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

Center For The Evaluation Of Risks To Human Reproduction

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

DECEMBER 2002 **NTP-CENTER-PG-02**

PREFACE

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:

CERHR
NIEHS EC-32
P.O. Box 12233
Research Triangle Park, NC 27709
919-541-3455

A Report of the CERHR Glycols Expert Panel:

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

With the Support of CERHR Staff:

NTP/NIEHS

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

Sciences International, Inc.

John Moore, DVM, DABT	Principal Scientist
Gloria Jahnke, MS, DVM	Toxicologist
Annette Iannucci, MS	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.

Abbreviations

ADH	alcohol dehydrogenase
ALDH	aldehyde dehydrogenase
ANOVA	analysis of variance
ATSDR	Agency for Toxic Substances and Disease Registry
bw	body weight
C	Celsius
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
d	day
dL	deciliter
DMBA	dimethylbenzanthracene
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
f	female
FDA	Food and Drug Administration
Fl oz	Fluid ounces
g	gram
GC	gas chromatography
gd	gestation day
GRAS	generally recognized as safe
h	hour
HCG	human chorionic gonadotrophin
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
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	molar
m	male
m^3	meters cubed
m^2	meters squared
mg	milligram
min	minute
mM	millimolar
mL	milliliter

mmol	millimole
mM	millimolar
MW	molecular weight
n, #	number
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 Co-operation and Development
OSHA	Occupational Safety and Health Administration
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
TLV	threshold limit value
USDA	United States Department of Agriculture
V_{max}	maximal velocity of metabolism
wk	week
µg	microgram
µmol	micromole

TABLE OF CONTENTS

LIