correlation between propylene glycol and lactate concentrations. For two patients, the authors had propylene glycol/creatinine clearance ratios and were able to calculate the serum half-life of propylene glycol. The authors estimated this to be 4.7 and 5.6 hours, respectively, for these patients. **[The dose was not stated, but because of the severe lactic acidosis, the results suggest that it must have been higher than the 2x41.4g dose/day for 3 days administered by Yu and Sawchuck *(50*), which did not cause lactic acidosis.]**

While it is clear that total body clearance of propylene glycol occurs by metabolism and by renal excretion of the parent compound, there are no data in humans from which to assess the percentage fate of propylene glycol by these mechanisms. In the rabbit, Yu and Sawchuck *(50*) observed that metabolic clearance accounts for 85.8−97.6% of total clearance at lower doses. Morshed et al. *(41*) provided evidence that the rate-determining step in the metabolic clearance of propylene glycol in the rat is the NAD-dependent alcohol dehydrogenase. Using the dehydrogenase inhibitor pyrazole, there was a dose-dependent inhibition of the dehydrogenase leading to a dose-dependent increase in urinary excretion of propylene glycol. They found that the maximum metabolizing capacity in the rat was 8.33 mmole of propylene glycol/kg bw/hour, which they stated would extrapolate to 1.06 kg bw/day for a 70 kg human.

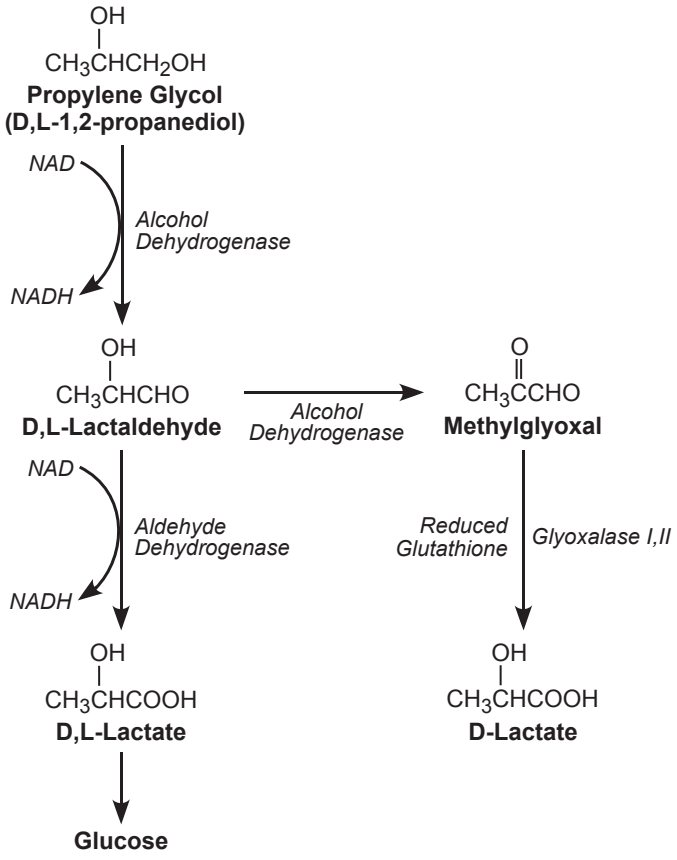
The Expert Panel believes that Speth et al. *(30*) supports the conclusion that humans clear propylene glycol similarly to rats and rabbits. However, saturation of metabolic clearance seems to occur at lower doses in humans than in rats and rabbits. Speth et al. (Table 2 of the study) *(30*) indicates that saturation of metabolic clearance seems to occur at a dose of about 7 g/day in some patients but not in others. Metabolic clearance does not seem to be affected at ~5 g/day (although no lower dose was used to prove it conclusively) and is uniformly decreased above 12.6 g/day. Speth et al. *(30*) provide evidence of metabolic saturation in propylene glycol metabolism at doses of approximately 7 g/day as seen by lengthening half-life and nonlinear increases in AUC and C_{max}. When this dose is converted to mmol/kg based upon the body weights reported for the three subjects receiving this dose, the value is 1.6 mmole/kg, which is considerably lower than the K_m reported by Morshed et al. in rats. Therefore, the half-life of propylene glycol before saturation of metabolic clearance when it would occur by a first order process is 1.6 ± 0.2 ($\pm$ SD) hours. This increased to above 3 hours after metabolic saturation of doses above 12 g/day, when metabolic clearance occurs by a zero order process. This is confirmed by Yu et al. *(29*) who found a "terminal elimination" half-life of ~4 hours in patients administered even higher doses (3 x 20.7 and 2 x 41.4 g/day) of propylene glycol. Unlike the half-life

of a compound cleared by a first order process, which is constant, the half-life of a chemical cleared by a zero order process is dose-dependent as is amply documented for propylene glycol.

2.1.3.1 Metabolism and Stereospecificity

Synthesis of propylene glycol results in a 1:1 ratio of D and L stereoisomer forms. There is some, although incomplete, information in the literature about stereospecificity of the enzymes in the propylene glycol metabolic pathways (Figure 2-1).

Figure 2-1. Propylene Glycol Metabolism in Mammals



From Christopher et al. (*39*)

In the main metabolic pathway, D and L forms of lactaldehyde and lactate are formed *(4, 39*). In the horse and rabbit, ADH will oxidize the L form of propylene glycol and lactaldehyde more efficiently than the D form *(52*). L-lactic acidosis has been observed in both humans and animals following exposure to propylene glycol *(39, 40*).

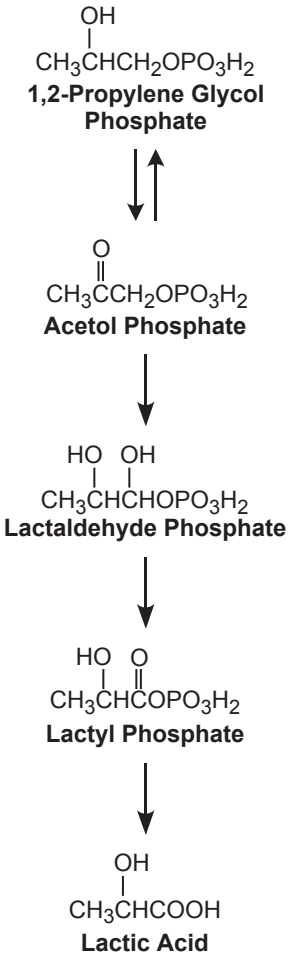
The conversion of lactaldehyde to methylglyoxal by ADH and then to D-lactate by glyoxalase and reduced glutathione is thought to be an alternate route of metabolism (Figure 2-1). D-lactate is cleared more slowly than L-lactate and is considered a poor substrate for gluconeogenesis.

Methylglyoxal synthetase can convert the substrate, dihydroxyacetone phosphate, to methylglyoxal. However, in conditions where ketone levels are high, such as diabetes or starvation, methylglyoxal

synthetase activity is increased, producing more methylglyoxal and D-lactate. Excessive production of D-lactate may result in its accumulation, especially in the brain, which has a low level of catabolizing enzymes *(39)*. Therefore, in cases of ketosis, excess levels of D-lactate may be exacerbated by propylene glycol.

In a third possible metabolic pathway, propylene glycol can be phosphorylated, converted to acetol phosphate, lactaldehyde phosphate, lactyl phosphate, and lactic acid (see Figure 2-2) *(49)*. Metabolism of D and L forms of propylene glycol in this pathway is species-specific. The rabbit converts the L-form of phosphorylated propylene glycol to lactic acid, whereas the rat and mouse can convert both forms *(52, 53)*.

Figure 2-2. Phosphorylated Propylene Glycol Metabolism in Mammals



From Ruddick (*49*).

A limited number of studies were summarized in detail since they demonstrate evidence of *in vitro* stereospecificity of ADH *(52)*, L-lactatemia in rabbits *(40)*, and increased D-lactate formation in cats *(39)*.

Stereospecificity of ADH was studied by Huff *(52)*. *In vitro* rabbit liver ADH K_s values were obtained

for ethanol, L-propylene glycol, and D-propylene glycol substrates and were 0.63, 3.6, and 33.3 μmoles/mL, respectively. K_s values obtained for acetaldehyde, L-lactaldehyde, and D-lactaldehyde were 3.6, 1.4, and 3.7 μmole/mL, respectively. A similar trend in values was observed with horse liver ADH. Therefore, ADH from horse and rabbit liver exhibited stereospecific preference for L-propylene glycol and L-lactaldehyde.

Strength/Weaknesses: Stereospecificity of metabolism should be considered because technical grade propylene glycol contains the stereoisomers in a 1:1 ratio. Huff *(52)* determined the K_m values for oxidation of the D- and L-forms by alcohol dehydrogenase and found that L-propylene glycol is 5−9 times more readily metabolized to L-lactaldehyde by rabbit and horse alcohol dehydrogenase than is the D-form. Therefore, it is plausible that D-propylene glycol will be cleared more slowly since this is the rate-determining step in the metabolic clearance of these compounds. Moreover, accumulation of D-lactate has been documented in cats *(39)* and humans *(54)*, which was partially attributed to D-lactate being a poor substrate for gluconeogenesis, a detoxification pathway for L-lactate. In addition, D, L-lactaldehydes are oxidized to methyl glyoxal with loss of the chirality center, which glyoxylase with GSH as co-substrate converts stereospecifically to D-lactate.

Another pathway occurs by phosphorylation of propylene glycol followed by oxidation steps without loss of the chirality center. Here, species differences were found; rabbits converted the L-form more readily to lactic acid, but rats and mice did it equally well with both forms *(52, 53)*. Due to incomplete time-point sampling and a lack of quantitative numbers regarding fluxes through the different pathways, it is not possible to put together a complete picture of stereospecific metabolism of D, L-propylene glycol.

It is of no toxicological consequence whether L- or D-lactatemia develops because both can contribute to the development of lactic acidosis. The longer half-life of D-lactate can be easily factored in via the Michaelis-Menten equation into a physiologically based pharmacokinetic (PBPK) model. The weakness of this approach is that D-lactate was shown to be efficiently utilized in man *(54)*, but its tubular reabsorption was shown to be retarded, particularly at higher concentrations (>3 meq/L). Since chirality is lost during oxidation of D, L-lactate, the preferential use of L-lactate must be due to a lower K_m of lactate dehydrogenase for L- than for D-lactate. In any event, reduced tubular reabsorption enhances overall clearance of D-lactate, whereas reduced utilization for gluconeogenesis runs counter to this effect, apparently outweighing both its reduced tubular reabsorption and its utilization in the Krebs cycle that produces CO_2.

The overall conclusion from all data is that acute exposure to D, L-propylene glycol can cause L-lactic acidosis (if the dose is very high) due to the more rapid biotransformation (alcohol dehydrogenase being the rate-determining step) of L-propylene glycol to L-lactate, whereas subchronic/chronic exposure leads to D-lactic acidosis due to accumulation of D-lactate derived from the glyoxylase/GSH pathway and from being a poor substrate for gluconeogenesis.

Utility (Adequacy) for CERHR Evaluation Process: The database is sufficient to understand and predict metabolic clearance of D, L-propylene glycol in man.

The role of propylene glycol metabolism in lactatemia in the rabbit was investigated by Morshed et al.

(40). Propylene glycol was administered to NZW rabbits by gavage in a single dose of 38.66 mmol/kg **[2.942 g/kg]** (1 mL 28.4% (v/v)) aqueous solution per 100 g bw. Whole blood was withdrawn from the marginal ear vein after a 24 hour fast and at 0.25, 1, and 3 hours after administration of propylene glycol. Blood pH and the levels of propylene glycol and D- and L-lactate and pyruvate were determined. The level of propylene glycol was estimated colorimetrically and the levels of lactate and pyruvate were estimated enzymatically. Data were evaluated by analysis of variance for repeated measures and were expressed as mean ± SD; a value of $P < 0.05$ was statistically significant. As noted in Table 2-1, blood propylene glycol concentrations were at a maximum 1 hour post-dosing.

Table 2-1. Levels of Propylene Glycol and its Metabolites in New Zealand White Rabbits after Oral Propylene Glycol (From Morshed et al. (40))

Parameter	*Fast*	*0.25 h*	*1 h*	*3 h*
Propylene Glycol	0 (0)	30.23 ± 12.45*** (0)	41.04 ± 9.98*** (0)	36.55 ± 8.0*** (0)
L-Lactate	1.04 ± 0.22 (1.08 ± 0.25)	2.55 ± 0.62** (1.12 ± 0.19)	2.03 ± 0.48** (1.0 ± 0.25)	1.77 ± 0.36** (1.07 ± 0.18)
D-Lactate	0.005 ± 0.005 (0.004 ± 0.003)	0.025 ± 0.004*** (0.005 ± 0.005)	0.10 ± 0.02*** (0.006 ± 0.004)	0.15 ± 0.03*** (0.10 ± 0.01)
Pyruvate	0.54 ± 0.10 (0.51 ± 0.08)	0.60 ± 0.14 (0.57 ± 0.10)	0.63 ± 0.13 (0.55 ± 0.12)	0.58 ± 0.10 (0.50 ± 0.14)
Lactate / pyruvate	1.92 ± 0.07 (2.12 ± 0.10)	4.27 ± 0.18*** (1.96 ± 0.09)	3.22 ± 0.05*** (1.82 ± 0.12)	3.05 ± 0.10*** (2.14 ± 0.08)

Note: Values are means ± SD obtained from four propylene glycol treated rabbits and are expressed as mmol/liter except the lactate/pyruvate, which is a ratio. This ratio was calculated using the data in this table and considering L-lactate as the total body lactate. Data in the parentheses indicate the values obtained from saline-administered control rabbits (n=4); ** $p < 0.01$; *** $p < 0.001$.

Treatment with propylene glycol significantly ($P < 0.01$) increased the concentration of L-lactate, which reached a plateau at 0.25 hours following exposure. D-lactate levels were significantly increased and reached maximum concentration at 3 hours after administration of oral propylene glycol. Although significant, the authors considered the increase in D-lactate to be negligible and noted that L-lactate levels were similar to total lactate levels. Levels of pyruvate remained unaffected before and after administration of propylene glycol. Blood pH was not significantly altered when compared to control values. The authors note that these findings are different than the results from oral administration of propylene glycol to the rat *(55)*.

Strength/Weaknesses: The Morshed et al. *(40)* paper provides some useful information about the early phase of metabolism of propylene glycol in rabbits; its usefulness for propylene glycol kinetics is limited because of poor sampling intervals. Blood levels of propylene glycol dropped from a maximum of 41.0 mM at 1 hour after dosing to 36.6 mM at 3 hours after dosing. A very rough estimate under the assumption of the first order one compartment model would indicate a half-life of about 12 hours in the rabbit. It must be emphasized that neither assumption may be correct, because the high dose and the very slow flux of L-lactate indicates that the system operated according to a zero order process. **[In any event, neither Morshed et al. *(40, 55)* paper is properly interpreted.]**

The study in rats *(55*) did not determine blood levels of propylene glycol although it used many doses and a sufficient number of time points. Lactate levels are plotted on an arithmetic scale, which allows half-life estimates by a visual inspection but no exact calculation. The statement "The elimination time ranged from 1.40 to 5.82 hour which followed apparent first order kinetics" is contradictory. The half-life of first order processes is a constant and independent of dose. Except for the two lower doses (0.4 and 0.8 mg/kg), which were below saturation of metabolic clearance, the higher doses (1.6, 3.2, and 6.0 ml/kg) were above saturation of metabolic clearance and therefore the metabolite (lactate) reflected the kinetics of the parent compound (saturation of alcohol dehydrogenase being the rate-determining step) with dose-dependent increase in its half-life.

The time course evaluated for propylene glycol-induced lactatemia in rabbits was too short to allow for any conclusions regarding D- or L-lactate half-life in the study of Morshed et al. *(40*). That study also contains contradictory data in that blood L-lactate concentrations peaked at the earliest time point (0.25 hours) and declined thereafter (see Table 2-1 above). However, the propylene glycol concentration peaked at 1 hour and fell only slightly by 3 hours. This irregular decline of primary metabolite in the face of increasing parent compound concentrations is not readily interpretable. One might conclude from this paper that L-lactate is orders of magnitude more important as a metabolite of propylene glycol than is D-lactate. However, it should be made clear that this may only be true for the rabbit, as Morshed et al. point out that rat ADH is more efficient in metabolizing D-propylene glycol than is rabbit ADH, which leads to slightly greater overall lactate levels from propylene glycol metabolism in rats than in rabbits. The lack of information of D- vs. L-lactate formation in humans makes it unclear whether humans are more like the rat or rabbit.

Utility (Adequacy) for CERHR Evaluation Process: The usefulness of the Morshed et al. *(40, 55*) data is limited for reproductive and developmental considerations. It is clear from these papers that high doses of propylene glycol will result in sustained hyperlactemia, probably without lactic acidosis, because of the efficient removal of lactate via gluconeogenesis.

In a study examining clinical chemistry abnormalities, 5 or 6 cats of each sex were fed a diet containing 12% propylene glycol (low dose, 1.60 g/kg bw/day) for 5 weeks (a dose equivalent to that found in commercial soft-moist cat foods), or a high dose diet containing 41% propylene glycol (8.00 g/kg bw/day) for 22 days *(39*). Propylene glycol (99.7% purity) was a racemic mixture of D- and L-isomers. Predosing observations were made such that each group of cats served as its own control. Clinical chemistry analyses were conducted on serum samples. L- (+) lactate was determined enzymatically using L-lactate dehydrogenase and D- (-) lactate was determined on days 0, 10, and 24 of the low-dose diet and days 0, 6, 10, and 24 of the high-dose diet. Data were analyzed by ANOVA and significance was at the $p<0.05$ level. Plasma levels of propylene glycol were measured in two of the low-dose cats. Propylene glycol levels on day 24 of dosing were 19.1 and 8.4 mM and propylene glycol was not detected in the control plasma. The authors reported a linear correlation between increases in anion gap **[anion gap = $(Na^+) - Cl^- + \text{total } CO_2$)]** and D-lactate in cats fed the low dose. Serum levels of D-lactate increased with days of propylene glycol ingestion and levels of L-lactate decreased in low-dose cats (Table 2-2). The authors noted previous observations where propylene glycol was found to produce L-lactic acidosis in humans and animals including cats shortly after exposure. Because their study first measured lactic acid exposure at 1 week following exposure, it is unknown if acute increases in L-lactate concentration occurred in the cats.

Table 2-2. Serum Lactate Levels in Cats Ingesting 1.6 g or 8.0 g Propylene Glycol/kg bw/day**

	0 days ingestion	***10 days ingestion***	***24 days ingestion***
D-lactate (1.6 g/kg)	0.08 ± 0.03 mmol/L	1.90 ± 0.80 mmol/L	1.96 ± 0.75 mmol/L
L-lactate (1.6 g/kg)	1.02 ± 0.18 mmol/L		0.60 (approx)*
D-lactate (8.0 g/kg)		4.21 ± 1.95 mmol/L	7.12 ± 0.14 mmol/L

* Value taken from graph; 0.32 ± 0.10 mmol/L lactate at 35 days ingestion.
** Christopher *(39)*.

Strength/Weaknesses: The Christopher et al. *(39)* paper is important because it links the anion gap with D-lactate levels in plasma in cats after repeated doses of propylene glycol. Plasma levels of propylene glycol were determined in two low dose (1.6 g/kg bw/day) cats, which in itself is not suitable for any kind of kinetic modeling. Nevertheless these data (19.1 and 8.4 mmol/L) are in agreement with the Morshed et al. *(41)* results, which showed that administration of a single dose (1.6 g/kg) of propylene glycol resulted in peak plasma concentration in the same concentration range (about 8 mmol/L). Thus, it appears that the half-life of propylene glycol is short in cats as well since there seems to be no accumulation of it after repeated administration.

Utility (Adequacy) for CERHR Evaluation Process: Christopher et al. *(39)* is a useful study linking human data *(54)* with animal data regarding D-lactatemia.

2.1.3.2 Overall Summary of Metabolism

It appears that high, acute doses of propylene glycol can lead to lactic acidosis. Unless the dose is very high, L-lactate is efficiently converted (detoxified) to glucose. However, D-lactate is not readily converted in the gluconeogenic pathway and therefore tends to accumulate after subacute/chronic dosing leading to D-lactic acidosis. Logically, lactate dehydrogenase must have a much higher affinity for L-lactate than for D-lactate because chirality is lost at the level of pyruvate and D- and L-lactate derived intermediates become indistinguishable upstream of pyruvate.

It may be more likely that at high propylene glycol doses and plasma lactate loads, lactate clearance via utilization in intermediary metabolism is saturated. Limited evidence for this is suggested in the D, L-lactate dosing study of Oh et al. *(54)*. Ten male volunteers received one of two different infusion rates (n = 5 per group) of D, L-lactate in which a doubling in the D-lactate blood level yielded only a 1.5-fold increase in D-lactate utilization rate but a 3.5-fold increase in D-lactate urinary excretion. The levels of D-lactate in this study were in the same range as those reported for total lactate at the high doses in rats *(55)*. The rate of L-lactate excretion and utilization were not reported in the human study *(54)*.

2.1.3.3 Developmental and Species Specific Variations in Metabolism and Enzyme Activities

Activities of enzymes such as ADH and ALDH can affect how fast propylene glycol is cleared from the body, thus affecting potential toxicity. A number of studies examined both the activities of these enzymes in human placenta and the age-related activity of the enzymes. Although most studies focused on ethanol metabolism, they are still relevant to propylene glycol metabolism, since ADH and ALDH activities are investigated. Therefore, CERHR conducted a brief review of the data.

Placental Metabolic Capacity
Studies in humans and rodents suggest that the placenta has extremely limited capacity to metabolize propylene glycol. Pares et al. *(56)* 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 *(57)* 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 activity *(58)*.

Developmental Aspects of Metabolic Capacity
Activity of ADH and ALDH was found to vary with developmental stage.

Sjoblom et al. *(58)* found that in Wistar rats ADH activity in 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: 53% of adult levels on postnatal (pnd) 1 with a continued gradual increase with age to 82% of adult activity on pnd 47. Similar developmental patterns were noted for ALDH in rat liver.

Pikkarainen and Raiha *(32)* 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, activity reached a level within ranges noted for adults. Great variation was noted in adult ADH activity.

Somewhat different results were reported subsequently by Smith et al. *(59)* who examined human 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 9−22-week-old fetal liver that was 30% of adult values, and in premature infants and children less than 1 year old, 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 greater 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 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.

Thus, it would appear that human liver ADH is expressed early in development and may well contribute to propylene glycol metabolic disposition. However, given the paucity of knowledge regarding isoform specificity towards propylene glycol, it is uncertain how these data on ethanol metabolism might be

extrapolated. Assuming the enzyme most active in ethanol metabolism, ADH1B, is also most active in propylene glycol metabolism, the significant fetal metabolism is not predicted to occur until later in gestational development (20−36 weeks).

Strength/Weaknesses: There are consistent data in both animals and humans showing that alcohol dehydrogenase is much lower prenatally. In humans, adult levels were reached by the age of 5 years and in rats on day 47 after parturition.

Utility (Adequacy) for CERHR Evaluation Process: D, L-lactate, metabolites of D, L-propylene glycols, are postulated to be associated with toxicity in mammalian species. Therefore a lack of *in situ* conversion in the fetus would seem to decrease the toxicity of propylene glycol. Since lactate distributes into total body water, the fetus will share the mother's metabolic load and associated acidosis, if present. The lower metabolism capability in newborns and infants, however, may partially protect them from metabolic acidosis after ingestion of propylene glycol.

Hepatic Metabolic Capacity in Humans Versus Rats

Zorzano and Herrera *(60, 61)* 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).

Inter-individual Variability Due to Generic Polymorphisms

Reviews by Agarwal *(62)*, Bosron and Li *(63)*, Pietruszko *(64)*, and Burnell et al. *(65)* 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 three loci (ADH1A, ADH1B, and ADH1C). Polymorphisms resulting in altered phenotypes are observed at the ADH1B and ADH1C loci.

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 an E_1, ALDHII, or $ALDH_1$) *(62-64)*. About 50% of Japanese and Chinese carry a phenotypically null variant of the ALDH2 enzyme.

2.1.4 Elimination

In mammals, part of the propylene glycol dose is eliminated unchanged by the kidney and part is metabolized by the liver to lactic acid and further metabolized to pyruvic acid; in mammals, with the exception of cats, the remainder is conjugated with glucuronic acid *(2)* and eliminated in the urine. The amount of propylene glycol eliminated by the kidneys has been estimated for humans at 45% *(48)*, for dogs at 55–88% *(49)*, and for rabbits at 2.4–14.2% *(50)*. Morshed et al. *(41)* provided evidence in the rat that increasing doses of propylene glycol increased elimination by the kidneys. Dosages of 19, 38, and 77 mmole/kg bw resulted in 2.3, 7, and 17% renal excretion of propylene glycol. Maximum urinary excretion of propylene glycol was determined using pyrazole (1.0 mmole/

kg bw), a competitive inhibitor of propylene glycol. High urinary clearance was observed with 75% excretion of the ingested dose within 24 hours.

2.1.4.1 Humans

In human adults receiving 20.7 or 41.4 g propylene glycol 2–3 times daily for a minimum of 3 days, the total body clearance was dependent on serum concentration and was approximately 0.1 L/kg bw/hour; elimination half-life in those same subjects was about 4 hours *(29)*. **[The influence of ethyl alcohol administration must be considered when interpreting results since it will compete with propylene glycol for the dehydrogenase enzymes.]** In a study where adults and children were rectally exposed once to ~123–173 mg/kg bw propylene glycol **[blood levels 1.6–2.2 mM],** the clearance rate was 0.2 L/hour/kg and half-life was 2.6–2.8 hours *(31)*. In 6 adults receiving propylene glycol intravenously, blood levels of propylene glycol were measured at 48–425 µg/mL **[0.63–5.6 mM]** and an average half-life of 2.3 hours was estimated *(30)*.

A small number of studies suggest that elimination of propylene glycol in infants is slower than in adults. In an 8-month-old infant exposed to propylene glycol through medication applied to burns, the propylene glycol blood level was 1.059 g/dL **[139 mM]** and the elimination half-life was measured at 16.9 hours *(35)*. Ten infants exposed to 10 mL **[10.36 g]** propylene glycol in a parenteral vitamin solution daily for 5 days had propylene glycol blood levels of ~65−950 mg/dL **[8.5–125mM]** and elimination half-lives of 10.8–30.5 hours, with a mean of 19.3 hours *(36)*.

Excretion of propylene glycol has been studied in patients with second and third degree burns over more than 20% of their total body surface *(34)*. According to ATSDR *(4)*, "Sulfadiazine preparations containing propylene glycol were applied dermally over a period of 3–7 days after admission to the hospital. Serum and urinary levels of propylene glycol were measured. Propylene glycol was detected in the serum of 24 of 45 patients and in the urine of 40 of 45 patients. Average urinary levels were 1.3 mg/mL with a range of 0–17.9 mg/mL for patients who lived, and 2.9 mg/mL with a range of 0–23 mg/mL for patients who died. Propylene glycol levels correlated with total burn surface area and total third degree burn surface area."

Strength/Weaknesses: Elimination kinetics of propylene glycol are well understood. Speth et al. *(30)* provides the major kinetic parameters needed for calculations. The saturation of metabolic clearance occurs in humans at about 7 g, which is somewhat lower than in animals. Kollöffel et al. *(31)* provide data in 10 adults which indicate that at a dose of 8.64 g, elimination of propylene glycol was zero order because it was nearly linear on an arithmetic scale. At a dose of 5.1 g/day the half-life of propylene glycol was 1.6 ± 0.20 hours, at doses of 7.2 to 7.7 g/day it was 1.9 ± 0.15 hours, and at doses of 12.6 to 21.0 g/day it was 3.2 ± 0.12 hours *(30)*. The data of Kollöffel et al. *(31)* provide 2.6 ± 0.2 hours as half-life in adults at a dose of 8.64 g/day. At a dose of 3 x 20.7 to 2 x 41.4 g/day, Yu et al. *(29)* estimated an elimination half-life of about 4 hours. Thus, the half-life of propylene glycol increased from 1.6 to 4 hours as the dose increased from 5.1 to 2 x 41.4 g/day. The half-life of chemicals eliminated by first order processes is independent of dose. Therefore, it is certain that in humans propylene glycol is eliminated by zero order kinetics at or above a dose of 5.1 g/day. Clearance data and AUCs verify this conclusion.

The infant studies suggest prolonged half-lives of propylene glycol *(35, 36)* in the range of 10.8−30.5

hours in infants receiving a dose of about 3 g propylene glycol. While such data are consistent with very low alcohol dehydrogenase activity perinatally *(32)*, they cannot be considered definitive due to confounding effect(s) associated with the disease treatment and the drugs associated with such therapy. In addition, the Kulick et al. *(34)* paper is not suitable for determination of elimination kinetics because only one time point was measured. Prolonged half-life of propylene glycol in infants is also supported by a recent report showing that it accumulated to very high levels (up to 2,000 μg/mL) in serum of these children *(66)*.

The infant studies suggest prolonged half-lives of propylene glycol *(35, 36)* in the range of 10.8−30.5 hours in infants receiving a dose of about 3 g propylene glycol. While such data are consistent with very low alcohol dehydrogenase activity perinatally *(32)*, they cannot be considered definitive due to confounding effect(s) associated with the disease treatment and the drugs associated with such therapy. In addition, the Kulick et al. *(34)* paper is not suitable for determination of elimination kinetics because only one time point was measured. Prolonged half-life of propylene glycol in infants is also supported by a recent report showing that it accumulated to very high levels (up to 2,000 μg/mL) in serum of these children *(66)*.

Utility (Adequacy) for CERHR Evaluation Process: There are sufficient data available on the elimination kinetics of propylene glycol in humans to model elimination in adults; data in infants and in the fetus are less certain.

2.1.4.2 Animals

"Dose-dependent elimination of propylene glycol is seen in rats, with saturation of the pathways at doses above 5.88 g/kg. An apparent maximum elimination rate of 8.3 mmol/kg/hour (0.63 g/kg/hour) was observed" *(4)*.

Yu and Sawchuk *(50)* studied the metabolism and elimination of propylene glycol after acute or chronic IV administration to NZW male rabbits. Rabbits were exposed acutely by IV injection to either 0.5, 1.0, or 2.0 g/kg bw (three rabbits per dose group). There was evidence of a saturation of propylene glycol metabolism at the 2.0 g/kg bw acute dose, as evidenced by the decreased metabolic clearance. The half-life and the terminal elimination phase rate constant was not significantly affected over this dose range. An additional few rabbits were exposed by continuous IV infusion to propylene glycol delivered at various rates (2.8−6.3 mg/min/kg bw) over the course of 51−52 hours. Both V_{max} and K_m were lower in the case of prolonged exposure, but the V_{max}/K_m ratio was approximately 3-fold greater than under acute dosing. Plots of metabolic clearance from single rabbits dosed acutely versus continuously indicate higher metabolic clearance rates from continuous exposure**. [This raises the possibility of the induction of a second, low K_m form of ADH during the 51–52 hours of infusion.]** The authors concluded that metabolism of propylene glycol was the dominant disposition pathway with a concentration-dependent metabolic clearance; renal excretion of propylene glycol was only 2.4–14.2% of the total dose after acute administration, most likely due to kidney reabsorption. Authors also concluded that for both acute and chronic administration of propylene glycol, the clearance of propylene glycol is lower at higher plasma concentrations and the rate of elimination of propylene glycol was dependent upon urine flow.

Ruddick *(49)* cited an earlier study by Lehman and Newman *(42)* where dogs were force fed 8 mL/kg

and 12 mL/kg of a 50% aqueous solution of propylene glycol. Blood concentrations were 1.3 g/dL **[171 mM]** 2 hours after dosing and 0.9 g/dL **[118 mM]** 4 hours after dosing. Recovery of 12–45% of the unchanged administered dose in the urine led the authors to conclude that the compound was eliminated by the kidney and a large portion of unexcreted chemical was metabolized.

Strength/Weaknesses: Animal data are consistent with human data regarding the elimination kinetics (practically the same elimination half-life before saturation of metabolic clearance) of propylene glycol, although minor species differences may be present. Saturation of metabolic clearance occurs at somewhat higher doses in animals; therefore, the half-life of elimination becomes dose-dependent (zero order) at higher doses.

Utility (Adequacy) for CERHR Evaluation Process: It is useful to have mechanistic insight into the process of elimination of propylene glycol as represented by the Yu and Sawchuk *(50)* paper on the urinary flow dependence of elimination as well as on the dose-dependence of metabolic clearance.

The Ruddick *(49)* and Lehman and Newman *(42)* papers are not suitable for quantitative kinetic evaluation.

2.2 General Toxicity

The majority of information in this section is summarized from the reviews by ATSDR *(4)* and LaKind et al. *(28)* and from the SIDS Initial Assessment Report for 11th SIAM *(27)* and the EPA Health and Environmental Effects Document on Propylene Glycol *(67)*. No toxicity studies have been located on propylene glycol subsequent to the 2001 SIDS Initial Assessment Report. A very limited number of toxicity studies included an examination of the reproductive organs and those studies are discussed in detail.

Propylene glycol has very low systemic toxicity in experimental animals and very high doses are used in most acute studies to determine a toxic level. It is primarily metabolized to lactic acid and pyruvic acid, both of which are normal constituents of the citric acid cycle. CNS, hematologic, hyperosmotic, and cardiovascular effects have been noted in humans and animals and high serum concentrations of propylene glycol may result in lactic acidosis and hyperosmotic changes in the blood *(4, 27, 49)*. Symptoms of acute propylene glycol intoxication in animals include CNS depression and narcosis. Individuals with compromised hepatic or renal function would be less apt to clear propylene glycol, and hence would be more susceptible to toxicity due to high blood levels *(2, 4, 68)*. No system or organ has been established as a target for the acute oral lethal effects of propylene glycol *(69)*.

Lactate can be detoxified into glucose and stored as glycogen as has been demonstrated by Wittman et al. *(47)* in propylene glycol-exposed rats. Doses of 0.5−2.0 g/kg of propylene glycol were administered to female rats and liver glycogen content and blood glucose were determined 90 minutes after dosing. Liver glycogen content nearly doubled and fasting blood glucose increased from 88 to about 140 mg%. Lactic acidosis was not reported. **[Lactic acidosis is not expected at these relatively low doses of propylene glycol. However, lactic acidosis can develop if these two detoxification pathways cannot remove excess lactic acid sufficiently.]**

ATSDR *(4)* stated that "The mechanism of action of propylene glycol is not well understood". **[In**

fact, much is known about the mechanism of action.] Lactatemia has been well documented in animals and there are supporting human data. Cats administered 12 (1.6 g/kg bw/day) or 41% (8.0 g/kg bw/day) propylene glycol in the diet (dry weight) for 22 days, showed a time-dependent increase in plasma lactate and in anion gap *(39*). Morshed et al. *(40, 43*) produced more data on the dose-dependence of blood lactate and/or pyruvate in rats and rabbits given propylene glycol orally. Finally, a human case report *(48*) demonstrated that repeated infusions of lorazepam dissolved in propylene glycol can lead to lactic acidosis with increased osmolar gap (21 mOsm/L). Furthermore, increased blood glucose (296 mg/dL) and elevated pyruvate level (1.01 mg/dL) indicate that the same metabolic pathways of detoxification occur in humans as in animals. Glasgow et al. *(36*) reported a good correlation between osmolality gap and serum propylene glycol concentrations in ten infants. The half-life was reported as 19.3 hours (range 10.8−30.5 hours), which is about 10 times longer than in adults. Alcohol dehydrogenase activity is up to 10 times lower in infants *(32*) than in adults providing an explanation for the prolonged half-life in the latter and at the same time further evidence that this enzyme is the rate-determining enzyme in the clearance of propylene glycol. Other endpoints of toxicity are anesthesia, probably by the same mechanism as other alcohols, and hemolysis, which may be due to the osmolality gap.

Strength/Weaknesses: There is an adequate database to assess the toxicity of propylene glycol *(4, 27, 28, 67*). Very high doses of propylene glycol cause CNS, hematologic/hyperosmotic, and perhaps cardiovascular effects, as well as lactic acidosis. Animals lethally intoxicated undergo CNS depression, narcosis, and eventual respiratory arrest.

Utility (Adequacy) for CERHR Evaluation Process: There are sufficient reliable reviews to obtain any information needed for informed toxicological judgment.

2.2.1 Humans

2.2.1.1 Oral Exposure

A lethal oral dose of propylene glycol has not been reported for humans *(28*), but it is estimated that the human lethal oral dose is >15 g/kg or >32 fl oz for a 150 lb person *(2*). In adults, serum levels of >180 mg/L **[2.37 mM]** have resulted in toxicity *(48*). In one case, an 11-year-old child receiving oral doses of 2−4 mL per day for 13 months as a component of a vitamin D preparation (estimated dose 4−8 g/kg bw/day) resulted in seizures and CNS depression *(28*). In acutely ill infants, death has occurred after repeated exposure to propylene glycol in medication; CNS depression and seizures have been reported after multiple oral doses *(36, 70*) **[see Section 2.5 Potentially Sensitive Subpopulations]**. According to HSDB *(2*), the acceptable daily intake of propylene glycol as a food additive is 25 mg/kg body weight.

2.2.1.2 Dermal Exposure

Contact dermatitis has been reported from propylene glycol exposure in a wide variety of topical preparations *(28*) and ingestion of propylene glycol in sensitized individuals has produced flares of dermatitis *(28*). Skin irritation resulting from topical exposure is manifest as erythematous reactions restricted to sites of exposure. The irritation potential is enhanced after prolonged dermal exposure, under dermal occlusion, and in combination with triethanolamine-stearate, a cosmetic emulsifier *(71, 72*). The nature of the skin reaction of propylene glycol-sensitive patients has been a matter of controversy *(73, 74*). In a study by Hannuksela and Forstrom *(73*), primary irritant reactions to the

skin and type IV delayed hypersensitivity reactions were observed following oral ingestion or topical application of propylene glycol. However, in most cases, the skin reaction was due to a primary irritation, not to an allergic reaction *(72)*.

2.2.1.3 Inhalation Exposure

Propylene glycol is a component of theatrical fog and is used for special effects. The Actors' Equity Association and the League of American Theaters and Producers sponsored a study which included an examination of the health effects of theatrical fog in response to actors' concerns about exposure *(75)*. The health endpoints selected for investigation were irritant effects to the respiratory tract and eyes. This study was conducted over 2 years with 439 actors from 16 musicals, and consisted of a baseline questionnaire, daily checklists, and medical evaluation. There was no clinically significant adverse impact on pulmonary function or in rates of asthma associated with exposure to propylene glycol. However, "peak exposures to elevated localized air concentrations following release of glycol smoke are associated with increased reporting of respiratory, throat, and nasal symptoms, and findings of vocal cord inflammation." The study authors recommended that exposures to propylene glycol by actors not exceed peak or ceiling concentrations of 40 mg/m^3.

NIOSH conducted a study in 1990 on the use of theatrical fog in Broadway theaters *(76)*. Personal breathing zone and general area air sampling and a questionnaire on irritant effects (130 questionnaires from productions with theatrical smoke, 90 questionnaires from productions without theatrical smoke) were collected from personnel from four productions using theatrical smoke and five productions without theatrical smoke. Air samples collected yielded propylene glycol concentrations <2.1 mg/m^3. However, there was a significant (.05) increase in the reporting of respiratory irritant symptoms such as runny nose, stuffy nose, and sneezing by personnel from productions using theatrical smoke.

In a study by Cohen and Crandall *(77)* [reviewed by LaKind et al. *(28)*], propylene glycol was recommended as a vehicle for administration of bronchodilator drugs. No adverse clinical effects were observed after subjects were exposed to an inhalant mist of isoproterenol-HCl containing 40% propylene glycol for 15 minutes at a temperature of 115–124° F.

Wieslander, Norbäck, and Lindgren *(23)* examined experimental exposure of volunteers to propylene glycol mist simulating concentrations routinely used in aviation emergency training. Twenty-seven non-asthmatic volunteers (22 males, 5 females) were exposed in an aircraft simulator to propylene glycol mist over a 1-minute period (average concentration 360 mg/m^3; range 176–851 mg/m^3). Average age was 44 ± 11 years. None of the subjects had previous occupational exposure to propylene glycol. Medical examinations were performed both within 15 minutes before and after the exposure. Exams included an estimate of tear film stability breakup time, nasal patency by acoustic rhinometry, lung function by dynamic spirometry, and a self-rated symptom questionnaire. After 1 minute of exposure there was a statistically significant difference when compared to pre-exposure levels in tear film stability (decreased; $p = 0.02$) and ocular and throat irritation ratings (both increased; $P < 0.001$) **[P values determined by Student's t test for paired comparisons]**. The forced expiratory volume in 1 second over the forced vital capacity was slightly reduced and the self-rating of severity of dyspnea increased. There were no apparent changes in nasal patency, vital capacity, forced vital capacity, nasal symptoms, dermal symptoms, smell of solvents, or any other systemic symptoms. The authors concluded that short exposure to propylene glycol mist from artificial smoke generators may

cause acute ocular and upper airway irritation.

2.2.1.4 Parenteral Exposure

Hemolysis, CNS depression, hyperosmolality, and lactic acidosis have been reported after IV administration of propylene glycol *(68)*. Rapid IV infusion of concentrated propylene glycol-containing drugs has been associated with respiratory depression, arrhythmias, hypotension, and seizures. Propylene glycol is used as a vehicle for IV administration of drugs such as lorazepam, etomidate, phenytoin, diazepam, digoxin, hydralazine, esmolol, chlordiazepoxide, multivitamins, nitroglycerin, pentobarbital sodium, phenobarbital sodium, and trimethoprim-sulfamethoxazole. Therefore, patients, especially children and infants, receiving IV drugs can be at risk for propylene glycol toxicity *(28)* **[see Section 2.5 Potentially Sensitive Subpopulations]**.

Information on the dose of propylene glycol necessary to induce toxicity is limited. Some reports describing the dose of propylene glycol given and the serum concentration measured in cases of toxicity in humans are contained in Table 2-8 in Section 2.5, Potentially Sensitive Subpopulations.

2.2.2 Experimental Animal Data

General toxicity studies in animals are discussed in the sections below and summarized in Table 2-4 on page II-35.

2.2.2.1 Oral Exposure

LD_{50} oral toxicity values are listed in Table 2-3.

Table 2-3. Propylene Glycol Oral Toxicity Values

Species	*LD50 (g/kg)*	*Reference*
Rat	8–46	ATSDR (4)
Mouse	25–32	ATSDR (4)
Rabbit	18–20	ATSDR (4)
Dog	19	HSDB (2)
Guinea Pig	18–20	ATSDR (4)
Human	>15 (estimated)	HSDB (2)

A wide range of LD_{50} values has been reported for the rat. In a study by Morshed et al. *(43)*, 6 male Wistar rats were dosed by gavage with saline or 2.942 g/kg bw/day propylene glycol in water for 10, 20, or 30 days. No deaths occurred over any of the time intervals. However, a 41% reduction in body weight was noted at 10 days and an increase in body weight was noted at 20 and 30 days in treated animals as compared to respective saline controls.

Strength/Weaknesses: This study by Morshed et al. *(43)* does not have strengths, only weaknesses. Controls gained 16.9 g during the first 10 days (1.69 g/day on average), 23.3 g after 20 days (1.17 g/day on average), and 40.15 g after 30 days (1.34 g/day on average). Well-maintained rats do not display such weight gain variability.

Utility (Adequacy) for CERHR Evaluation Process: None.

In a study by Weatherby and Haag *(78)* [reviewed by OECD *(27)*] in rats, only minimal kidney changes were observed and the LD_{50} value was determined to be 33.5 g/kg.

Strength/Weaknesses: This is an older study *(78)* which characterized acute toxicity of propylene glycol in rats and rabbits by various routes of administration. As expected, propylene glycol was most toxic when administered IV. Toxicity decreased IV > IM > subcutaneous > oral. There was no apparent species difference. Information provided on the chronic administration of propylene glycol is sparse but the hemolysis experiment with human blood *in vitro* demonstrates conclusively the hemolytic potential above 0.111 M.

Utility (Adequacy) for CERHR Evaluation Process: This study by Weatherby and Haag *(78)* is useful for the characterization of acute toxicity, but is less useful for chronic toxicity.

Acute oral toxicity in rabbits was studied by administering a 20% aqueous solution of propylene glycol by stomach tube over a 1-hour period (15.75−21.00 g/kg) *(79)* [reviewed in LaKind et al. *(28)*; OECD *(27)*]. Animals exhibited an increased respiratory rate, loss of equilibrium, depression, analgesia, coma, and died by 36 hours post dosing. The minimum fatal dose was determined to be 18.9 g/kg (3 of 9 deaths), with 100% mortality at a dose of 21 g/kg (4 of 4 deaths).

Strength/Weaknesses: The Braun and Cartland *(79)* paper predates the Weatherby and Haag *(78)* publication and represents a less extensive but nevertheless reliable documentation of the acute toxicity of propylene glycol administered IM and subcutaneously to rats and orally to rabbits. Results of the two studies are very similar. Data on chronic toxicity are scant.

Utility (Adequacy) for CERHR Evaluation Process: This report is useful for the characterization of acute, but not chronic, toxicity.

Chronic toxicity studies reflect that propylene glycol has a very low order of toxicity. In the following toxicity studies by Morris et al. *(80)* and Gaunt et al. *(81)*, reproductive tissues were examined.

Albino rats (inbred strain, male and female, 20 rats/group) were administered 0, 2.45, and 4.9% of propylene glycol in the diet (0, 1.23, and 2.45 g/kg bw/day, respectively) for 2 years. Other glycol chemicals were also part of this chronic study. Body weights and food consumption were determined at weekly intervals. No changes were noted when compared to control animals for growth rate, food and water consumption, and animal survival. There were no differences between control and propylene glycol groups in gross and microscopic lesions in the lung, heart, liver, spleen, kidney, adrenal glands, and testes **[individual data or summary tables not reported]**. The authors noted that there were no bladder stones or signs of chronic kidney damage and no change in the gross morphology of the testes when compared to control animals. "Slight liver damage" **[authors' words]** was observed in the propylene glycol exposed group *(80)* [reviewed in LaKind et al. *(28)*; OECD *(27)*]. **[No statistical analyses were performed and the histopathology of the liver is not described.]**

Strength/Weaknesses: The Morris et al. *(80)* paper predates standardized chronic toxicity test protocols and some may view it as poorly controlled. However, the experiment is well-described including the limitations. Therefore, it appears reasonable to accept that daily doses of 4.9% propylene glycol

in the diet (~3 g/kg) caused centrilobular atrophy, bile duct proliferation, and fatty degeneration in the liver even though it is not stated in the paper at which dose slight liver damage was observed. The highest doses (1.7 to 2.1g/kg) used by Gaunt et al. *(81*) were close to the lower dose in this study and no liver effect was reported by Gaunt et al. Therefore, the lower dose probably did not cause liver damage. Failure to conduct statistical analyses weakens this study.

Utility (Adequacy) for CERHR Evaluation Process: The Morris et al. *(80*) study can only serve as a modest indicator that 3 g/kg propylene glycol chronically might cause slight liver injury.

In 2-year and 15-week toxicity studies in rats given propylene glycol in the diet *(81*), body weight, renal concentration tests, organ weights, histology, and incidence of neoplasms were described. Necropsy at the end of the study included gross and microscopic examination of the male and female reproductive tracts. Charles River CD rats from a Specific Pathogen Free (SPF) breeding colony were used in this study. At the start of the study, the weight range of the males was 120–150 g and of the females was 120–140 g. **[Statistical methods were not described and standard errors for treatment groups were not presented.]** The studies were run concurrently.

For the short-term study, groups of 15 male and 15 female rats were fed diets containing 0, or 50,000 ppm propylene glycol [Shell Co. Ltd., >99% purity] for 15 weeks. Body weights and food consumption were not recorded. During the last week of treatment, renal concentration tests were estimated over a 6-hour water deprivation period. At necropsy, blood was collected for hematology and blood concentrations of urea, glutamic-oxalacetic, and glutamic-pyruvic transaminases were determined. At necropsy, brain, heart, liver, spleen, kidneys, adrenals, gonads, and pituitary were weighed. In the short-term study, the authors reported no differences between the control rats and those fed the 50,000 ppm diet for the parameters measured, including the urine and serum analyses, blood chemistry, and organ weights **[data not reported].**

In the long-term study, groups of 30 male and 30 female rats were fed diets containing either 0, 6,250, 12,500, 25,000, or 50,000 ppm propylene glycol for 2 years. Animals and food consumption were monitored daily and body weights recorded at 2 week intervals. Blood was collected from the tail vein of 8 male and 8 female rats in the 0, 25,000, and 50,000 ppm dose groups at 13, 21, 52, and 80 weeks of the study; and in the 0, 6,250, and 12,500 ppm groups at week 54 of the study. A urinary concentration test was done on selected rats from the 0, 25,000, and 50,000 ppm dose groups. Measurements were made of both specific gravity and urine volume over a 6-hour water deprivation period, during a 2-hour period after a 25 mL/kg water load, and then during a 4-hour period beginning 16 hours after the water load. At necropsy, brain, heart, liver, spleen, kidneys, adrenals, gonads**,** stomach, small intestine, and cecum were weighed. Samples of these organs, the following organs, and any tissue that appeared abnormal were preserved in 10% buffered formalin: salivary gland, trachea, aorta, thymus, lymph nodes, pituitary, urinary bladder, colon, rectum, pancreas, uterus, and muscle.

In the 2-year study, the mean daily intakes of propylene glycol were approximately 0, 0.2, 0.4, 0.9, and 1.7 g/kg in males and 0, 0.3, 0.5, 1.0, and 2.1 g/kg in females for the 0, 6,250, 12,500, 25,000, or 50,000 ppm propylene glycol dose groups, respectively. **[The authors did not provide daily food consumption or bi-monthly animal weight data.]** No abnormalities were observed among groups in deaths, behavior, or food consumption. The authors reported no significant differences between the

control and treated groups with respect to blood chemistry or renal concentration tests. Organ weights (including gonads) and organ weights relative to terminal body weight were similar between control and treated groups. Incidences of histological findings and the incidence of neoplasms in various tissues were presented, but the tabulated data did not include reproductive organs. Abnormalities cited were similar for the control and treated groups. The authors noted that the changes observed were consistent with those of aging rats and concluded that a "no-untoward-effect level" found in this study was 2.1 g/kg for male rats and 1.7 g/kg for female rats **[highest dose used]**.

Strength/Weaknesses: Gaunt et al. *(81*) is a well-conducted carcinogenicity bioassay which clearly demonstrates that an average daily dose of 1.7 g/kg in male rats and an average daily dose of 2.1g/kg in female rats had no adverse effect (NOAEL) on body weight gain, mortality, hematology, urinary cell excretion, renal function, serum chemistry, or absolute and relative organ weights. The histopathological changes were consistent with those expected in aging rats. No malignancy could be attributed to treatment. Although reference is made in the text to "no statistically significant differences," it is not stated what statistical methods were used. However, the reputation of the British Industrial Biological Research Association (BIBRA) and of the authors of this paper lend credibility to the statement. It is unfortunate that a higher dose was not used, because as conducted, the Panel did not learn anything about the chronic toxicity in rats, only about propylene glycol's safety. Up to 78 weeks there is no discernible effect on body weight but thereafter, there might have been a slight body weight effect. Unfortunately, no standard error is given and mortality was high in all groups, which was at least partially due to a high rate of pulmonary infection.

Utility (Adequacy) for CERHR Evaluation Process: This study by Gaunt et al. *(81*) establishes a highly credible NOAEL for propylene glycol in terms of chronic toxicity in both male and female rats. This information could be very useful when evaluating reproductive/developmental toxicity (i.e., a maternal NOAEL).

Propylene glycol administered in the drinking water of rats at doses >13.2 g/kg bw/day for 140 days resulted in CNS depression and minor liver injury (reviewed by Mortensen *(72*) and LaKind et al. *(28*)). In a 2-year drinking water study in rats (dosed up to1.834 g/kg bw/day), no renal pathology and very slight liver damage was found *(28*).

The Seidenfeld and Hanzlik *(82*) paper predates all other publications thus far evaluated. It includes detailed observation of the animals. A mix of acute and subchronic studies was conducted in rats and rabbits. Acute studies provided the dose ranges for the later, more detailed experiments of Braun and Cartland *(79*) and Weatherby and Haag *(78*). **[The Panel notes that even though the style of the Seidenfeld and Hanzlik publication may appear outdated, the data seem reliable. In fact, the dose x time product for slight vacuolization of the liver is 1,862g x day in this study and 2,160g x day in the Morris et al. *(80*) report. Thus, it can be concluded that slight hepatic injury could be expected in rats at a daily intake of 2 g/kg bw of propylene glycol. The study by Seidenfeld and Hanzlik is useful because now the Morris et al. *(80*) report can be viewed as confirmatory evidence for the slight liver damage as a high dose effect.]**

Utility (Adequacy) for CERHR Evaluation Process: This study is useful because now the Morris et al. *(80*) report can be viewed as confirmatory evidence for the slight liver damage as a high dose effect.

Propylene glycol was fed to dogs as a carbohydrate source in the diet at a concentration of 8% (2 g/kg bw/day) and 20% (5 g/kg bw/day) for 2 years; a control group was fed an equal caloric amount of dextrose and a second control group did not receive the dextrose. No adverse effects were observed in the low-dose group. In the high-dose group, there was evidence of RBC destruction (packed cell volume and hemoglobin values were lower and reticulocytes were higher than control values). There were no differences in kidney weights compared to the control group and no other indications of toxicity *(67, 83*).

Strength/Weaknesses: Weil et al. *(83*) studied the toxicity of propylene glycol in beagle dogs fed in the diet at 2 and 5 g/kg bw/day for 2 years. A roughly isocaloric diet to the propylene glycol containing dextrose was fed to a positive control group. After appropriate statistical evaluation, the authors concluded that 5 g/kg bw/day of propylene glycol in the diet resulted in enhanced erythrocyte destruction with signs of increased erythropoiesis. Use of a positive control group was useful to identify this effect as caused by propylene glycol. The NOAEL for chronic toxicity in dogs (2 g/kg bw/day) was essentially identical to the rat NOAEL.

Utility (Adequacy) for CERHR Evaluation Process: This paper is very useful because it has a dose that was actually toxic, which allows judgement of the ratio between LOAEL and NOAEL.

No effects were found on the kidneys in studies by VanWinkle and Newman *(84*) in dogs. Female dogs were administered 5% propylene glycol in drinking water two times a day for up to 9 months; male dogs were allowed to drink 600 mL of 10% propylene glycol daily. Kidney function was measured by phenosulfonphthalein excretion and liver function by rose bengal in the blood and galactose and uric acid in the urine. No pathological changes were found in these organs *(28*).

Strength/Weaknesses: In these experiments *(84*), liver and kidney function of dogs provided drinking water containing 5% propylene glycol (5 .1 cm^3=5.3 g/kg body weight) were determined and found not to be effected. However, dogs given water with 10% propylene glycol died and those provided with 10% propylene glycol containing water in the morning and clean water in the evening showed impaired renal function as indicated by increased blood urea. Authors stated that control values ranged from 14 to 24 mg% and after drinking the glycol for 6 months the range was 12−33 mg%. Statistical analysis was not performed and if it had been, it certainly would have shown no difference. There are no hematology measurements.

Utility (Adequacy) for CERHR Evaluation Process: The studies of Van Winkle and Newman *(84*) may be considered inadequate by today's standards, but they still provide useful data as confirmatory evidence for the NOAEL of 2 g/kg bw/day established by Weil et al. *(83*) in dogs.

Table 2-4. Summary of Toxicity of Propylene Glycol in Experimental Animals (data from OECD (27) and ATSDR (4))

Species	*Route*	*Dose/Duration*	*Findings (g/kg bw/day)*	*Study*
Rat	Oral	1%–50% in drinking water for 140 d	NOAEL 13.2 (equiv to 10% in water)	Seidenfeld and Hanzlik (*82*)
	Oral	0.625%–5% in feed for 103 wk	NOAEL 1.70 (m) NOAEL 2.10 (f) (equiv to 5% in feed)	Gaunt et al. (*81*)
	Inhalation	321 ppm for 90 d	Enlarged goblet cells/ thickened tracheal epithelium	Suber et al. (*85*)
	Inhalation	0.17−0.35 mg/L for 18 months continuous exposure	LOAEL 112 ppm (50% increase in body weight)	Robertson (*86*)
Rabbit	Dermal	0.52 g/one time (~0.17 g/kg bw)	Neat material not irritating	Clark et al. (*87*)
	Inhalation	10% for 20 min or 120 min	Increased degenerated goblet cells @ 20 min and 120 min	Konradova et al. (*88*)
Monkey	Inhalation	32–112 ppm 13 months	LOAEL 112 ppm	Robertson (*86*)
Cat	Oral	0.080–4.24 g/kg bw/day in feed for 2–3 months	LOAEL 0.424 NOAEL 0.080 (Heinz body formation)	Reviewed by OECD (*27*)
	Oral	6 or 12% in feed for 117 d	LOAEL 0.741–1.60 (Heinz body formation) NOAEL < 0.741–1.60	Bauer et al. (*89*)
	Oral	1.6 g/kg bw/day for 5 wks or 8.0 g/kg bw/day for 22 d	Low dose, anion gap; high dose polyuria/polydipsia, ataxia, depression	Christopher et al. (*39*)
Dog	Oral	8 or 20% in feed for 104 wks	LOAEL 5.00 (equiv 20% feed) (anemia) NOAEL 2.00 (equiv 8% feed)	Weil et al. (*83*)

2.2.2.2 Dermal Exposure

Propylene glycol was tested on the clipped skin of NZW rabbits according to three protocols (the cosmetic protocol, the Association Francaise de Normalization protocol, and the OECD protocol); in all three tests, propylene glycol was classified as a nonirritant *(28*).

Strength/Weaknesses: Irritation potential of propylene glycol, although minimal, has been established in man.

Utility (Adequacy) for CERHR Evaluation Process: None.

2.2.2.3 Inhalation Exposure

The ATSDR review *(4*) states that studies available on inhalation exposure of animals to propylene glycol are inconclusive. An acute inhalation study with 10% propylene glycol **[mg/L not stated]** for 20 or 120 minutes in rabbits resulted in degenerated goblet cells in the trachea *(88*). However, a subchronic exposure study in rats *(85*) did not support these findings. Rats exposed to 321 ppm over 90 days had thickened respiratory epithelium and enlarged goblet cells *(85*). Monkeys (n=29) and rats **[number not specified]** were continuously exposed to propylene glycol vapor at doses of 32–113 ppm for 13 months. At 113 ppm, hemoglobin levels were slightly increased; there were no adverse effects noted on body weight or on the renal, respiratory, gastrointestinal, hepatic, or endocrine systems *(4*).

Strength/Weaknesses: Konradova et al. *(88*) demonstrated that a 10% propylene glycol mist inhaled by rabbits resulted in enhanced mucolytic activity (+69%) of respiratory goblet cells. This is not surprising from a surface tension lowering agent. In fact, the effect of pure propylene glycol was less pronounced than that of clinically used mucolytics (Broncholysin, Histabron). Other conclusions regarding ciliated cells are difficult to assess because of the smallness of the effect. Moreover, a much more thorough study of inhalation of a propylene glycol aerosol did not confirm these findings *(85*).

Utility (Adequacy) for CERHR Evaluation Process: None

The Suber et al. *(85*) paper appears to be a well-conducted subchronic, nose-only inhalation study by a contract laboratory. Nominal doses were 0.0, 0.16, 1.0, and 2.2 mg/L of propylene glycol with an air flow rate of 1.0−1.5 L/min to each animal. Absorption was not determined, but system toxicity could not be expected even if 100% of the highest dose had been absorbed. As is clear in Bau et al. *(38*), only a fraction of inhaled propylene glycol will be absorbed into the systemic circulation through the lungs. Nasal hemorrhage is compatible with the known irritation potential of propylene glycol. Goblet cell score was significantly increased in the nasal turbinates, which is plausible for a surface-active agent facilitating the discharge of mucous from the swollen goblet cells.

Utility (Adequacy) for CERHR Evaluation Process: This is a useful study *(85*) that confirms the view arrived at for kinetic reasons that exposure by inhalation to propylene glycol does not pose a significant toxicological problem.

Robertson et al. *(86*) examined the chronic toxicity of propylene glycol by inhalation in Rhesus monkeys and rats. This is a very interesting study because both rats and monkeys were exposed

continuously to saturated/supersaturated air of propylene glycol (55 –113 ppm) for up to 1 year. At the highest dose, hemoglobin levels seemed to have increased. However, since no standard error is given and no statistical analysis was performed, it is uncertain whether this is a real effect. Otherwise, no adverse effects were found in spite of extensive gross and histopathologic examination. In fact, both rats and monkeys inhaling propylene glycol gained more weight than the controls. The health status of monkeys was poor, which was not uncommon in 1947. Assuming Rhesus monkeys inhale about 2 m^3 of air per day, the data indicate that primates may safely inhale about 1 g of propylene glycol per day. Although this paper uses unusual reporting methods by today's conventions, it certainly appears reliable and interpretable.

Utility (Adequacy) for CERHR Evaluation Process: Continuous exposure to propylene glycol vapor (without vehicle) in a primate species provides important evidence.

2.2.2.4 Hematological effects

Results from animal studies indicate that intermediate and chronic exposure to propylene glycol may lead to hemolysis of RBCs. After a 90-day inhalation exposure to 321 ppm of propylene glycol, female rats had decreased white blood cell count, while exposure to 707 ppm of propylene glycol decreased hemoglobin concentrations. No dose-related changes in RBCs were observed in male rats *(85*). After exposure of rats to 5% propylene glycol in the diet for 2 years, there were no hematological effects noted *(81*). However, Saini et al. *(90*) [reviewed by OECD *(27*)] found that a single oral dose of either 0.73 or 2.94 g/kg bw given to female Wistar rats, produced a reversible, statistically significant decrease in hemoglobin, packed cell volume, and RBC counts for 2 days. Electron microscopy revealed a rough RBC surface. However, in an early study by Robertson et al. *(86*), Rhesus monkeys continuously exposed to concentrations of propylene glycol in air up to 112 ppm for 13 months had a slightly greater increase **[statistical analyses not reported]** in RBCs and hemoglobin content than the control animals.

Cats exposed to oral administration of propylene glycol developed Heinz bodies in RBCs and experienced decreased RBC survival *(89, 91*). Heinz bodies are composed of denatured proteins, primarily hemoglobin. Cats exposed orally to 1.2, 1.6, 2.4, and 3.6 g/kg bw/day of propylene glycol for 2, 5, or 17 weeks developed increased numbers of RBCs with Heinz bodies. The cat is very sensitive to propylene glycol toxicity, with a 0.44 mg/kg bw/day dose reported to result in Heinz body formation in erythrocytes (reviewed by OECD *(27*)). This sensitivity occurs at concentrations that were present in soft moist cat foods and lead the FDA to remove propylene glycol from cat foods in 1996 *(9*).

In a study by Weil et al. *(83*) dogs were fed propylene glycol at 2 and 5 g/kg bw/day through the diet. Significant hematological changes were noted in the high dose group after two years; hemoglobin, hematocrit, and total erythrocyte counts were lower, whereas, poikilocytes and reticulocytes were increased.

Strength/Weaknesses: There are few and inconsistent changes in hematologic parameters in the Suber et al. *(85*) study. No inferences can be made for erythropoiesis.

Utility (Adequacy) for CERHR Evaluation Process: None

Strength/Weaknesses: Saini et al. *(90*) reported hematologic effects of propylene glycol in rats

administered single doses of 0.7 or 3 g/kg bw by gavage. There is sufficient experimental detail given to deem the results reliable. However, Gaunt et al. *(81*) did not find any hematologic effect after feeding about 2 g/kg bw/day for 2 years. It is very likely that the acute changes seen by Saini et al. *(90*) have been overcome by 2 years due to adaptation.

Utility (Adequacy) for CERHR Evaluation Process: This is a useful report *(90*) that confirms that the hematopoietic system is also a target of propylene glycol in rats, albeit at higher chronic doses than in cats, dogs, and probably monkeys.
Strength/Weaknesses: The Robertson et al. *(86*) study has a very large uncertainty attached to it, as discussed earlier, and provides marginal evidence of a hematologic effect in non-human primates.

Utility (Adequacy) for CERHR Evaluation Process: The hemolytic capability of propylene glycol has been demonstrated *in vitro* in human erythrocytes *(78*). However, the primate data presented by Robertson et al. *(86*) do not provide evidence of a hematological effect of propylene glycol on primates.

Strength/Weaknesses: Christopher et al. *(91*) reported D-lactic acidosis and Heinz body formation in cats administered daily 1.6 or 8 g/kg propylene glycol for up to 35 days. Authors conclusively demonstrated a dose-dependent reduction of erythrocyte survival. Bauer et al. *(89*) confirms in essence the findings of Christopher et al. *(91*) and refines the dose response on Heinz body formation and erythrocyte survival.

Utility (Adequacy) for CERHR Evaluation Process: Christopher et al. *(91*) provide an excellent study that establishes a plausible mechanism for propylene glycol-induced hemolysis and the Bauer et al. *(89*) study provides important confirmatory evidence for the impairment of hematopoiesis by propylene glycol. Thus, the hemolysis potential of high doses of propylene glycol, which is a plausible effect, is firmly established in two species (cat and dog) and reasonably well substantiated in other species including man.

2.3 Genetic Toxicity

2.3.1 Humans

No studies were located regarding *in vivo* genotoxic effects in humans or animals.

2.3.2 Experimental systems

2.3.2.1 In Vitro

ATSDR *(4*) states that "Propylene glycol was not mutagenic in *S. typhimurium* strains TA 98, TA100, TA1535, TA1537, and TA1538 with and without metabolic activation. Propylene glycol was negative for sister chromatid exchange and changes in alkaline elution rate using Chinese hamster cells or human fibroblasts" (Table 2-5).

Table 2-5. Genotoxicity of Propylene Glycol In Vitro (from ATSDR (4))

Species (test system)	*Endpoint*	*Results with activation*	*Results without activation*	*Reference*
Prokaryotic organisms:				
S. typhimurium	Gene mutation	Negative	Negative	Clark et al. (87)
S. typhimurium	Gene mutation	Negative	Negative	Pfeiffer and Dunkelberg (92)
Mammalian cells:				
Human fibroblasts	Chromosome aberrations	Negative	Negative	Tucker et al. (93)
Chinese hamster cells	Chromosome aberrations	Negative	Negative	Tucker et al. (93)
Chinese hamster lung cells	DNA damage	Negative	Negative	Swenberg et al. (94)

Propylene glycol was one of a number of chemicals evaluated for mutagenicity in a study of chemicals used and formed after the fumigation of foodstuffs *(92)*. A modified Ames test used histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537. Propylene glycol (98 % purity, diluted in water, test volume, 0.1 mL) was added to 2 mL distilled water and 0.1 mL (10^8) bacteria. This mixture was added to 2 mL Topagar and poured into a Petri dish containing histidine-free agar, incubated for 48 hours at 37°C, and revertant colonies counted. Liver microsomes were not incorporated into the test mixture. The authors concluded that propylene glycol, as well as ethylene glycol and diethylene glycol, showed no mutagenic activity with any of the four *Salmonella* strains **[data not shown by authors]**. All experiments were performed 6–10 times **[controls and statistics are not described].**

Strength/Weaknesses: Pfeiffer and Dunkelberg *(92)* studied mutagenicity of ethylene oxides, propylene oxide, various halo-alcohols, and several glycols. The test systems used were those normally used for *S. typhimurium* strains TA98, T4100, TA1535, and TA1537 without metabolic activation. The reaction mixture was modified to accommodate the low water solubility of ethylene oxide and propylene oxide. As expected, the epoxides gave strong positive results, the halo-alcohols variable responses, and the glycols were uniformly negative. There are no weaknesses apparent in these experiments.

Utility (Adequacy) for CERHR Evaluation Process: This study *(92)* provides experimental confirmation of the expected and the plausible.

Propylene glycol was one of the chemicals evaluated by Swenberg et al. *(94)* using an *in vitro* assay to assess DNA damage and predict carcinogenic potential. Chinese hamster lung fibroblast (V79) cells were grown in tissue culture to which radioactive thymidine was added for 20–24 hours, then the radioactivity was removed and the cells were incubated for 4 –20 hours in a non-radioactive medium. Cells were then exposed to test chemicals for up to 4 hours with or without the presence of a liver microsomal enzyme activation system (S-9). Cell viability was assessed by measurement of cellular ATP levels. DNA damage was measured by an increase in elution rate under alkaline conditions of single-stranded fibroblast DNA from polyvinyl filters. Propylene glycol exposure for 1, 2, or 4 hours

with or without a rat microsomal activation system did not cause a significant increase in the elution rate from that of non-treated cells **[statistical method not described or referenced**].

Strength/Weaknesses: Clastogenicity of a large number of compounds was tested by an *in vitro*/ alkaline DNA elution assay *(94*). The complete lack of experimental detail regarding propylene glycol diminishes its value.

Utility (Adequacy) for CERHR Evaluation Process: The study *(94*) is of very little use to CERHR, although it confirms the expected and the plausible.

Propylene glycol is listed as a chemical giving negative results in the sister chromatid exchange assay using normal human fibroblast cells. The highest concentration tested was 0.1 M *(93*). **[Details of this assay were not given.]**

Strength/Weaknesses: Sister chromatid exchange was tested with a high number of chemicals as reviewed by Tucker et al. *(93*). Propylene glycol was found to be negative in this test system.

Utility (Adequacy) for CERHR Evaluation Process: It is helpful to know that propylene glycol was negative in still another chromosomal test.

Propylene glycol was included in the primary mutagenicity screening of food additives used in Japan *(95*). *Salmonella*/microsome tests (Ames tests) and chromosomal aberration tests using a Chinese hamster fibroblast cell line were performed. Propylene glycol (99% purity) was negative in the Ames test (dimethyl sulfoxide [DMSO] solvent, 32 mg/mL maximum non-cytotoxic dose) and positive in the chromosomal aberration test (maximum dose 32 mg/mL). A chemical is positive in the chromosomal aberration test if the total incidence of cells with aberrations is 10% or higher. For propylene glycol in saline, 38% of cells had aberrations after 48 hours and the incidence of polyploid cells was reported to be 1%. These results were not discussed further by the authors.

Strength/Weaknesses: A high number of food additives was screened for mutagenicity and clastogenicity *(95*). The Ames test was conducted in the usual *S. typhimurium* strains and chromosomal aberrations were tested in a Chinese hamster fibroblast cell line. There is sufficient experimental detail to deem the results reliable. Once again, propylene glycol was negative in the Ames test but positive in the clastogenicity test.

Utility (Adequacy) for CERHR Evaluation Process: This study *(95*) is not useful because the biological significance of these *in vitro* data are unclear.

The FDA *(96*) submitted propylene glycol for mutagenic evaluation **[discussed in the *In Vivo* Section 2.3.2.2]**. Along with the *in vivo* assays, one *in vitro* cytogenetics study was performed. WI-38 cells (human embryonic lung cells) were exposed to concentrations of propylene glycol at 0.001, 0.01, and 0.1 µg/mL. Concentrations of 0.1 µg/mL resulted in complete destruction of the cells. A negative control of saline and a positive control of 0.1 µg/mL triethylene melamine were used. The authors concluded that propylene glycol produced no significant aberrations in the anaphase **[sic]** chromosomes of the cells at the dosage levels employed in this study.

Strength/Weaknesses: This is a comprehensive evaluation of the mutagenicity of propylene glycol *in vitro* and *in vivo (96*). There is sufficient experimental detail to satisfy doubts that propylene glycol is neither mutagenic nor clastogenic.

Utility (Adequacy) for CERHR Evaluation Process: The study *(96*) confirms the expected and is plausible.

2.3.2.2 In Vivo

Propylene glycol was tested using the mouse micronucleus test with 38 other food additives *(97*). The micronucleus test was conducted in 8-week-old ddY mice (6/dose group). Animals were dosed by intraperitoneal (IP) injection, once/day for 5 days with propylene glycol. Femoral marrow cells were flushed with fetal bovine serum. Slides were fixed in methanol and stained with Giemsa. Preparations were coded so that the scorer was not aware of the treatment. One thousand polychromatic erythrocytes (PCE) per mouse were scored under 100x power and the number of micronucleated polychromatic erythrocytes (MNPCE) was recorded. Results were compared with control groups and historical negative control groups. The frequency of MNPCEs in each treatment group was compared with the binomial distribution specified by historical control data from that laboratory. Dose-response relationships were tested by the Cochran-Armitage trend test. A positive result was recorded when one or more treatment groups showed a statistically significant difference ($P < 0.01$). Dose groups and results with propylene glycol are given in Table 2-6 below. Test results were negative.

Table 2-6. Results of the Micronucleus Test Using Mouse Bone Marrow Cells (97)

Propylene glycol, saline, IP	*MNPCEs (%)*	*PCEs (%)*	*Mortality*	*Trend Test*
0 mg/kg bw	0.20±0.19	43.9±12.2	0/6	NS*
2,500	0.20±0.18	53.6±9.2	0/6	
5,000	0.17±0.10	52.8±6.3	0/6	
10,000	Mortality	Mortality	6/6	

*NS: non-significant

Strength/Weaknesses: Propylene glycol was negative in the micronucleus test *(97*). A wide dose range (2.5–15.0 g/kg bw) was used, which covered the whole spectrum of effects including 50% mortality at the highest dose. The study was conducted blind and analyzed by appropriate statistics. Chemicals expected to have a positive response did indeed show a statistically significant increase in micronuclei. There are no apparent weaknesses to this study.

Utility (Adequacy) for CERHR Evaluation Process: This study *(97*) provides *in vivo* confirmation for the lack of clastogenicity of propylene glycol.

The FDA submitted propylene glycol for mutagenic evaluation *(96*) in three genotoxicity test systems: host mediated assay, dominant lethal assay, and *in vivo* cytogenetic studies. The three *in vivo* assays are discussed below (Table 2-7) and the *in vitro* cytogenetics study is discussed in the *In Vitro* Section 2.3.2.1.

In the host-mediated assay (*in vivo*, mice), doses of propylene glycol at 30, 2,500, and 5,000 mg/kg bw

and negative control of saline, and positive controls of 350 mg/kg bw ethyl methane sulfonate and 100 mg/kg bw dimethyl nitrosamine were tested. Acute studies (1 dose by gavage of chemical, followed by IP injection with *S. typhimurium* 30 min after dosing) produced no significant increases in mutation frequencies with *Salmonella* TA1530 and with all levels of *Salmonella* G46, except the 5,000 mg/kg bw level, which produced a weak questionable positive response. *Saccharomyces* D3 showed increased recombinant frequencies at all levels except the acute high dose. Subacute studies (dosing once/day by gavage for 5 days, inoculating IP 30 minutes after last dose) produced increased recombinant frequencies at all levels. While some statistically-significant differences were noted in the mid- and high-dose animals from both phases of the investigation, comparison with historic data demonstrated that this was a consequence of unrepresentative low control data rather than a substance-specific effect. Therefore the authors concluded that propylene glycol has no capacity to induce mutations.

For the dominant lethal assay (*in vivo*, rats), propylene glycol was administered by gavage at 30, 2,500, and 5,000 mg/kg bw and a negative control of saline and a positive control of 0.3 mg/kg bw triethylene melamine were tested. Propylene glycol was considered non-mutagenic in rats in this assay at these doses.

For cytogenetics studies (*in vivo*, rats), propylene glycol was administered by gavage at 30, 2,500, and 5,000 mg/kg bw, and a negative control of saline and a positive control of 0.3 mg/kg bw triethylene melamine were tested. Propylene glycol produced no significant increases in aberrations of bone marrow cells when administered orally at these doses.

Table 2-7. In Vivo Genotoxicity Results (96)

Assay	*Dose of Propylene Glycol*	*Endpoint*	*Result*
Host Mediated Assay, mice	30, 2,500, 5,000 mg/kg bw	Increase in mutation frequencies: *Salmonella* TA1530 and G46 *Saccharomyces* D3	Negative
Dominant Lethal Assay, male rats treated	30, 2,500, 5,000 mg/kg bw	Increase in % dead implants in pregnant, untreated female	Negative
Cytogenetics studies, rats	30, 2,500, 5,000 mg/kg bw	Chromosome aberrations (bone marrow)	Negative

Strength/Weaknesses: The Litton Bionetics, Inc. *(96*) report is a detailed and comprehensive *in vitro* and *in vivo* evaluation of propylene glycol for genotoxicity. There are no apparent weaknesses in this report.

Utility (Adequacy) for CERHR Evaluation Process: The data in this report *(96*) demonstrate propylene glycol's lack of genotoxicity.

2.4 Carcinogenicity

2.4.1 Human Data

No data on carcinogenicity in humans were identified

2.4.2 Experimental Animal Data

2.4.2.1 Oral Exposure

In a long-term dietary toxicity study in rats by Gaunt et al. *(81)* **[see Section 2.2.2.1, General Toxicity]**, rats were fed propylene glycol up to 5% (2,500 mg/kg bw/day) in their diet for 103 weeks. Death rate, body weight gain, food consumption, hematology, and renal clearance were monitored. No significant differences were noted between control and treated rats for the parameters examined. There were no treatment-related increases in neoplasms.

Charles River CD rats from a SPF breeding colony were used in this study. At the start of the study, the weight range of males was 120–150 g and of females was 120–140 g. **[Statistical methods were not described and standard errors for treatment groups were not presented.]** In a 2-year study, the mean daily intakes of propylene glycol were approximately 0, 0.2, 0.4, 0.9, and 1.7 g/kg bw in males and 0, 0.3, 0.5, 1.0, and 2.1 g/kg bw in females for the 0, 6,250, 12,500, 25,000, and 50,000 ppm propylene glycol dose groups, respectively. **[The authors did not provide daily food consumption or bi-monthly animal weight data.]** No abnormalities were observed among groups in deaths, behavior, or food consumption. The authors reported no significant differences between the control and treated groups with respect to blood chemistry or renal concentration tests. Organ weights (including gonads) and organ weights relative to terminal body weight were similar between control and treated groups. Necropsy at the end of the study included gross and microscopic examination of the male and female reproductive tracts. Incidences of histological findings and the incidence of neoplasms in various tissues were presented, but the tabulated data did not include reproductive organs. Abnormalities cited were similar for the control and treated groups. The authors noted that the changes observed were consistent with those of aging rats and concluded that a "no-untoward-effect level" found in this study was 2.1 g/kg bw for male rats and 1.7 g/kg bw for female rats **[highest dose used].**

Strength/Weaknesses: Gaunt et al. *(81)* reported on a state-of-the-art carcinogenicity bioassay (four different doses) with propylene glycol. Average body weights of males were about 12% and those of females about 10% below controls, in the highest dose groups, although there is no statistical analysis of the data to know for sure if these are real differences. There were no treatment-related malignancies.

Utility (Adequacy) for CERHR Evaluation Process: It is clear that propylene glycol does not cause cancer at or near a toxic level administered in the diet.

2.4.2.2 Dermal Exposure

In skin-painting studies, Stenback and Shubik *(98)* examined the potential carcinogenicity and toxicity of several commonly used cutaneous agents including propylene glycol. Seven-week-old female Swiss mice (50/concentration) were treated with 10, 50, and 100% propylene glycol in acetone over the lifetime of the animal. Propylene glycol (0.02 mL) was dropped onto the shaved dorsum (1-inch square area) twice a week. Animals were allowed to die naturally or were sacrificed moribund. Complete necropsies were performed and all tumors were examined histologically. The skin tumor incidence seen in the treated animals (2–4%) was comparable to the values obtained with acetone controls (50 animals) and with untreated animals (135 animals). DMBA (10 μg 2 times/wk) treatment (positive control, 50 animals) resulted in a 78% skin tumor incidence. The method of statistical evaluation was cited but not

described in the text by the authors. The authors concluded that there was no increase in dermal tumors or change in longevity in female Swiss mice after chronic treatment with propylene glycol.

Strength/Weaknesses: Stenback and Shubik *(98)* conducted a skin-painting experiment with, among other chemicals, propylene glycol. A uniform protocol was followed, which is problematic for compounds as different in their kinetics and dynamics as propylene glycol and DMBA. The dose was 0.02 mL pure propylene glycol or 50 and 10% solutions in acetone twice a week. It is in agreement with propylene glycol's low irritation potential that there were no skin tumors in treated mice, although this strain of mice (Swiss females) is exquisitely sensitive to the induction of skin tumors. The highest dose translates to approximately 0.8 g/kg bw twice a week. Systemic effects would not be expected from this dose rate even if absorption was 100%.

Utility (Adequacy) for CERHR Evaluation Process: This is a useful study *(98)*, with highly predictable outcomes.

2.5 Potentially Sensitive Subpopulations

A few case reports have been published suggesting exacerbation of clinical signs in hospitalized individuals as a result of propylene glycol. These cases are primarily associated with individuals with compromised liver or kidney function, with burn patients, and with overdosage of premature infants. As discussed in Section 2.1.4, after absorption, the kidneys eliminate 45% of propylene glycol with the remainder metabolized by the liver to lactic acid, pyruvic acid, or acetone. Therefore, patients with impaired liver or kidney function would be at increased risk for developing propylene glycol toxicity *(48)*. In patients with renal insufficiency, high propylene glycol levels have been associated with lactic acidosis (hyperlactemia) *(99, 100)*. Propylene glycol has been found in the blood of alcoholics with cirrhosis of the liver without detectable measurable blood alcohol levels *(101)*.

Propylene glycol toxicity has been suspected in patients with an abnormal serum osmolal gap[1]. In some cases, a review of the patient's medication history identified propylene glycol as a vehicle in the medications administered. The following information has been taken primarily from data presented in complex clinical case studies the cause of medical symptoms observed undetermined. Some examples of clinical cases of suspected propylene glycol toxicity are summarized in Table 2-8 below.

2.5.1 Oral and Intravenous Use

Oral or IV administration of propylene glycol may exacerbate dermatitis in some individuals *(102)*.

Propylene glycol is used as a vehicle for IV administration of drugs such as lorazepam, etomidate, phenytoin, diazepam, digoxin, hydralazine, esmolol, chlordiazepoxide, nitroglycerin, pentobarbital sodium, phenobarbital sodium, and trimethoprim-sulfamethoxazole and is a vehicle for some IV vitamin preparations. Serum concentrations of propylene glycol received through IV medications have been shown to correlate with serum lactate concentrations in patients with normal renal and hepatic function *(51)*. In children, seizures and respiratory depression have occurred after taking liquid medications containing propylene glycol *(103, 104)*.

[1] osmolal gap = measured serum osmolality – calculated serum osmolality; normal gap < 10; calculated osmolality = $2[Na^+]$ + glucose/20 + BUN/3 ; an increased osmolal gap can be indicative of increased solute in the blood.

Table 2-8. Some Clinical Complications Associated with Propylene Glycol (PG) Use

Patient; Reference	*Route*	*Findings*
8-month-old male infant; Fligner (*35*)	Dermal; silver sulfadiazine therapy in propylene glycol for burns, 78% surface area, 10.6 g/L **[139.5 mM]** PG serum level	Cardiopulmonary arrest, respiratory acidosis, increased osmolal gap
3.4 kg infant, cardiac surgery, heart failure; Huggon (*70*)	IV, PG vehicle in enoximone and glyceryl trinitrate infusions	Hyperosmolality
Premature infants; MacDonald (*106*)	IV, propylene glycol as part of a daily multivitamin preparation, 3g/day PG (alternative product delivering 0.3g/day PG had no effect on other premature infants)	Seizures
Premature infant, 27 wk gestation; Glasgow (*36*)	IV, propylene glycol as part of a daily multivitamin preparation, 9.3 g/L **[122.4 mM]** PG serum level	Serum hyperosmolality, acute renal failure
11-year-old boy, candidiasis-endocrinopathy syndrome with hypoparathyroidism; Arulanantham (*104*)	Oral, PG vehicle in dihydrotachysterol	Seizures
16-year-old boy, onset of seizures; Yorgin et al. (*107*)	IV, PG vehicle in pentobarbital and phenobarbital	Exacerbation of seizures, reversible acute renal failure
39-year-old woman, history of seizures; Lolin (*108*)	Most likely ingestion, 4 g/L **[52.6 mM]** PG serum level	Status epilepticus, metabolic acidosis, plasma hyperosmolality, respiratory depression
45-year-old man, respiratory distress, on ventilator; Arbour (*109*)	IV, PG vehicle in lorazepam, 1.7 g/L **[22.4 mM]** PG serum level	Hyperosmolality, metabolic acidosis
58-year-old man, renal disease, chronic schizophrenia; Cate (*68*)	Most likely ingestion, 0.7 g/L **[9.21 mM]** PG serum level	Unconscious, lactic acidosis, azotemia
60-year-old man, respiratory distress, on ventilator; Arbour (*48*)	IV, PG vehicle in lorazepam, infused for 5 d at 2.5 g PG/hr	Hyperosmolality
70-year-old woman, complications with surgery; Bedichek & Kirschbaum (*110*)	IV, 479 g PG administered with etomidate and other medications over a 24 hr period	Seizures, status epilepticus

2.5.2 Infants

The decreased size of premature infants and an increased serum half-life **[see Section 2.1.4.1]** for propylene glycol in premature infants *(35, 36*) predispose them to a greater probability of toxic effects from over administration of propylene glycol. There is particular concern for very small infants and those receiving multiple IV medications containing propylene glycol. Absorption of propylene glycol from ointments applied to burns and injection of multivitamin products in infants has resulted in

serum hyperosmolality *(36, 70*), which was associated with cardiorespiratory arrest in one case *(70*).

In one report, propylene glycol was shown to have a longer (16.9 h) half-life in a premature infant when compared with the half-life in adults (5 h) *(35*). Glasgow *(36*) measured the serum half-life in infants. Ten infants received 10 mL IV of daily multivitamin preparation (containing 30% propylene glycol) once a day for at least 5 days. Four infants had a serum level >3.0 g/L **[39.5mM]** propylene glycol. The range of serum propylene glycol values was 0.65–9.5 g/L **[8.55–125mM]**. In the control group, propylene glycol was not detected in six infants; two other infants had propylene glycol serum levels of 0.7g/L **[9.21mM]**. The propylene glycol levels in the serum of the control infants were attributed to Mycostatin cream usage for diaper rash and phenobarbital therapy. Thirty-six hours later, serum levels were taken. The mean half-life in these infants was calculated to be 19.3 hours with a range of 10.8–30.5 hours.

Propylene glycol serum concentration, serum lactate, and osmolar gap were measured in 11 intubated pediatric intensive care patients [1−15-month-old, 6 females] on continuous lorazepam infusion *(66*). Differences in propylene glycol concentration, serum lactate concentration, and osmolar gap at the beginning of therapy, 48 hours into therapy, and at the end of therapy were compared using repeated measures analysis of variance. Lorazepam infusion rates ranged from 0.1 to 0.33 mg/kg bw/hour and lasted 3–14 days. All patients in this study had normal renal function. At the end of therapy, serum levels of propylene glycol ranged from approximately 0.2 to 2 mg/mL. A significant correlation between the cumulative dose of lorazepam and propylene glycol serum concentration at the end of therapy was demonstrated ($p < 0.005$). However, propylene glycol accumulation was not associated with an elevation in serum lactate concentrations or osmolar gap. The authors caution that although "continuous lorazepam infusion seems to be a safe option for sedating patients with normal renal function in the pediatric intensive care unit, it would be prudent to monitor for lactic acidosis and hyperosmolality... ".

Propylene glycol is commonly used as a vehicle in topical, oral, or injectable medications *(16*). The American Academy of Pediatrics recommends mandatory labeling of inactive ingredients **[classified by the FDA as pharmaceutical excipients]** for all prescription and over-the-counter products *(105*). At present, labeling is voluntary for prescription drugs, and since 1998, is required for over-the-counter drugs. This requirement was a result of the FDA Modernization Act of 1997 *(8*).

2.6 Summary

2.6.1 Toxicokinetics and Metabolism

The absorption, distribution, metabolism, and excretion of propylene glycol have been studied in humans, cats, rats, mice, and rabbits. The studies reviewed by the Panel identified no major differences between humans and animals in the toxicity of propylene glycol. Toxic effects of propylene glycol occur only at very high doses. The domestic cat is the most sensitive species to propylene glycol toxicity, producing Heinz body anemia in response to propylene glycol as an additive (at 6% w/w or above) to its diet. The toxicokinetic properties are very similar across species studied. A consideration in the selection of experimental species is the metabolism of D- and L-optical isomers. Commercial propylene glycol is a 1:1 D, L mixture of both stereoisomers, and species differences in the rate of metabolism and excretion of D- and L- forms of propylene glycol are noted by the Panel. However, due to incomplete time point sampling and a lack of quantitative numbers regarding fluxes through

the different pathways, it was not possible for the Panel to provide a complete description of the stereospecific metabolism of D, L propylene glycol in different species. However, there are sufficient data in humans to conclude that acute exposure to D-, L-propylene glycol can cause L-lactic acidosis (if the dose is very high) due to the more rapid biotransformation (ADH being the rate determining step) of L-propylene glycol to L-lactate. However, with subchronic/chronic exposure to propylene glycol, D-lactic acidosis occurs due to the accumulation of D-lactate. D-lactate is derived from the glyoxylase/GSH pathway and since it is a poor substrate for gluconeogenesis, there would be a greater accumulation of the D-lactate than L-lactate with chronic exposures.

Dermal absorption studies in humans have shown that absorption of propylene glycol through intact skin is very limited. However, once the dermal layers are disturbed (such as with burns or irritation), dermal absorption can be a significant source of exposure.

In humans, absorption of propylene glycol after oral exposure reached maximum plasma concentrations within 1 hour of dosing and the average serum half-life was estimated to be from 1 to 4 hours. From rectal absorption studies, the half-life of propylene glycol was determined to be 2.8 0.7 hours in adults and 2.6 ± 0.3 hours in children (5 –12 years) *(31)*. The similarity in the half-life for adults and children in this age range is in agreement with alcohol dehydrogenase reaching adult levels by 5 years of age *(32)*. Glasgow et al. *(36)* reported an average half-life in 10 infants of 19.3 hours (range 10.8–30.5 hours), which is about 10 times longer than in adults. Alcohol dehydrogenase activity is up to ten times lower in infants *(32)* than in adults, providing an explanation for the prolonged half-life of propylene glycol in infants.

There are excellent data on the determination of the apparent volume of propylene glycol distribution in humans and animals; these data demonstrate that it distributes into total body water. In human studies, volumes of distribution were measured at 0.52 L/kg with oral dosing *(29)*, 0.77– 0.79 L/kg with rectal exposure *(31)*, and approximately 0.55– 0.94 L/kg with IV exposure *(30)*. Therefore, it can be concluded that propylene glycol will distribute into the water compartment of the placenta and fetus.

Since lactate distributes into total body water, the fetus will also experience the mother's metabolic acidosis if present and lactate would be present in breast milk. However, newborns and infants may be protected from metabolic acidosis after ingestion of propylene glycol due to a slower metabolic conversion to lactate.

Except for the amount entering the nasopharynx and being swallowed, under normal exposure conditions propylene glycol exposure by inhalation is not toxicologically relevant due to its low vapor pressure (0.07 mm Hg).

Total body clearance occurs by metabolic clearance and by renal excretion. Morshed et al. *(41)* provide evidence in the rat that the rate-determining step in the metabolic clearance of propylene glycol is NAD-dependent alcohol dehydrogenase. The Panel concludes from the data of Speth et al. *(30)* that humans clear propylene glycol similarly to rats and rabbits, but saturation of metabolic clearance occurs at lower doses in humans than in rats and rabbits. From the data of Speth et al. *(30)* and Yu et al. *(29)* the Panel determined that metabolic clearance follows a first-order process (up to doses of approximately 12 g/day) with a constant half-life of 1.6 ± 0.2 h (± SD). Beyond this dose, the

serum half-life becomes dose dependent (zero order process) with a serum half-life above 3 hours. Propylene glycol is converted to lactic acid by ADH and further to pyruvate, which provides energy through the Krebs cycle; lactate can be detoxified into glucose and stored as glycogen, providing other sources of energy *(47*).

The Panel concluded that the toxicokinetic data for propylene glycol are sufficient for evaluating the potential for propylene glycol to pose a risk to human reproduction.

2.6.2 General Toxicity

Propylene glycol has very low systemic toxicity in experimental animals and very high doses are required to determine a toxic level *(4, 27, 28*). CNS, hematologic, hyperosmotic, and cardiovascular effects have been noted in humans and animals and high serum concentrations of propylene glycol may result in lactic acidosis and hyperosmotic changes in the blood. Animals lethally intoxicated undergo CNS depression, narcosis, and respiratory arrest. In humans, a lethal oral dose has been estimated to be >15 g/kg for an adult *(2*). Mortality has occurred in hospitalized infants after repeated exposure to propylene glycol in medication **(see Potentially Sensitive Subpopulations)**.

Acute oral toxicity has been well characterized in the rat, mouse, rabbit, dog, and guinea pig with LD_{50} values, 8–46 g/kg bw (See Table 2-3), reported at very high oral doses.

In a 2-year study by Gaunt et al. *(81*), an average daily dose of 1.7 g/kg bw in male rats and 2.1 g/kg bw in female rats had no adverse effect on body weight gain, mortality, hematology, urinary cell excretion, renal function, serum chemistry, or absolute and relative organ weights. Weil et al. *(83*) studied the toxicity of propylene glycol fed in the diet to dogs at 2 and 5 g/kg bw/day for 2 years. No adverse effect was noted in the low-dose group; there was evidence of RBC destruction in the high-dose group. The Panel concluded that in assessing toxicity from chronic exposure, 2 g/kg bw/day is a NOAEL for dogs and rats; 5g/kg bw/day is a LOAEL for dogs.

In a continuous inhalation study, Robertson et al. *(86*) examined chronic toxicity of propylene glycol (55–113 ppm) in Rhesus monkeys and rats for up to 1 year. Both rats and monkeys inhaling propylene glycol gained more weight than the control group; no adverse effects were noted. The Panel estimates that the monkeys inhaled approximately 1 g of propylene glycol per day.

Results from animal studies indicate that intermediate and chronic exposure to propylene glycol may lead to changes in hematological parameters and hemolysis of RBCs. Cats exposed to oral administration of propylene glycol developed Heinz bodies in RBCs and decreased RBC survival. Doses as low as 0.424 g/kg bw/day have resulted in Heinz body formation in cat erythrocytes *(27*). In a study in dogs fed 5 g/kg bw/day for 2 years *(83*), evidence of RBC destruction was noted. The Panel concluded that there is sufficient data on the hemolytic potential of high doses of propylene glycol in the cat and dog, and limited substantiated data in other species, including humans.

The Panel concluded that there are sufficient data to characterize the acute and chronic toxicity of propylene glycol in laboratory animals, including non-human primates. In humans, information on toxicity is limited to medical case studies. However, because of the similarities in the toxicokinetic profile of propylene glycol across species, the toxicity data from the animal studies can be extrapolated

to human exposures.

2.6.3 Genetic Toxicity

No studies were located regarding *in vivo* genotoxic effects in humans. Propylene glycol was consistently negative in *in vitro* and *in vivo* animal tests.

2.6.4 Carcinogenicity

No data on carcinogenicity in humans were identified.

Gaunt et al. *(81)* reported a 2-year bioassay where rats were fed up to 5% (2,500 mg/kg bw/day) propylene glycol in their diet. No treatment-related neoplasms were noted. The Panel concluded that dietary administration of propylene glycol does not cause cancer at or near a toxic level.

2.6.5 Potentially Sensitive Subpopulations

There have been reports of propylene glycol toxicity in individuals with compromised liver or kidney function and in infants who have inadvertently received an overdose of propylene glycol in conjunction with drug therapies. Serum half-life of propylene glycol in infants is longer than in adults. Fligner et al. *(35)* reported a half-life of 16 hours for a premature infant as compared to 5 hours in adults. Glasgow *(36)* measured serum half-life in ten infants. The range of serum values was 0.65–9.5 g/L **[8.55–125mM]**. Mean half-life of propylene glycol was calculated to be 19.3 hours with a range of 10.8–30.5 hours which is about 10 times longer than in adults. Alcohol dehydrogenase can be up to ten times lower in infants, which would account for the prolonged half-life in infants.

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 and Perinatal Toxicity Studies

A prenatal oral developmental toxicity study of propylene glycol was performed by Bushy Run Research Center *(111)*. The results are summarized in Table 3-2 (pg. 52). This study was conducted in compliance with Good Laboratory Practices. Propylene glycol was administered by gavage to Charles River CD-1 mice from gd 6 to 15 *(111)*. Pregnant CD-1 mice (4 dose groups, 30 mice/dose group, pregnancy determined by presence of copulation plug [gd 0]) received undiluted propylene glycol (99.9% purity) once a day in the following dosage volumes: 0.5, 5.0, or 10.0 mL/kg bw/day [0.52, 5.2, or 10.4 g/kg bw day, respectively]. A control group of 30 pregnant females received 10.0 mL/kg bw/day of Milli-Q™ water. Females were approximately 46 days old and males were 49 days old upon receipt. Animals were acclimated for approximately 2 weeks before cohabitation. The mice were maintained on a 12-hour, light-dark cycle and food and water were available throughout the study. Pregnancy rate ranged from 93.3 to 100% and there were no unscheduled deaths during the study. Maternal weight and food and water consumption were monitored at 3 day intervals. After initiation of treatment, all animals were observed twice daily for morbidity and mortality.

At scheduled necropsy on gd 18, maternal body weight, liver and kidney weights, gravid uterine weight, number of corpora lutea, and number of implantation sites/resorptions were noted. All live and dead fetuses were sexed, weighed, and examined for external malformations; all live fetuses were examined for visceral malformations using a modification of methods described by Staples; 1/2 of live fetuses in each litter were decapitated and the heads were examined for craniofacial malformations using sectioning methods modified from Wilson; all live fetuses (1/2 intact and 1/2 decapitated) were stained with Alizarin Red S and examined for skeletal malformations **[cartilage not stained]**.

Maternal Parameters

Pregnancy rate ranged from 93.3 to 100% and there were no unscheduled deaths during the study. No treatment-related clinical signs were noted in any dose group; there were no treatment-related effects on maternal body weights or food consumption throughout gestation. Water consumption from gd 6 to 15 showed a significant ($p < 0.01$) increase over control values in the 10 mL/kg bw/day dose groups and for gd 15-18 in the 5 mL/kg bw/day ($p < 0.05$) and 10.0 mL/kg bw/day dose groups ($p < 0.05$). Water consumption was also significantly higher in the high-dose group from gd 6 to 9 ($p < 0.05$) and gd 6 to 15 ($p < 0.01$) (See Table 3-2 for water consumption data on gd 0-6, 6-15, 15-18). There were no treatment-related necropsy findings in the dams; no effects on body weight, gravid uterine weight, corrected body weight, corrected weight change, or relative and absolute liver and kidney weights. No effect was noted on the number of corpora lutea, resorptions, dead fetuses, or sex ratio. The percentages of preimplantation loss and live fetuses were similar across treatment groups.

Table 3-1. Summary of Gestational Water Consumption (g/animal/day) (111)

Group	Doses (mL/kg bw/day) [mean ±SD]			
	0	0.5	5.0	10.0
Gd 0–6 (pre-treatment)	10.90±4.298	10.91±4.952	11.13±2.207	11.08±2.075
Gd 6–15 (treatment period)	12.83±3.675	12.14±2.167	13.80±2.253	15.35±2.925**
Gd 15–18 (post-treatment)	11.41±1.574	11.46±1.897	13.02±4.503*	13.06±2.149*

*p<0.05; **p<0.01

Fetal Parameters

A statistically significant (p<0.05) decrease (3%) in fetal body weight in the high-dose group was not considered biologically relevant due to the magnitude of the change and the lack of a dose-related trend. No differences were noted in malformations by category (external, visceral, skeletal) or in total malformations among all treatment groups. An increase in unossified cervical centra was noted in the high-dose group (p<0.05, 9/29 litters vs 2/28 in control group). However, this was not considered biologically relevant by the authors, as this finding was similar to historical control values in this laboratory. Significant increases in fetal atelectasis, poorly ossified supraoccipital bone, and a decrease in the extra ossification site in the nasal fontanel in the low-dose group were not considered to be biologically relevant due to the lack of a dose-effect relationship.

Statistical analyses used the pregnant dam or the litter as the unit of comparison. The authors of the Bushy Run Research Center Report noted that "the data for a quantitative continuous variables were inter-compared for the three treatment groups and the control group by use of Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. The t-tests were used when the F value from the ANOVA was significant. When Levene's test indicated equal variances, and the ANOVA was significant, a pooled t-test was used for pairwise comparisons. When Levene's test indicated heterogeneous variances, all groups were compared by an ANOVA for unequal variances followed, when necessary, by the separate variance t-test or pairwise comparisons. Nonparametric data were statistically evaluated using the Kruskal-Wallis test, followed by the Mann-Whitney U test when appropriate. Incidence data were compared using the Fisher's Exact Test. For all statistical tests, the probability value of <0.05 (two-tailed) was used as the critical level of significance."

The authors concluded that ". . . dosages up to 10.0 mL/kg bw/day were not associated with any treatment-related effects on endpoints such as clinical signs, body weight, body weight gain, food consumption, or pregnancy outcome. Increases in water consumption were observed in dams from the 5.0 and 10.0 mL/kg bw/day groups and were probably a physiologic response to the high dosages given by gavage. There was no evidence of treatment-related effects on developmental parameters. Therefore, in this study, the "no-observed-effect level" (NOEL) **[NOAEL[2]]** for maternal effects was 0.5 mL/kg bw/day. The NOEL **[NOAEL]** for developmental toxicity was at least 10.0 mL/kg bw/day."

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

Table 3-2. Summary of Developmental Toxicity Study of Propylene Glycol Given by Gavage to CD-1 Mice on GD 6–15 (111)

Effect	*Doses (mL/kg bw/day) [mean ±SD]*			
	0	***0.5***	***5.0***	***10.0***
Maternal corrected body weight change (body weight at sacrifice minus gravid uterine weight, g)	6.21 ± 1.87	6.46 ± 2.05	6.89 ± 1.57	6.67 ± 2.35
Maternal liver weight (% of corrected body weight)	7.585 ± 0.5711	7.595 ± 0.7563	7.540 ± 0.6111	7.681 ± 0.7679
Maternal kidney weight (% of corrected body weight)	1.217 ± 0.1047	1.202 ± 0.1248	1.202 ± 0.0838	1.261 ± 0.1235
% Live fetuses/litter	93.5 ± 8.08	91.5 ± 18.76	94.3 ± 6.73	89.6 ± 19.45
Fetal body weight/litter (g)	1.351 ± 0.0734	1.315 ± 0.0799	1.361 ± 0.0947	1.306 ± 0.0733*
Total # litters with live malformed fetuses/# examined	9/28	8/28	10/28	13/29

Dams were sacrificed on gd 18 and fetuses from 28–30 litters/group were evaluated for prenatal developmental toxicity.
* $p < 0.05$

Strengths/Weaknesses: The Driscoll et al. *(111)* study is GLP-compliant with adequate numbers of animals per group and follows a design that permits evaluation of dose-response relationships. The Panel concurs that developmental and maternal NOAELs were determined under the conditions used in the study.

Utility (Adequacy) for CERHR Evaluation Process: This study *(111)* is useful for risk extrapolation purposes, with the caveat that exposures were delivered as bolus doses by means of gavage administration, which is unlikely to mirror expected human exposure. The study design limits developmental toxicity conclusions to exposure during the prenatal period.

The FDA *(112)* sponsored a "Teratologic evaluation of FDA 71–56 (Propylene Glycol) in mice, rats, hamsters and rabbits." These prenatal studies were conducted under contract for FDA by the Food and Drug Research Laboratories, Inc. in East Orange, NJ. **[This NTIS available report does not give detailed experimental protocol information (such as chemical purity, stability, or dose analysis). Protocol details such as gross necropsy and examination of uterine contents methods are not given.]**

Mice

Timed-mated outbred CD-1 albino mice (25/group) were dosed by oral intubation with propylene glycol as a water solution from gd 6 to 15. Observation of the vaginal sperm plug occurred on gd 0. Dose groups were 0, 16, 74.3, 345, and 1,600 mg/kg bw/day. Aspirin at a dose of 150 mg/kg bw was used as a positive control. Body weights of the dams were recorded on gd 0, 6, 11, 15, and 17. Food consumption and clinical signs were also monitored **[stated in text, but data not reported]**. All but one pregnant dam in the 74.3 mg/kg bw/day dose group survived to term. **[No maternal deaths were reported in the other dose groups.]** On gd 17 all dams were anesthetized and a Cesarean section performed. There were no apparent treatment-related differences in the number of implantation sites, resorptions,

fetal body weight, and viability among the dose groups. All fetuses were examined for external abnormalities, 1/3 of the fetuses from each litter were Wilson sectioned for visceral examination, the remaining 2/3 of each litter were examined for skeletal defects by clearing the tissue with potassium hydroxide and staining the bone with Alizarin Red S dye. **[Cartilage was not stained.]**

The following conclusion was reported for mice by the study authors *(112)*:

> "The administration of up to 1,600 mg/kg body weight of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Results in mice are listed in Tables 3-3 and 3-4.

Table 3-3. Mouse Maternal and Fetal Toxicity Data for PG [no statistical analyses reported] (112)

	Sham	***Aspirin (mg/kg bw)***	***PG (mg/kg bw)***			
		150	***16.0***	***74.3***	***345.0***	***1,600.0***
Pregnancies						
Total #	22	23	22	22	20	23
Died/aborted (before gd 17)	0	0	0	1	0	0
To term (on gd 17)	22	23	22	21	20	23
Live Litters						
Total #	22	22	22	21	20	21
Implantation Sites						
Avg/dam	11.8	12.5	11.8	11.8	11.3	11.0
Resorptions						
% dams with partial resorptions	45.5	34.8	31.8	14.3	50.0	17.4
% dams with complete resorptions	–	4.35	–	–	–	4.35
Live Fetuses						
Avg/dam	10.4	11.5	11.4	11.4	10.5	10.2
Sex ratio (M/F)	0.78	0.74	0.80	0.79	0.86	0.86
Avg Fetus wt, in grams	0.90	0.84	0.88	0.90	0.91	0.96
Dead Fetuses						
% litters with dead fetuses	31.8	–	9.09	19.1	20.0	4.35
% litters with all dead fetuses	–	–	–	–	–	4.35

–: No data presented in FDRL report. The reason for the lack of data was not specified.

Table 3-4. Summary of Mouse Fetal Skeletal and Soft Tissue Findings for PG (112)*

	Sham	*Aspirin (mg/kg bw)*	*PG (mg/kg bw)*			
		150	*16.0*	*74.3*	*345.0*	*1,600.0*
Live Fetuses Examined	161/22	185/22	173/22	170/21	145/20	165/21
Sternebrae						
Incomplete ossification	66/16	34/15	62/18	75/16	39/11	28/12
Bipartite	–	9/7	2/2	–	6/4	3/3
Extra	–	–	3/2	–	–	–
Missing	22/10	26/11	14/7	11/7	33/10	13/6
Ribs						
Incomplete ossification	–	1/1	–	–	–	1/1
Fused/split	–	–	–	1/1	–	–
More than 13	37/13	41/18	30/16	34/16	24/13	38/18
Vertebrae						
Incomplete ossification	3/2	8/6	2/1	1/1	10/4	9/4
Skull						
Incomplete closure	3/3	–	–	–	–	1/1
Extremities						
Incomplete ossification	–	7/6	–	–	7/3	3/2
Other						
Hyoid, missing	23/10	37/15	37/12	20/11	35/13	17/10
Hyoid, reduced	19/10	11/7	19/11	27/13	27/11	16/12
Soft Tissue						
Gastroschisis	1/1	–	–	–	1/1	–
Meningo-encephalocele	–	1/1	–	–	–	–

* Number of fetuses affected/Number of litters affected

–: No data presented in FDRL report. The reason for the lack of data was not specified.

Rats

Timed-mated Wistar albino rats (25/group) were dosed by oral intubation with propylene glycol as a water solution from gd 6 to 15. Observation of the vaginal sperm plug was gd 0. Dose groups were 0, 16, 74.3, 345, and 1,600 mg/kg bw/day. Aspirin at a dose of 250 mg/kg bw was used as a positive control. Body weights of the dams were recorded on gd 0, 6, 11, 15, and 20. Food consumption and clinical signs were also monitored **[stated in text, but data not reported]**. All dams survived to term. On gd 20 all dams were anesthetized and a Cesarean section performed. There were no apparent treatment-related differences in the number of implantation sites, resorptions, fetal body weight, and viability among the dose groups. All fetuses were examined for external abnormalities, 1/3 of the fetuses from each litter were Wilson sectioned for visceral examination, the remaining 2/3 of each litter were examined for skeletal defects by clearing the tissue with potassium hydroxide and staining

the bone with Alizarin Red S dye. **[Cartilage was not stained.]**

The following conclusion was reported for rats by the study authors *(112)*:

> "The administration of up to 1600 mg/kg/ (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Results in rats are listed in Tables 3-5 and 3-6.

Table 3-5. Rat Maternal and Fetal Toxicity Data for PG [no statistical analyses reported] (112)

	Sham	***Aspirin (mg/kg bw)***	***PG (mg/kg bw)***			
		250	***16.0***	***74.3***	***345.0***	***1,600.0***
Pregnancies						
Total #	22	21	23	22	20	24
Died / aborted (before gd 20)	0	0	0	0	0	0
To term (on gd 20)	22	21	23	22	20	24
Live Litters						
Total #	22	20	23	22	20	24
Implantation Sites						
Avg/dam	11.4	10.7	11.2	11.1	12.3	10.7
Resorptions						
% dams with partial resorptions	18.2	42.9	17.4	4.55	10.0	–
% dams with complete resorptions	–	4.76	–	–	–	–
Live Fetuses						
Avg/dam	11.1	9.43	11.0	11.0	12.1	10.7
Sex ratio (M/F)	0.90	1.06	1.02	1.05	0.83	0.98
Avg fetus wt, in grams	3.39	2.68	3.91	3.73	3.91	3.75
Dead Fetuses						
Total	–	–	–	–	–	–

–: No data presented in FDRL report. The reason for the lack of data was not specified.

Table 3-6. Summary of Rat Fetal Skeletal and Soft Tissue Findings for PG (112)*

	Sham	Aspirin (mg/kg bw)	PG (mg/kg bw)			
		250	16.0	74.3	345.0	1,600.0
Live Fetuses Examined	173/22	137/20	179/23	169/22	167/20	180/24
Sternebrae						
Incomplete ossification	82/20	91/20	92/19	64/18	35/11	31/12
Bipartite	3/3	5/4	–	2/1	–	1/1
Missing	2/2	86/19	13/5	5/5	–	8/5
Ribs						
Incomplete ossification	–	1/1	–	–	–	–
Fused/split	–	1/1	–	–	–	–
Wavy	1/1	46/16	23/9	27/11	11/5	15/8
Less than 12	2/2	2/1	–	–	–	–
More than 13	7/3	91/19	3/1	1/1	6/4	3/3
Vertebrae						
Scoliosis	1/1	–	–	–	–	–
Incomplete ossification	–	101/19	1/1	13/7	3/3	18/9
Skull						
Incomplete closure	26/14	47/16	27/15	23/11	22/11	25/13
Missing	–	6/2	–	–	–	–
Extremities						
Incomplete ossification	–	3/1	–	–	–	–
Other						
Hyoid, missing	15/8	65/18	19/10	16/8	13/9	15/7
Hyoid, reduced	20/9	19/10	17/9	9/6	16/8	15/8
Soft Tissue						
Gastroschisis	–	1/1	–	–	–	–
Exophthalmos	–	2/1	–	–	–	–
Encephalo-myelocele	–	8/3	–	–	–	–
Meningo-encephalocele	–	4/2	–	–	–	–
Hydrocephalus	–	1/1	–	–	–	–

* Number of fetuses affected/Number of litters affected

–: No data presented in FDRL report. The reason for the lack of data was not specified.

Hamsters

Timed-mated outbred Golden hamsters (25/group) were dosed by oral intubation with propylene glycol from gd 6 to 10. Observation of motile sperm in the vaginal smear was gd 0. Dose groups were 0, 15.5, 72, 334.5, and 1,550 mg/kg bw/day. Aspirin at a dose of 250 mg/kg bw/day was used as

a positive control. On gd 14, a Cesarean section was performed. There were no apparent treatment-related differences in the number of implantation sites, resorptions, fetal body weight, or viability among the dose groups. All fetuses were examined for external abnormalities, 1/3 of the fetuses from each litter were Wilson sectioned for visceral examination, the remaining 2/3 of each litter were examined for skeletal defects by clearing the tissue with potassium hydroxide and staining the bone with Alizarin Red S dye. **[Cartilage was not stained.]**

The following conclusion was reported for hamsters by the study authors *(112)*:

> "The administration of up to 1550 mg/kg/ (body weight) of the test material to pregnant hamsters for 5 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Results in hamsters are listed in Tables 3-7 and 3-8.

Table 3-7. Hamster Maternal and Fetal Toxicity Data for PG [no statistical analyses reported] (112)

	Sham	*Aspirin (mg/kg bw)*	*PG (mg/kg bw)*			
		250	*15.5*	*72.0*	*334.4*	*1,550.0*
Pregnancies						
Total #	21	21	24	25	22	22
Died/aborted (before gd 14)	0	2	0	0	0	1
To term (on gd 14)	21	19	24	25	22	21
Live Litters						
Total #	21	19	24	25	22	21
Implantation Sites						
Avg/dam	14.3	15.2	13.8	13.8	14.2	13.7
Resorptions						
% dams with partial resorptions	4.76	21.1	12.5	20.0	4.55	28.6
% dams with complete resorptions	–	–	–	–	–	–
Live Fetuses						
Avg/dam	14.2	14.6	13.5	13.5	14.1	12.4
Sex ratio (M/F)	0.95	0.79	1.12	1.07	0.91	0.94
Avg fetus wt, in grams	1.74	1.78	1.79	1.80	1.84	1.79
Dead Fetuses						
% litters with dead fetuses	4.76	10.5	8.33	8.00	4.55	14.3
% litters with all dead fetuses	–	–	–	–	–	–

–: No data presented in FDRL report. The reason for the lack of data was not specified.

Table 3-8. Summary of Hamster Fetal Skeletal and Soft Tissue Findings for PG (112)*

	Sham	*Aspirin (mg/kg bw)*	*PG (mg/kg bw)*			
		250	*15.5*	*72.0*	*334.4*	*1,550.0*
Live Fetuses Examined	207/21	193/19	228/24	233/25	214/22	184/21
Sternebrae						
Incomplete ossification	67/18	167/19	51/17	58/19	63/16	57/15
Bipartite	23/14	26/14	23/15	15/10	30/15	17/11
Extra	1/1	1/1	1/1		1/1	6/4
Missing	37/13	45/15	47/17	20/11	24/10	27/12
Ribs						
Fused/split						1/1
More than 13	41/17	30/14	37/14	47/21	63/19	31/13
Vertebrae						
Scoliosis	1/1	–	–	–	–	–
Incomplete ossification	4/3	5/3	4/2	3/2	2/2	1/1
Skull						
Incomplete closure	–	2/2	–	–	–	–
Extremities						
Incomplete ossification	–	1/1	2/2	4/4	3/2	1/1
Other						
Hyoid, missing	4/4	2/2	5/5	2/2	1/1	–
Hyoid, reduced	9/6	25/10	7/5	1/1	5/3	–
Soft Tissue						
Hydrocephalus	1/1	–	–	–	–	–
Atelocardia		1/1	–	–	–	–
Fetal monster	–	–	–	1/1	–	–
Umbilical Hernia	2/2	–	–	–	–	–
Dephallia	1/1	–	–	–	–	–
Meningo-encephalocele	–	–	2/1	1/1	–	–

* Number of fetuses affected/Number of litters affected
–: No data presented in FDRL report. The reason for the lack of data was not specified.

Rabbits

Dutch-belted female rabbits were dosed by oral intubation with propylene glycol from gd 6 to 18. Dose groups were 0, 12.3, 57.1, 267, and 1,230 mg/kg bw/day. 6-Aminonicotinamide (2.5 mg/kg) dosed on gd 9 was a positive control. On gd 0, each doe received an injection of human chorionic gonadotropin (400 IU) and 3 hours later was artificially inseminated with diluted donor buck semen. On gd 29 a Cesarean section was performed. There were no apparent treatment-related differences in the number of corpora

lutea, implantation sites, resorptions, fetal body weight, and viability among dose groups. All fetuses were examined for external abnormalities. The live fetuses from each litter were placed in an incubator for 24 hours for evaluation of neonatal survival. All surviving pups were sacrificed at the end of that time and examined by dissection for visceral abnormalities. All fetuses were cleared with potassium hydroxide and stained with Alizarin Red S dye and examined for skeletal defects. **[Cartilage was not stained.]**

The following conclusion was reported for rabbits by the study authors *(112)*:

> "The administration of up to 1230 mg/kg/ (body weight) of the test material to pregnant rats **[sic]** for 13 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Results in rabbits are listed in Tables 3-9 and 3-10.

Table 3-9. Rabbit Maternal and Fetal Toxicity Data [no statistical analyses reported] (112)

	Sham	*6 AN* (mg/kg bw)*	*PG (mg/kg bw)*			
		2.5	*12.3*	*57.1*	*267.0*	*1,230.0*
Pregnancies						
Total #	11	10	11	12	14	13
Died/ aborted (before gd 29)	0	0	2	1	2	0
To term (on gd 29)	11	10	9	11	12	13
Corpora Lutea						
Total #	156	176	182	190	198	199
Avg/dam	11.1	11.7	10.1	13.6	10.4	13.3
Live Litters						
Total #	11	10	9	11	12	13
Implantation Sites						
Avg/dam	6.36	6.90	7.67	6.36	5.25	7.54
Resorptions						
% dams with partial resorptions	45.5	50.0	22.2	45.5	16.7	15.4
% dams with complete resorptions	–	–	–	–	–	–
Live Fetuses						
Avg/dam	5.91	5.00	7.33	5.00	5.08	7.31
Sex ratio (M/F)	0.81	0.79	1.13	1.29	0.69	1.11
Avg fetus wt, in grams	42.3	32.5	36.4	39.9	42.9	39.0
Dead Fetuses						
Total	–	–	–	–	–	–

* 6-Aminonicotinamide, positive control

–: No data presented in FDRL report. The reason for the lack of data was not specified.

*Table 3-10. Summary of Rabbit Fetal Skeletal and Soft Tissue Findings for PG** (112)*

	Sham	*6 AN* (mg/kg bw)*	*PG (mg/kg bw)*			
		2.5	*12.3*	*57.1*	*267.0*	*1,230.0*
Live Fetuses Examined	65/11	50/10	66/9	55/11	61/12	95/13
Sternebrae						
Incomplete ossification	1/1	5/2	1/1	–	2/2	10/6
Bipartite	–	–	1/1	–	2/2	–
Fused	–	6/36	–	–	–	–
Extra	1/1	1/1	2/2	3/3	1/1	
Missing	–	3/2	1/1	–	–	11/3
Ribs						
Incomplete ossification	–	–	–	–	–	–
Fused/split	–	14/7	–	–	–	–
Vertebrae						
Fused	–	1/1	–	–	–	–
Scoliosis	–	10/4	–	–	–	–
Tail Defects	–	48/9	–	–	–	–
Scrambled	–	22/6	–	–	–	–
Soft Tissue						
Anopia, short tail	–	1/1	–	–	–	–
Encephalocele	–	7/1	–	–	–	–
Med Rotation of Hindlimbs	–	17/6	–	–	–	–
Umbilical Hernia	–	1/1	–	–	–	–
Scoliosis	–	1/1	–	–	–	–
Harelip	–	2/2	–	–	–	–

* 6-Aminonicotinamide, positive control

** Number of fetuses affected/Number of litters affected

–: No data presented in FDRL report. The reason for the lack of data was not specified.

Based upon the conclusions of the study authors, the NOAELs for maternal and fetal toxicity of propylene glycol were at the highest dose tested are summarized in Table 3-11.

Table 3-11. NOAEL Levels for Maternal and Fetal Toxicity of PG (summarized from (112))

Species	*NOAEL (mg/kg bw/day) (at highest dose tested)*
Mice	≥1,600
Rats	≥1,600
Hamsters	≥1,550
Rabbits	≥1,230

Strengths/Weaknesses: In general, adequate numbers of animals (25 dams per treatment group) were employed in these studies *(112*). In most cases, average and percent summaries were provided without associated standard errors, which prevented an assessment of the statistical significance of differences reported. Differences between the negative control and dose groups were small and not likely to be statistically different, but there were a few cases where formal analysis would have been helpful. The report provided detailed information only on fetal weights and resorptions but no corresponding information on malformations, nor was detailed information on maternal body weights over the course of the study presented. No historical control data were presented to allow assessment of the importance of observance of specific malformations. A variety of endpoints were assessed, including both maternal and fetal endpoints. Multiple doses of test compound were used in each species, so dose-response relationships could be assessed.

Aspirin was used as the positive control treatment for mice, rats, and hamsters and 6-aminonicotinamide was used for rabbits. Results indicate that aspirin is only mildly teratogenic for mice and hamsters but is strongly teratogenic for rats. 6-aminonicotinamide is clearly teratogenic for rabbits. The use of a weak positive control makes clear conclusions for mice and hamsters more difficult.

The major limitation in the study is in the presentation. Very few experimental details were presented, and it is not clear if any formal statistical analysis was performed. For example, the rationale for the selection of the positive control and the doses used is not given. The sequence for necropsy of the dose groups is not known. Whether the necropsy was done on an entire dose group within the same time period or over the entire necropsy period can affect the findings of minor developmental delay (such as delayed ossification and wavy ribs). Such findings can be apparent in the first groups sacrificed, but not as apparent in later groups. Detailed necropsy information such as the number of unossified vertebrae is not reported. In some cases, it is not possible to reconstruct litter incidences of effects from the data presented. The same endpoints were not collected across all species; for example, the number of corpora lutea were apparently only recorded for rabbits and not for the other three species. While the average numbers of implantation sites across test groups in mice, rats, and hamsters suggest that propylene glycol did not have a large impact on pre-implantation loss, it would have increased confidence in the data if corpora lutea had also been counted.

At the highest dose tested, propylene glycol did not seem to affect mice or hamsters in the parameters examined (maternal weight, number of implants per litter, fetal weight, death and resorptions, and malformations). A large number of malformations were observed in mice across all treatments, including positive and negative controls, causing concern about the validity of the whole study. Similar concerns are not present for hamsters. With rats, higher numbers of wavy ribs and incomplete ossification of the vertebrae were observed at the same level as the positive control, suggesting a propylene glycol effect. The incidences of these defects did not appear to be dose-related. No propylene glycol effects were observed in rabbits.

Utility (Adequacy) for CERHR Evaluation Process: These data *(112*) would appear to be of limited use for the CERHR evaluative process. The lack of detail presented in the report as well as the lack of statistical analysis makes it difficult to form solid conclusions. The lack of formal statistical analysis suggests that these data might be more useful to help confirm results demonstrated in other studies. In two of the four studies, the choice of the positive control compound does not appear to be

appropriate. Generally, propylene glycol did not appear to have had major adverse effects in any of the four species tested and, when effects were present, they did not appear to be dose-responsive. The study suggests that the NOAEL level for mice, hamsters, and rabbits is at least 1,600, 1,550, 1,230 mg/kg bw/day, respectively; the levels are given in Table 3-11. The appropriateness of the NOAEL level for rats (1,600 mg/kg bw/day) given in Table 3-11 depends on the importance attributed to the rib and vertebrae malformations observed. The general lack of effect gives some measure of comfort, but important observations may not have been made. The Panel judges the data in this report insufficient to predict human health effects.

Kavlock et al. *(113*) employed an *in vivo* teratology screening procedure to evaluate propylene glycol along with 45 other chemicals. Timed-pregnant CD-1 mice (approx. 60-days-old) were dosed with propylene glycol in water **[% purity not stated]** by oral gavage on gd 8–12 at a dose of 10,000 mg/kg bw/day. In this assay, pregnant females were dosed at a level predicted to induce a mild degree of maternal toxicity or at a level stated in the literature to be teratogenic. In the propylene glycol experimental block, a control group was dosed with water (40 mice) and groups of 30 mice were exposed to propylene glycol or another substance (sucrose). Maternal toxicity endpoints examined were number pregnant, mortality, and number of animals with resorptions. For fetal toxicity, the number of live pups and their weights on pnd 1 and 3 were recorded. Data analysis was performed using the General Linear Models procedure on SAS. When a significant effect of treatment was detected by ANOVA analysis, individual group means were compared with a Student's t-test on least-squares means.

For propylene glycol, maternal and fetal parameters were not significantly different from values of control animals. Out of 30 animals dosed with propylene glycol, 83% were pregnant; no dams died and there were no resorptions. For 40 control animals dosed with vehicle, 68% were pregnant; no dams died and there were no resorptions. Neonatal values for pup survival and weight are included in Table 3-12.

Table 3-12. Pup Survival and Weight after Treatment of Pregnant CD-1 Mice by Gavage with Propylene Glycol (10 g/kg bw/day) from gd 8 to 12 (113)

Compound	*PND 1*		*PND 3*	
	# live	*wt (g)*	*# live*	*wt (g)*
Control (water)	*10.08 ± 0.46	1.59 ± 0.02	10.00 ± 0.45	1.88 ± 0.04
Propylene Glycol (in water)	10.60 ± 0.44	1.53 ± 0.03	10.52 ± 0.44	1.84 ± 0.03

*mean ± standard error of the mean

Strengths/Weaknesses: An adequate number of mice were used in this study *(113*) in the group exposed to propylene glycol. Only a single dose of propylene glycol was used, and the endpoints evaluated and the dosing period used are not those commonly evaluated in a comprehensive developmental toxicity study.

Utility (Adequacy) for CERHR Evaluation Process: These data appear to be of limited value for the CERHR evaluative process. A high dose of propylene glycol was used with no apparent adverse effects on the offspring, which is reassuring. However, the lack of a dose-response, as well as the differences in measured endpoints, make these data less convincing.

3.2.2 Injection

3.2.2.1 Prenatal toxicity

Chick eggs

In an early study by Gebhardt *(114)*, propylene glycol was found to be teratogenic when injected into chick eggs. Eggs (avg wt 59 g) from White Leghorn chickens were used in this study. Propylene glycol **[0.05 mL, >99% purity]** was injected into the air chamber or yolk sac of the egg. Control eggs had the same size needle inserted into the egg for 2 seconds, but were not injected. Eggs (18–30) were injected on one of incubation days 0 through 7 with either propylene glycol or sham treatment. Eggs were rotated hourly and incubated at 38°C and 55% relative humidity. Candling was done on the fourth and sixth days of incubation and all unfertilized eggs and eggs with dead embryos were recorded and removed. Gross morphology was studied on the 15th day of egg incubation by clearing the skeleton and staining with Alizarin Red S. **[Statistical methods were not reported.]** The number of embryos that died within the first 15 days of development were recorded and malformations in the surviving embryos were determined. The authors noted that the embryos were most sensitive to propylene glycol injection into the air chamber on day 4 of development, when 90% of the embryos died within 2 hours and 20% of the surviving embryos had asymmetric malformations of the limbs **[time/percent mortality graph provided, no other data provided]**. In a second experiment, propylene glycol or propylene glycol diluted 1:1 and 1:2 in water was injected into the air chamber of day 4 chick embryos **[see Table 3-13 below, controls were not described by the authors]**. The authors speculated that the apparent toxic effect of propylene glycol on day 4 may be due to disruption of the embryo vasculature.

Table 3-13. Teratogenic Effect of Propylene Glycol Injected into the Air Chamber of 4-Day-Old Chick Embryos (114)

Dilution	*# of Eggs*	*% Mortality*	*% Malformed Surviving Embryos*
Undiluted	227	90	21
Diluted 1:1	165	82	27
Diluted 1:2	144	57	8

Strengths/Weaknesses: The study *(114)* was performed in a non-mammalian species. An adequate number of embryos were evaluated in each group.

Utility (Adequacy) for CERHR Evaluation Process: These data appear to be of little use in the CERHR evaluative process. Experiments performed in chick embryos are not relevant to assessing risks to humans. Additionally, the data in this study conflict with those reported by Landauer and Salam *(115)*, further weakening their relevance.

Propylene glycol and dimethyl sulfoxide were compared with water as solvents for teratogens in chick embryos *(115)*. Chick embryos (White Leghorn chicken eggs) were injected (0.2 mL) into the yolk sac with teratogen on day 4 of incubation and fetuses examined on day 19. Teratogens tested were: bidrin, 6-aminonicotinamide, 3-acetylpyridine, sulfanilamide, 3-amino-1,2,4 triazole, physostigmine sulfate, and nicotine sulfate. The authors found less teratogenicity of known human teratogens when

the solvent was either dimethyl sulfoxide or propylene glycol as compared to water. Although the data for solvent injection alone are not presented in this paper, the authors stated that they did not find propylene glycol toxic to day 4 chick embryos as Gebhardt *(114*) previously reported.

Strengths/Weaknesses: The study *(114*) was performed in a non-mammalian species.

Utility (Adequacy) for CERHR Evaluation Process: These data appear to be of little use in the CERHR evaluative process. Experiments performed in chick embryos are not relevant to assessing risks to humans.

3.2.3 Mechanistic and In Vitro Studies

3.2.3.1 Embryo culture

Kowalczyk et al. *(116*) examined by *in vitro* culture the effects of propylene glycol, glycerol, and several alcohols on mouse preimplantation development. Random-bred mice (Harlan Sprague-Dawley) were superovulated (5 IU PMSG IP followed in 48 hours with 5 IU HCG) and paired with B6SJL/J males. Female mice were sacrificed on gd 2 (day of vaginal plug = gd 1) for collection of two-cell embryos or on gd 3 for collection of eight-cell morulae. Oviducts were flushed with M2 medium and embryos were cultured in Ham's F-10 media. Embryos at the two-cell stage were washed 3x in Ham's F-10 and cultured 24 hours in medium containing 6−131 mM propylene glycol (0, 0.05, 0.1, 0.2, or 1.0%). Embryos were then washed in Hams F-10 (three times) and cultured in propylene glycol-free medium for 5 days to observe development to the blastocyst stage. Embryos collected at the morulae stage were exposed to propylene glycol for 24 hours. The percentage of embryos cavitating and the blastocoel volume was recorded at 0, 4, 8, and 24 hours after removal of the propylene glycol from the medium. All experiments were repeated at least 3 times (43 embryos/treatment group, avg.). Differences in the control and treatment groups were tested for significance ($p < 0.01$) using Chi-Squared analysis. Embryos exposed to propylene glycol or glycerol exhibited development to the blastocyst stage that was comparable with controls. Morulae cultured 24 hours in medium with 0, 0.05, 0.1, 0.2, or 1.0% propylene glycol or glycerol cavitated at a rate that was comparable with stage-matched controls **[data not shown]**. Blastocoel volume expansion was unaffected **[method referenced, but not described]**. The authors concluded that the progression of preimplantation embryo development to the blastocyst stage is not affected by propylene glycol at 0.05, 0.1, 0.2, or 1.0%. The authors found that ethanol stimulated embryo development and cavitation whereas the other alcohols tested (methanol, 2-propanol, 1-propanol, and 1-butanol) were toxic to blastocyst formation.

Strengths/Weaknesses: Several doses of propylene glycol were tested for their effects on blastocyst formation and cavitation rate using a mammalian species.

Utility (Adequacy) for CERHR Evaluation Process: Although reassuring in that the doses of propylene glycol used in the study had little effect on murine preimplantation development, these data appear to be of little use in the CERHR evaluative process.

3.2.3.2 Cryoprotectant

Propylene glycol is a permeating cryoprotectant used to depress the temperature at which intracellular ice forms and to stabilize the plasma membrane. It is routinely used as a cryoprotectant in the cryo-

preservation of human oocytes. In an effort to optimize cryopreservation of oocytes, a number of studies examined methods to improve cryopreservation techniques *(117-124*) **[not reviewed in this report]**.

Studies by Damien et al. *(125*) evaluated the usage of propylene glycol with faster ultra rapid embryo freezing protocols. The purpose of this study was to identify the maximal concentration of propylene glycol and sucrose that will not adversely alter the development of the mouse pronuclear stage embryo and to determine the mechanism by which propylene glycol mediates embryotoxicity. Pronuclear mouse zygotes from superovulated B6D2Fi mice were evaluated. Each series of experiments was replicated 3–5 times. In both the control and 1.5 M propylene glycol-treated group, 78% of the zygotes developed into 2-cell embryos. With 3 M propylene glycol, 7% of the zygotes developed into 2-cell embryos (ANOVA, $P < 0.05$). The zygotes were observed over a 20-minute period at 22°C under phase optics. In a second series of experiments, pronuclear mouse zygotes were incubated in either fluorescein diacetate or Acridine Orange and then transferred to either phosphate buffered saline or propylene glycol in water **[% purity not reported]**. Fluorescence is maintained as long as the cell membrane is not damaged and was retained in 98% of the zygotes exposed to 1.5 M propylene glycol, 81% (Chi-Squared test, $P < 0.05$) exposed to 3.0 M propylene glycol, and 5% (Chi-Squared test, $P < 0.05$) exposed to 6.0 M propylene glycol (Table 3-14). A shift in fluorescent wavelength at 3.0 M propylene glycol also indicated that the pH of the embryos had decreased. The authors concluded that a 20-minute exposure to 1.5 M propylene glycol did not affect embryonic development, while concentrations greater than or equal to 3.0 M inhibited embryonic development through cell membrane damage and pH changes.

Table 3-14. The Effect of a 20-Minute Exposure of Propylene Glycol (PG) on the Percentage of Zygotes Showing Fluorescein Diacetate and Acridine Orange Fluorescence (125)

DYE	*0M PG*	*1.5M PG*	*3.0M PG*	*6.0M PG*
Fluorescein diacetate	100(52)	98(50)	81(53)*	5(64)*
Acridine orange	95(56)	95(40)	7(46)*	0(32)*

The total number of embryos is given in parentheses.
*The percentage of embryos that maintained fluorescence was significantly reduced ($P < 0.05$).

Strengths/Weaknesses: The strengths of this study *(125*) are that 3 or 4 concentrations of propylene glycol covering a 12-fold concentration range were used *in vitro* to determine the effect of this compound on early embryonic development. Also, the authors carefully monitored volume changes and fluorescence. However, various concentrations of sucrose were also added to the zygotes. To determine the percentage of zygotes that developed into 2-cell embryos, the number of zygotes were pooled across sucrose concentrations, thereby ignoring any effect of sucrose. In addition, widely different numbers of zygotes were used in the experiments, from 66 in the 1.5 M propylene glycol group to 155 in the 0 M propylene glycol group.

Utility (Adequacy) for CERHR Evaluation Process: These data are of little use to the Panel in the CERHR evaluative process.

3.2.3.3 Hydra Screening Assay

In an evaluation of the utility of the hydra prescreening developmental assay to predict experimental

findings in laboratory animals *(126)*, propylene glycol was one of 14 glycols and glycol ethers evaluated and compared to published animal data. Adult polyps of *Hydra attenuata* are grown under conditions **[not specified]** such that they will reproduce by asexual budding. For each assay, approximately 700–1,000 adult hydra are dissociated mechanically into component cells and randomly re-associated into small pellets (~20 pellets) by gentle centrifugation. After 92 hours of incubation **[conditions not specified]**, approximately 10–20 adult hydra will form from each pellet and form free-standing polyps. By incubation of adult hydra or pellets in the presence of test chemical at log increment dilutions, the minimum effective concentrations (MEC) of the test substance capable of producing adult (A) and developmental (D) toxicity can be determined. The A/D ratio will increase in size as embryo toxicity increases over adult toxicity **[controls or further experimental details were not reported, no statistical methods were reported]**. The A/D ratio reported by the authors for propylene glycol was 1.3. **[Results for propylene glycol and how toxicity detected or measured were not discussed by the authors.]** Although no animal data or rank order are given, the authors conclude, "The results of these hydra assays of glycols and glycol ethers typify results to be expected in mammals" (Table 3-15).

Table 3-15. Developmental Toxicity of Glycols and Glycol Ethers in Hydra (126)

Test Chemical	*A=MEC (adult) mL/L*	*D=MEC('embryo') mL/L*	*A/D*
Ethylene glycol (EG)	50	30	1.7
Propylene glycol	40	30	1.3
Hexylene glycol	20	6	3.3
EG monomethyl ether	40	30	1.3
EG monoethyl ether	30	6	5
EG monobutyl ether	4	0.9	4.4
EG monophenyl ether	1	0.3	3.3
EG monomethyl ether monoacetate	0.7	0.7	1.0
EG monoethyl ether monoacetate	0.6	0.6	1.0
EG diacetate	0.2	0.2	1.0
Diethylene glycol	30	30	1.0
Diethylene glycol monomethyl ether	30	20	1.5
Diethylene glycol dibutyl ether	0.9	0.4	2.2

Strengths/Weaknesses: A major weakness in the Johnson et al. *(126)* study is the use of an invertebrate animal.

Utility (Adequacy) for CERHR Evaluation Process: These data *(126)* are of little use in the CERHR evaluative process. These experiments were performed in artificial 'embryos' created from dissociated marine invertebrates. Data from this assay are not relevant to assessing risks to humans.

3.3 Utility of Data

No human data on developmental toxicity in humans were identified. Based upon the Driscoll et al. *(111)* study, the Panel concluded that the available data are sufficient to evaluate prenatal developmental

toxicity by the oral route in mice. Although data for other experimental animal species by the same route are inadequately presented, the finding of a lack of an effect is consistent with results of the Driscoll study. There were no acceptable postnatal developmental studies identified by the Panel. However, the lack of an effect on postnatal survival and reproductive performance after oral exposure of the pregnant mouse was determined for propylene glycol in a continuous breeding study addressed in Section 4.2 *(127)*. These data are sufficient to assess prenatal, but insufficient to assess postnatal, developmental toxicity in humans.

3.4 Summary

3.4.1 Human Data

No human data on developmental toxicity were identified.

3.4.2 Experimental Animal Data

A prenatal developmental toxicity study was conducted in mice orally exposed to propylene glycol at the Bushy Run Research Center *(111)*. Under the conditions used in this study, which was performed in compliance with EPA GLP regulations, a developmental toxicity NOEL **[NOAEL[3]]** for fetuses of 10.0 g/kg bw/day was reported; this was the highest dose tested. The maternal NOEL **[NOAEL]** was 0.50 g/kg bw/day based on increases in water consumption observed at 5.0 and 10.0 g/kg bw/day. It is reasonable to speculate that this effect was a physiological response to the high doses of propylene glycol administered.

Prenatal developmental toxicity studies were conducted in mice, rats, hamsters, and rabbits orally exposed to propylene glycol at the Food & Drug Research Laboratories, Inc. *(112)* under contract for the FDA. NOAEL levels determined for maternal and fetal toxicity were at the maximum doses used: 1.6 g/kg bw/day for rats and mice, 1.55 g/kg bw/day for hamsters, and 1.23 g/kg bw/day for rabbits. Propylene glycol did not appear to have any major adverse effects in any of the four species tested. Unfortunately, detailed information on study design is not presented in this report, and no statistical information is presented. Although propylene glycol is apparently without detrimental effect to the fetus, the Panel concluded that these data, as presented, are inadequate to be used as the sole study to interpret developmental toxicity.

Propylene glycol was also tested in a CD-1 mouse screening assay by Kavlock et al. *(113)*. Timed-pregnant CD-1 mice were dosed with propylene glycol in water by oral gavage on gd 8–12 at a dose of 10 g/kg bw/day. Endpoints examined were number of dams pregnant, mortality, and the number of dams with resorptions; the number of live pups and their weights on pnd 1 and pnd 3 were recorded. No significant adverse effects were noted for the maternal and fetal parameters evaluated. The Panel concluded that although an adequate number of animals were used, the endpoints evaluated and the dosing period used were not adequate for a comprehensive developmental toxicity study.

The Panel concluded that the available data are sufficient to evaluate the developmental toxicity of propylene glycol in mice. Data from the Driscoll et al. *(111)* study indicate that oral prenatal exposure

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

to propylene glycol is not a developmental toxicant at doses of up to 10 g/kg bw/day in mice. Data in several other species, while inadequately presented, are consistent with the findings in the mouse. The only data available to evaluate postnatal effects of propylene glycol are those from the continuous breeding study in mice conducted for an assessment of possible fertility effects *(128*). When mice were exposed throughout gestation and lactation and to 34 weeks of age with estimated doses as high as 10 g/kg bw/day, no adverse effects were observed on fertility indices. These data suggest that development was not significantly impaired.

4.0 REPRODUCTIVE TOXICITY DATA

4.1 Human Data

No human reproductive toxicity studies were identified.

4.2 Experimental Animal Data

An early report examined the toxicology and reproductive performance of rats **[strain not specified]** fed propylene glycol **[purity not specified]** or glycerol in the diet *(129)*. Minimal experimental information is reported. However, some data are provided from this multigeneration reproductive study in which the animal diets were formulated so that an isocaloric amount of propylene glycol (from 0–30% (w/w)) replaced cornstarch in the feed. Animals were monitored and continued on the diet through three successive generations. Animals were fed *ad libitum* and body weights were measured weekly. Six dose groups and one control group (three males and six females per group) were used. Two females were housed with one male **[length of time not reported]**; a weekly record was made of the average amount of diet consumed. At 70–80 days of age, females were monitored for pregnancy and removed to individual cages before litter delivery. The number, date, and average weight of the young were recorded. Less thrifty pups were culled if the number exceeded six pups per litter. Litters were weighed at weekly intervals until weaning. Three males and six females were chosen per dose group from the first litter animals and retained on the same diet. The study was continued through three successive generations. The authors provide a summary table of "Composite responses of three generations of female rats produced on each of several diets" **[food consumption data are not provided]** (see Table 4-1).

Table 4-1. Composite Responses of Three Generations of Female Rats Produced on PG in the Diet (129)

% PG (w/w)	*# females*	*# females with litters*	*# litters born*	*# pups born*	*Avg wt of pups (g)*	*# of pups weaned*	*Avg # of pups/ dam*	*Avg # of pups/ litter*
0	36	36	91	689	6.0	422	19.1	7.4
2.5	18	16	38	260	5.5	147	16.3	6.8
5.0	18	18	40	315	5.4	193	17.5	7.8
7.5	18	18	40	229	5.8	144	12.7	5.7
10.0	18	16	46	280	5.8	158	17.5	6.1
20.0	18	16	38	204	6.0	120	12.7	5.4
30.0	18	9	18	113	6.0	77	12.5	6.3

This data table shows that the percentage of females reproducing ranged from 88–100% for the 0-20% propylene glycol dose groups and 50% for the 30% propylene glycol dose group, and the average number of young born per litter ranged from 5.4 to 7.8 pups for the 0–30% propylene glycol dose groups. The authors noted that in the 30% propylene glycol dose group, 18 females had 11 litters born from the first generation females, 6 litters from the second, and one litter from the third generation, and that "Rats receiving the 30% propylene glycol diet failed to produce the third generation of

young." The authors conclude that "In view of the limited data available, it is difficult to state with any degree of certainty what effect the composition of the diet had on the ability of the females to reproduce." **[This report does not identify the specific statistical methods used.]**

Following this study, some of the progeny from the third generation (9 males and 18 females each from the 10 and 20% propylene glycol dose groups) were continued through three additional generations. The animals from each of these groups were subdivided into three subgroups containing three males and six females. The animals of one subgroup were continued on the original diet of either 10 or 20% propylene glycol; the animals of the second subgroup were changed to control diet (0% propylene glycol); the animals of the third subgroup were changed to a corresponding dose of glycerol. Data reported for subgroups one and two are presented below (see Table 4-2).

Table 4-2. Composite Responses of Three Generations of Female Rats Produced on PG in the Diet (129)

%PG[a]/%PG[b]	*# females*	*# females with litters*	*# litters born*	*# pups born*	*Avg wt of pups (g)*	*# of pups weaned*	*Avg # of pups/dam*	*Avg # of pups/litter*
10/0	14	14	32	226	5.5	158	16.1	7.1
10/10	16	16	35	223	5.4	158	14.0	6.4
20/0	18	18	39	361	5.6	197	20.6	9.3
20/20	18	18	35	237	5.6	154	13.2	6.8

[a] Previously fed diet for three generations
[b] Diet during three-generation test period

These data show that the percentage of females reproducing was 100% for the 0–20% propylene glycol dose groups and the average number of young born per litter ranged from 6.4 to 9.3 pups for the 0–20% propylene glycol dose groups. **[The authors did not comment on these data and failed to provide information on their statistical analyses.]**

Strengths/Weaknesses: The rat study cited above *(86, 129*) was conducted more than 50 years ago, prior to GLP. Many experimental details (e.g., animal strain, statistics, and even some reproductive data) were not provided.

Utility (Adequacy) for CERHR Evaluation Process: Since many experimental details are not provided, this study *(129*) is not useful in assessing reproductive hazard.

There has been one other multigeneration reproductive study on propylene glycol *(127*).

NTP tested propylene glycol for reproductive/developmental toxicity in conjunction with testing of glycol ethers in order to examine structure-activity correlations. Using the reproductive assessment by continuous breeding (RACB) protocol, Lamb *(127*) investigated the reproductive function of male and female mice (COBS crl:CD-1 (ICR) BR outbred albino) exposed to propylene glycol in drinking water. A quality assurance audit was done on all study records. Propylene glycol (>99% purity) was chemically characterized. Stability studies and mixing studies were performed; aliquots

of all formulations were analyzed. Concentrations were within 5% of the nominal value. Standard statistical analyses were done on the reproductive and fertility data. Statistical significance was at the P = 0.05 level. Reproductive data were evaluated by the Cochran-Armitage test for dose related trends in fertility and mating indices; pairwise comparisons between the control and dose groups were made using Fisher's Exact test. Pup and litter data were evaluated by the Kruskal-Wallis test and Jonckheere's test. Pairwise comparisons were made with Wilcoxon's rank-sum test. All analyses were performed on males, females, and both sexes combined; to remove any potential effect of number of pups in litter on pup weight, an analysis of covariance was performed.

A dose range-finding study (Task 1) was done with mice exposed to propylene glycol in drinking water for 14 days. Dose groups (8 male and 8 female mice/group; 2 mice of the same sex housed per cage) were 0, 0.5, 1.0, 2.5, 5.0, and 10.0% (w/v) propylene glycol. During the testing period, there was no mortality in any of the dose groups. However, in the high-dose group, males and females gained weight over control animals (2 and 7% heavier, respectively) and animals in the 10% dose group drank more water than the control group (60% more for males and 58% more for females). **[Food consumption not reported; caloric intake among dose groups not standardized.]**

Task 2 is designed to determine the effect of the chemical on fertility and reproduction. Animals were exposed to propylene glycol (> 99% purity) in drinking water for a total of 18 weeks: one week prior to cohabitation, 14 weeks during cohabitation, and 3 weeks after cohabitation. A vehicle control group (40 males/40 females) and 3 dose groups of 20 males and 20 females per dose group were used. Based upon the results of Task 1, Task 2 drinking water concentrations were set at 0, 1, 2.5, 5% (w/v) propylene glycol. Chemical consumption estimates in this study were 0, 1.82, 4.80, and 10.1 g/kg bw/day for each of the respective dose groups; body weights of F_0 parents were monitored on study days 0, 7, 28, 56, 84, and 112. Live litters born during the cohabitation phase were weighed, sexed, and examined for external abnormalities and then sacrificed. Approximate delivery time and number of dead and cannibalized pups were noted. Offspring from the last litter (5^{th} litter) of the control and high-dose groups were allowed to mature and reproductive performance was evaluated (Task 4). During the cohabitation phase, no chemical-related deaths and no significant chemical-related clinical signs of toxicity were noted. Propylene glycol had no significant effect on any of the following reproductive parameters in F_0 animals: number of litters per pair, number of live pups per litter, sex ratio, pup weights, number of days to litter, and dam weights at delivery. F_0 parents were not necropsied.

F_1 pup survival and body weights through pnd 14 were monitored in the control (34/39 litters/breeding pairs) and the high dose groups (19/20 litters/breeding pairs) from the final litter (5^{th} litter). Propylene glycol had no effect on F_1 pup survival or body weight gain **[note that dams were still being exposed to propylene glycol from the drinking water during the preweaning period].**

A Task 3 crossover study is done to determine the affected sex. Since there was no effect of propylene glycol on fertility, this study was not conducted.

Task 4 was designed to evaluate the reproductive performance of the last litter (5^{th} litter) from Task 2. F_1 males and females (20 each/dose group) were randomly selected from the control and high-dose groups (5% propylene glycol in drinking water) in Task 2 and mated on pnd 64–84 to animals from

the same dose group. Breeding pairs were separated after 7 days of cohabitation or after detection of a copulatory plug; the male and female were then housed singly. F_1 animals were weighed at weaning, first day of cohabitation, and then weekly. Water consumption was monitored weekly starting the first week after cohabitation. The high-dose group animals received exposure to propylene glycol throughout Task 2: from their dosed dam and then continuous exposure from drinking water (author-estimated daily dose of propylene glycol, 14.4 g/kg bw/day). There were no differences between the control and high-dose groups with respect to body weights or water consumption. The mating index for control and treated groups was 85%; the fertility index was 75% for control and 80% treated groups (nonsignificant). There were no significant differences in F_2 litter size, number of live pups, sex ratio, or pup weights. After delivery of the F_2 pups, the F_1 adults were necropsied. Sperm morphology and vaginal cytology evaluations **[on females that did not have pups]** were conducted. There were no significant differences in body or kidney and liver weights or serum calcium concentrations (both sexes). In males, there were no significant differences in the average weights of seminal vesicles, right cauda, prostate, right testis, and right epididymis. Sperm motility, sperm counts, or incidence of abnormal sperm did not significantly differ from control animals. In females, there was no difference in estrual cyclicity when compared to control animals. No organs were examined histologically. **[Note that for Task 2 and Task 4 food consumption not reported; caloric intake among dose groups was not standardized.]**

From the NTP studies, the authors concluded that propylene glycol administered in the drinking water at up to the 5.0% dose level had "no effect on the fertility and reproduction in adult or second generation CD-1 mice. Furthermore, there was no apparent effect with respect to body and organ weights (both absolute and adjusted), sperm motility, sperm counts per g caudal tissue, incidence of abnormal sperm, estrual cyclicity, and calcium levels in blood-serum of second generation mice."

The results of this NTP study are briefly summarized and compared to 47 other continuous breeding studies in a publication by Morrissey et al. *(128*).

Bolon et al. *(130*) assessed differential follicle counts in mouse ovaries (ten mice/group) in animals that had been exposed to propylene glycol using the NTP continuous breeding protocol and reported that it had no effect on follicular counts.

Strengths/Weaknesses: The NTP multi-generational study *(127*) provided an acceptable toxicological protocol, and found that propylene glycol administered in the drinking water at up to a 5% dose level had no effect on fertility and reproduction in adult and second generation mice. Only the mouse and the rat have been studied, and findings from the two rat studies were inconclusive. The NTP study using mice reported no reproductive toxicity.

Utility (Adequacy) for CERHR Evaluation Process: This GLP study *(127*) is adequate for assessing reproductive hazard.

4.3 Utility of Data

There are no available data on the reproductive toxicity of propylene glycol in humans. An NTP multigeneration study *(127*) in mice concluded that propylene glycol administered in concentrations up to 5% (w/v) in the drinking water of mice did not cause reproductive toxicity in males or females

or their progeny. These data were judged by the Panel to be relevant to consideration of human risk.

4.4 Summary

4.4.1 Human Data

No human reproductive toxicity studies were identified.

4.4.2 Experimental Animal Data

The study by Guerrant et al. *(129)* in rats were conducted more than 50 years ago, prior to GLP protocols. Many experimental details were not provided and the results are judged inconclusive by the Panel.

In the NTP multi-generation study *(127)*, propylene glycol was administered in the drinking water to mice at 0, 1, 2.5, and 5% (w/v) dose level; there was no effect on fertility or reproduction in the first and second generation mice. There was no apparent effect with respect to body, kidney, and liver weights, pup survival, sperm motility, sperm counts, incidence of abnormal sperm, or estrual cyclicity. During the cohabitation phase, no chemical-related deaths and no significant chemical-related clinical signs of toxicity were noted. Propylene glycol had no significant effect on any of the following reproductive parameters in F_0 animals: number of litters per pair; number of live pups per litter; sex ratio; pup weights; number of days to litter; and dam weights at delivery.

The Panel concluded that there is adequate evidence in mice that propylene glycol does not cause reproductive toxicity in males and females when exposure is up to 5% propylene glycol in drinking water over an 18-week exposure period (1 week prior to cohabitation, 14 weeks during cohabitation, and 3 weeks after cohabitation) or in their progeny. The Panel judged these data relevant for assessing human risk.

5.0 SUMMARIES, CONCLUSIONS AND CRITICAL DATA NEEDS

5.1 Summary and Conclusions of Reproductive and Developmental Hazards

5.1.1 Developmental Toxicity

Prenatal developmental toxicity was assessed in CD-1 mice (gd 6–15), Wistar rats (gd 6–15), Golden hamsters (gd 6–10), and Dutch-belted rabbits (gd 6–18) by daily oral intubation. Neither developmental nor maternal toxicity was detected at the highest dose used in each of these studies (mice – 1.6 g/kg bw/day or 10.4 g/kg bw/day; rats – 1.6 g/kg bw/day; hamsters – 1.55 g/kg bw/day; rabbits – 1.23 g/kg bw/day). These data are sufficient to conclude that propylene glycol is not a developmental toxicant in these species under these treatment conditions. These data are assumed relevant for assessing human hazard. No human developmental toxicity data were identified.

5.1.2 Kinetics

The pharmacokinetics of propylene glycol are reasonably well understood in humans as well as animals. Data indicate rapid and extensive absorption followed by rapid distribution into total body water. The rate-determining step in its metabolism is alcohol dehydrogenase which, when saturated, switches from a first order process into a zero order process. Saturation of metabolism appears to occur in rats and rabbits at a dose of about 1.6 to 2 g/kg bw, whereas in humans this seems to happen at a dose of about 0.2 g/kg bw. Since alcohol dehydrogenase activity is not fully developed in infants, saturation of metabolism occurs at lower doses. In accordance with a zero order process, the half-life of propylene glycol in humans and rats increases from about 1.5 hours to more than 5 hours with increasing doses above metabolic saturation. By a NAD-dependent reaction, alcohol dehydrogenase converts propylene glycol to lactaldehyde, which is further metabolized to lactate. Since propylene glycol has a chiral center, technical grade propylene glycol results in the formation of 50/50 D, L-lactate. L-lactate is indistinguishable from endogenous lactate, which is a good substrate for gluconeogenesis. D-lactate is less readily converted to glucose than L-lactate, which prolongs its half-life leading, under conditions of prolonged exposure (e.g., IV infusion), to D-lactic acidosis. It is difficult to cause L-lactic acidosis even with very high doses of propylene glycol because of its efficient detoxification via gluconeogenesis.

The second reason for lack of development of L-lactic acidosis is the saturation of alcohol dehydrogenase, which results in a constant rate of lactate production. Due to removal of L-lactate by gluconeogenesis, a further increase in lactate levels is not possible after saturation of metabolism.

The excretion of propylene glycol is species-dependent. Humans clear about 45% of propylene glycol via kidney, and in dogs, up to 88%. In rats and rabbits, very little of the parent compound is excreted by the kidney until saturation of metabolism occurs. Inhibition of alcohol dehydrogenase by pyrazole increases urinary excretion of propylene glycol to 75% in rats, as expected.

Since propylene glycol has very low intrinsic toxicity, saturation of metabolism plays a protective role in its toxicity since the conversion of propylene glycol to the more toxic lactate (particularly D-lactate) is slowed. Because of low alcohol dehydrogenase activity in infants and children, this protective effect is more pronounced in infants and up to 5 years of age.

There are few uncertainties in the kinetics of propylene glycol. They all relate to the expression of various isoforms of alcohol dehydrogenase in various species and in different tissues. An investigation of the above question almost certainly will provide the answer for the 8−10 times lower dose required for saturation of metabolism in humans compared to rats and rabbits.

5.1.3 Reproductive Toxicity

In an NTP continuous breeding study *(127)*, propylene glycol was administered to mice in the drinking water at up to 5% (w/v) **[10.1 g/kg bw/day].** This dose had no effect on fertility of either males or females in either the first or second generation. These data are sufficient to conclude that propylene glycol is not a reproductive toxicant in males or females or in their progeny under the conditions of this study. These data are assumed relevant for assessing human hazard. No data on human reproductive toxicity were found.

5.2 Summary of Human Exposure

In 1999, 1,083 million pounds of propylene glycol were produced in the U.S. with apparent consumption of 854 million pounds *(5)*. Of the apparent amount consumed, uses included, in million pounds and percentages: unsaturated polyester resins (228, 26.7%); cosmetics and personal care products; pharmaceuticals, and human food (170, 19.9%); liquid detergents (135, 15.8%); deicing fluids (85, 10%); antifreeze/engine coolant (55, 6.4%); paints and coatings (40, 4.7%); tobacco humectant (25, 2.9%); other fluids (32, 3.8%); and other applications (84, 9.8%) *(5)*.

The general population is exposed to propylene glycol by oral intake, dermal contact, and inhalation. The average daily intake of propylene glycol from food products in the United States has been estimated at 2,400 mg/day **[34 mg/kg bw/day for a 70 kg person]** *(13)*. Propylene glycol is an inert ingredient in some pharmaceutical preparations. Propylene glycol is also found in many pharmaceuticals that are administered intravenously, which represents a unique exposure route for certain subpopulations.

Occupational exposure to propylene glycol may occur through dermal contact or through inhalation of airborne propylene glycol from heating or spraying processes. An AIHA WEEL Guide of 50 ppm (total exposure) and an inhalation aerosol exposure of 10 mg/m^3, each an 8-hour TWA, have been recommended *(18)*. Propylene glycol occupational exposure data are limited to several small studies. Laitinen et al. *(20)* measured propylene glycol exposure in motor servicing workers. Propylene glycol was not detected in air, and urinary propylene glycol levels did not differ between exposed workers and unexposed controls. Norbäck et al. *(19)* measured airborne propylene glycol exposure (geometric mean 350 µg/m^3, maximum 12,700 µg/m^3) among Swedish painters during indoor application of water-based paints. Propylene glycol levels were measured in urine samples collected pre- and post-shift from aircraft deicing workers (range: 0.72–13.44 mg/L; 0.41–10.58 mg/g creatinine); and in urine samples from a comparison group (range 0.29–10.7 mg/L, 1.18 mg/g creatinine) *(22)*. In a NIOSH HHE of aircraft deicing workers, personal breathing zone air samples for propylene glycol over a 6-hour period ranged from 10 to 21 mg/m^3, with a mean of 15 mg/m^3 *(24)*.

5.3 Overall Conclusions

No human data on reproductive or developmental toxicity are available. Although the serum half-life of propylene glycol is greater in infants and children than in adults, the concern for postnatal developmental toxicity in infants and children younger than 5 years of age is diminished by low levels

of alcohol dehydrogenase. Furthermore, published data documenting high blood levels of propylene glycol during continuous therapeutic infusion in pediatric intensive care patients 15 months of age and younger were not associated with any acute toxicity *(66)*. The knowledge that human metabolism of propylene glycol saturates at an 8–10 times lower dose than in rats or rabbits provides further confidence that human developmental or reproductive risks are of negligible concern.

Available data are sufficient to conclude that this compound is not a reproductive or developmental toxicant in mice, rats, hamsters, or rabbits. The oral dose levels identified from animal studies are:

- NOAEL ≥ 10 g/kg bw/d in mice, highest dose tested
- NOAEL ≥ 1.6 g/kg bw/d in rats, highest dose tested
- NOAEL ≥ 1.55 g/kg bw/d in hamsters, highest dose tested
- NOAEL ≥ 1.23 g/kg bw/d in rabbits, highest dose tested

There are no major differences in general toxicity between humans and animals (except the cat), and toxicity only occurs at very high doses (LD_{50} values of 8 –46 g/kg in rats, and is estimated to be >15 g/kg bw in humans).

Based on these findings, the Panel concludes that current estimated exposures to propylene glycol are of negligible concern for reproductive or developmental toxicity in humans.

5.4 Critical Data Needs

Although the Panel has only negligible concern for developmental and reproductive effects, it suggests that there is a critical data need for long-term follow up of children and pregnant women exposed to high dose propylene glycol from continuous IV infusion.

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

Center For The Evaluation Of Risks To Human Reproduction

PUBLIC COMMENTS ON THE EXPERT PANEL REPORT ON PROPYLENE GLYCOL

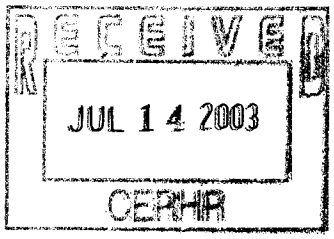
Appendix III

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council

July 10, 2003

via US Mail and e-mail



Dr. Michael Shelby
CERHR
P.O. Box 12233
MD EC-32
Research Triangle Park, NC 27709

Re: Comments of the Propylene Oxide/Propylene Glycol Panel on the NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Propylene Glycol (NTP-CERHR-PG-03, May 2003) 68 Federal Register:26325 (May 15, 2003).

Dear Dr. Shelby:

The Propylene Oxide/Propylene Glycol Panel (PO/PG Panel) of the American Chemistry Council submits the attached comments in response to the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) *Expert panel Report on the Reproductive and Developmental Toxicity of Propylene Glycol* (Report) and the May 15, 2003 Federal Register Announcemnt. The member companies of the PO/PG Panel comprise the major domestic producers of propylene glycol in the United States.[1]

The PO/PG Panel commends the CERHR and expert panel participants for conducting an open and collegial review meeting, focused on the robust and voluminous scientific literature relevant to evaluation of the reproductive and developmental toxicity of the glycols. The PO/PG Panel agrees with the expert panel's conclusion that "*current estimated exposures to propylene glycol are of negligible concern for reproductive or developmental toxicity in humans*" (p. 76). While agreeing with the overall conclusions, the PO/PG Panel also notes that many of the deficiencies of the December 2002 draft Report, especially those in Chapter 2 ("General Toxicology and Biological Effects") remain unchanged in the May 2003 Report. As a result of the failure to heed the earlier comments, the expert panel's conclusions are not as strongly supported as the data demonstrate.

Appendix III

[1] Members of the Propylene Oxide/Propylene Glycol Panel are The Dow Chemical Company, Huntsman Corporation, and Lyondell Chemical Company.

In lieu of reiterating previously submitted comments, the PO/PG Panel incorporates the comments of January 23, 2003 into these comments by reference, with a request that special attention be paid to the review by Dr. Mark Udden, a clinical hematologist at Baylor College of Medicine, of the failure to heed our earlier comments.

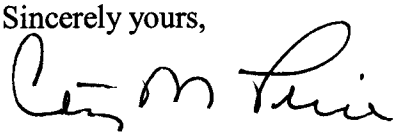
The comments included in the following submission expand on three significant deficiencies in the May 2003 Report that remain little changed from the December 2002 draft:

- Analysis of the data relating to wavy ribs and incomplete ossification of the vertebrae noted in the older FDA developmental toxicity study conducted in rats indicates that this common finding is not attributable to propylene glycol.
- Interpretation of information on the hematological effects of propylene glycol is inconsistent. Overall, evidence that propylene glycol adversely affects red blood cells in rats and humans is lacking.
- Evidence for the existence of potentially sensitive sub-populations is inconsistent, and the CERHR conclusion that such sub-populations exist is not justified.

Unfortunately, the CERHR does not contemplate further revision of the expert panel's Report. The PO/PG Panel requests therefore, that the deficiencies of the Report be addressed in the *NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Propylene Glycol* that will be prepared to complete the CERHR review process.

If you or your staff has any questions, please contact the PO/PG Panel Manager, Dr. Anne P. LeHuray at (703) 741-5630 or *anne_lehuray@americanchemistry.com.*

Sincerely yours,



Courtney M. Price
Vice President, CHEMSTAR

Enclosures

Appendix III

U.S. NATIONAL TOXICOLOGY PROGRAM (NTP)
CENTER FOR THE EVALUATION OF RISKS TO HUMAN REPRODUCTION (CERHR)

NTP-CERHR EXPERT PANEL REPORT
ON THE
REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF PROPYLENE GLYCOL
MAY 2003
NTP-CENTER-PG-03

COMMENTS
OF THE
PROPYLENE OXIDE/PROPYLENE GLYCOL PANEL
OF THE
AMERICAN CHEMISTRY COUNCIL

COMMENTS SUBMITTED: JULY 10, 2003

Appendix III

INTRODUCTION

The Propylene Oxide/Propylene Glycol Panel (PO/PG Panel) of the American Chemistry Council (ACC) submits these comments in response to the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) notice (68 Federal Register:26325, May 15, 2003) indicating that CERHR released the *Expert panel Report on the Reproductive and Developmental Toxicity of Propylene Glycol* (Report). As described in the Federal Register notice, the Report is intended to reflect "*the summaries and conclusions of the expert panel's evaluation of the scientific data for potential reproductive and/or developmental hazards associated with exposure to ... propylene glycol.*"

The expert panel met in February 2003 to review and evaluate reproductive and developmental toxicities of propylene glycol. In response to the draft Report released in preparation for the February meeting (67 Federal Register:236, Dec. 9, 2002), the PO/PG Panel submitted detailed comments on the draft Report on January 23, 2003. CERHR posted these comments on its web site (http://cerhr.niehs.nih.gov/news/egpg/pg_pubcomm.html) prior to the expert panel meeting.

The PO/PG Panel commends the CERHR and expert panel participants for conducting an open and collegial review meeting, focused on the robust and voluminous scientific literature relevant to evaluation of the reproductive and developmental toxicity of the glycols. The PO/PG Panel agrees with the expert panel's conclusion that "*current estimated exposures to propylene glycol are of negligible concern for reproductive or developmental toxicity in humans*" (p. 76). While agreeing with the overall conclusions, the PO/PG Panel also notes that many of the deficiencies of the December 2002 draft Report, especially those in Chapter 2 ("General Toxicology and Biological Effects") remain unchanged in the May 2003 Report. As a result of the failure to heed the earlier comments, the expert panel's conclusions are not as strongly supported as the data demonstrates.

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This set of comments expands on three significant deficiencies in the May 2003 Report that remain little changed from the December 2002 draft:

- Analysis of the data relating to wavy ribs and incomplete ossification of the vertebrae noted in the older FDA developmental toxicity study conducted in rats indicates that this common finding is not attributable to propylene glycol.
- Interpretation of information on the hematological effects of propylene glycol is inconsistent. Overall, evidence that propylene glycol adversely affects red blood cells in rats and humans in lacking.
- Evidence for the existence of potentially sensitive sub-populations is inconsistent, and the CERHR conclusion that such sub-populations exist is not justified.

Unfortunately, the CERHR does not contemplate further revision of the expert panel's Report. The PO/PG requests therefore, that the deficiencies of the Report be addressed in the NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Propylene Glycol that will be prepared to complete the CERHR review process.

COMMENTS

1. Analysis of the data relating to wavy ribs and incomplete ossification of the vertebrae noted in the older FDA developmental toxicity study conducted in rats indicates that this common finding is not attributable to propylene glycol.

In two instances, the Report contains discussion of a purported effect of propylene glycol on wavy ribs and incomplete ossification of the vertebrae in the older FDA developmental toxicity study conducted in rats:

Page 61:

> With rats, higher numbers of wavy ribs and incomplete ossification of the vertebrae were observed at the same level as the positive control, suggesting a propylene glycol effect. The incidences of these defects did not appear to be dose-related.

Page 62:

> The appropriateness of the NOAEL level for rats (1,600 mg/kg bw/day) given in Table 3-11 depends on the importance attributed to the rib and vertebrae malformations observed.

Table 3-6 of the Report contains the data used to support these statements. Using the data in Table 3-6, the incidence and percentages for wavy ribs and incomplete ossification of the vertebrae have been calculated. The data is presented in the table below.

ENDPOINT - RATS	DOSE GROUP					
	CONTROL	POS. CONTROL	16 MG/KG	74.3 MG/KG	340 MG/KG	1600 MG/KG
Wavy ribs						
pup incidence	1/173	46/137	23/179	27/169	11/167	15/180
litter incidence	1/22	16/20	9/23	11/22	5/20	8/24
% pup	0.60%	34%	13%	16%	7%	8%
% litter	5%	80%	39%	50%	25%	33%
Vertebrae – incomplete ossification						
pup incidence	0/0	101/137	1/179	13/169	3/167	18/180
litter incidence	0/0	19/20	1/23	7/22	3/20	9/24
% pup	0%	74%	0.50%	8%	2%	10%
% litter	0%	95%	4%	32%	15%	38%

As demonstrated in the table, the data do not support the statement that "*With rats, higher numbers of wavy ribs and incomplete ossification of the vertebrae were observed at the same level as the positive control....*" Clearly the positive control values (80%, 95%) are more than twice as high as those reported even in the 1,600 mg/kg high dose group (33%, 38%).

One may question whether there was an effect in these endpoints based upon comparison with the negative control ("Control") group. Given the differences in the strains of rats used in the 1973 FDA study and those currently available, it would be inappropriate to conduct a quantitative comparison of the incidence of wavy ribs and incomplete ossification of the vertebrae from historical databases currently available in testing laboratories. However, as a qualitative comparison, it is important to note that historically, wavy ribs and incomplete ossification of the vertebrae are very common findings in developmental toxicity studies conducted with rats. The most striking finding, evident in the data in the table above, is the unusually low incidence of these lesions in the negative control population.

Additional support for discounting the significance of these findings is the lack of any dose-response relationships for these endpoints. For example, in the case of the wavy ribs, the lowest dose level tested (16 mg/kg; 100-fold lower than the high dose group) had a higher incidence that the highest dose level tested (1,600 mg/kg).

2. Interpretation of information on the hematological effects of propylene glycol is inconsistent. Overall, evidence that propylene glycol adversely affects red blood cells in rats and humans in lacking.

The PO/PG Panel previously (January 23, 2003) submitted extensive comments to CERHR on the interpretation of hematological data for propylene glycol presented in the December 2002 draft Report. The previous submission included an expert evaluation of publications cited in the draft Report, prepared by Dr. Mark Udden, a clinical hematologist at Baylor College of Medicine. The May 2003 Report contains little evidence that Dr. Udden's review has been taken into account. Rather than repeat these comments (which remain valid and are presented again to CERHR for consideration), the focus here is instead on the inconsistencies in the Report text noted in the paragraphs that follow.

Section 2.2.2.4, page 38, fourth paragraph

> Christopher et al. (*91*)[1] provide an excellent study that establishes a plausible mechanism for propylene-induced hemolysis and the Bauer et al. (89) study provides important confirmatory evidence for the impairment of hematopoiesis by propylene glycol. Thus the hemolysis potential of high doses of propylene glycol, which is a plausible effect, is firmly established in two species (cat and dog) and reasonably well substantiated in other species including man.

This statement is an over-generalization of the available scientific data, and ignores the several established metabolic and physiological factors that predispose red blood cells from the cat to propylene glycol-induced toxicity. These are well known to the expert panel, because they are discussed at length by Christopher *et al.* (91) and also summarized by Dr. Udden. It is therefore difficult to understand why the expert panel Report appears to conclude that results obtained in the cat are indicative of a potential for propylene glycol to cause adverse hematological effects in humans: overall scientific evidence to support this is lacking. The work of Christopher, Bauer and co-workers focused on the susceptibility of the cat to propylene glycol-induced Heinz body formation with no mention of 'hemolysis' (which appears to be the

[1] Numbers in parentheses refer to the reference numbers of citations in the Report.

main end-point of interest to the expert panel). There also does not appear to be any evidence from either study for any inhibition of hematopoiesis (cited by the expert panel Report as a toxic response to propylene glycol). The PO/PG Panel strongly recommends that CERHR reconsider the basis for these conclusions, which are at variance with the published data.

Indeed, the only evidence presented by the expert panel in support of the "hemolytic capability" of propylene glycol in humans is a 65-year old *in vitro* study by Braun and Cartland (79). The expert panel is referred to Dr. Udden's report which includes several more up-to-date references and which also serves to put these *in vitro* findings into context: the findings are an osmotic, not a toxiciological, phenomenon seen at propylene glycol concentrations of 30% or greater. This contrasts with human *in vivo* data cited elsewhere by the expert panel (*e.g.*, Chicella *et al.*; (66)), which indicates that the maximum concentration of propylene glycol in serum from patients undergoing intravenous (IV) drug therapy is 763±660 mg/l (*i.e.*, approximately 0.08-0.14%), which is several hundred fold lower than that shown to cause hemoloysis *in vitro*. This data suggest that red blood cell hemolysis in patients exposed to propylene glycol by IV infusion is unlikely to be an issue.

With regard to the expert panel's view that the information from Saini *et al.* (90) "*confirms that the haemotopoietic system is also a target of propylene glycol in rats*," the PO/PG Panel again questions the scientific reliability of hematological data obtained from animals subjected to four retro-orbital bleeds within two days with no concurrent control group. Certainly there was a clear effect on some parameters that were measured, but with no controls it cannot be determined if this was due to propylene glycol or day-to-day experimental variability.

Overall, the expert panel's conclusions on the potential hematotoxicity of propylene glycol in humans is unsupported.

3. Evidence for the existence of potentially sensitive sub-populations is inconsistent, and the CERHR conclusion that such sub-populations exist is not justified.

The PO/PG Panel previously submitted (January 23, 2003) extensive comments on the expert panel's view that individuals with compromised liver or kidney function, burn patients or premature infants may be "at increased risk for developing propylene glycol toxicity." There is little evidence that the previous comments have been taken into account in revising the Report, apart from inclusion of some moderating words (*i.e.*, "overdosage of premature infants"; "complex clinical case studies") in the introductory paragraph of Section 2.5 (page 44). Rather than repeat the previously submitted comments (which remain valid) the focus here instead is on inconsistencies in the report text described in paragraphs that follow.

Section 2.5, paragraph 1, line 6 (page 44)

> Patients with impaired liver or kidney function would be at increased risk for developing propylene glycol toxicity (*48*).

This is a logical statement given the importance of hepatic metabolism and renal excretion in overall clearance of propylene glycol from the body. It also seems self-evident that the toxicokinetics and toxicodynamics of propylene glycol will be altered in individuals with pre-existing liver or kidney disease or who develop hepatic or renal insufficiency as a result of clinical treatment. Against this background, interpretation of case reports of lactic acid acidosis,

increased osmolality and other clinical sequelae requires careful objective assessment: while propylene glycol may have been present in the circulation, there is only anecdotal evidence that propylene glycol was responsible for the effects reported in these individuals. This should be made clear in the Report, and the individual references cited by the expert panel should be assessed to determine if the references are scientifically reliable.

Section 2.5.1, page 44, final sentence

> Serum concentrations of propylene glycol received through IV medications have been shown to correlate with serum lactate concentrations in patients with normal renal and hepatic function (*51*).

The expert panel cites Kelner and Bailey (51), who reported serum propylene glycol and lactate concentrations in 5 subjects (two middle aged adults, three infants aged 4 months or younger) with 'normal' renal and liver function and who concluded that "propylene glycol administration may be an important cause of lactic acid acidosis" as support for this statement. In reaching this conclusion, Kelner and Bailey developed a regression model for relating serum concentrations of propylene glycol and lactate in their patients *i.e.,*

$$Y = 0.034X + 2.97$$

where Y is the lactate concentration in serum (mEq/l) at a propylene glycol concentration of X (mg/l). This suggests there is a clear (numerically positive) relationship between propylene glycol and lactate levels in serum.

However, other data cited in the Report, namely the publication by Chicella *et al.* (66; not present in the earlier version of the Report) lead to a very different conclusion. In Chicella *et al.* (66), no correlation was observed between serum propylene glycol and lactate in 11 patients (aged 1-15 months) receiving IV medication containing propylene glycol. Since Chicella *et al.* was a prospective study designed specifically to address issues of interest to the CERHR, it is unclear why its findings have been overlooked in favor of other less reliable data.

As part of their investigation, Chicella *et al.* recorded serum propylene glycol concentrations in their subjects at various time points leading to mean values of 85, 519 and 763 mg/l at (roughly) the start, middle and end of treatment. These are consistent with measured serum propylene glycol concentrations reported by Kelner and Bailey (roughly in a range of 50-400 mg/l). Based on the data and modeling of Kelner and Bailey, equivalent mean serum lactate values of 5.9, 21 and 29 mEq/l would be predicted for the Chicella subjects. In fact Chicella *et al.* reported very modest measured lactate values of 1.9, 1.7 and 1.7 mmol/l, respectively (consistent with their conclusion that no lactic acid acidemia was present in these patients).

The expert panel should consider the data of Chicella *et al.*, rather than those of Kelner and Bailey, to support the conclusions of section 2.5: that propylene glycol is a low concern for lactic acid acidemia.

Section 2.5.2, first line, page 45

> The decreased size of premature infants, and an increased serum half-life for propylene glycol in premature infants (*35, 36*), predispose them to a greater probability of toxic effects from over administration of propylene glycol.

Studies reported by Fligner *et al.* (35), Glasgow *et al.* (36) and Huggon *et al.* (70) are used to support this and related statements present in this section of the Report. As discussed in

the PO/PG Panel's January 23, 2003 submission, patients included in the cited case reports were already suffering from pre-exisiting clinical conditions that likely had a fundamental impact on the disposition of propylene glycol in the body, or they were administered powerful, potentially toxic pharmaceutical treatments as part of their therapy, or a combination of the two. This presumably explains in part the expert panel's observation (page 13, line 7) that data from Fligner *et al.* and Glasgow *et al.* reflect "circumstances that preclude confident extrapolation to a healthy general population." Huggon *et al.* also add a warning that impaired renal failure may have contributed to the increased osmolality recorded in their study. Given the unreliability of this information, the expert panel should re-evaluate whether these studies support the conclusion that premature infants are at greater risk of propylene glycol toxicity.

It is also clear from data presented in Chicella *et al.* (66) that no adverse responses were reported in slightly older infants (age 1-15 months) following IV drug therapy that resulted in a mean serum propylene glycol concentration as high as 763 mg/ml. Therefore, the basis of the expert panel's decision (Section 2.5.2, paragraph 3, final sentence) to "caution" that it may be necessary to monitor lactic acidosis and hyperosmolality in pediatric patients given IV infusions containing propylene glycol is unknown.

Given the considerable uncertainties in these studies (detailed in the PO/PG Panel's January 23, 2003 submission) the conclusion of the May 2003 Report, that the studies provide evidence of a sub-population with an enhanced sensitivity to propylene glycol related toxicity is questionable.

Section 2.5.2, first paragraph, last line (page 45):

> .....propylene glycol.....was associated with cardiopulmonary arrest in one case (*70*).

This statement, citing Huggon *et al.* (70) is factually incorrect: circulatory problems were present in this individual before any exposure to propylene glycol. There is no mention of propylene glycol-induced "cardiopulmonary arrest" in the publication.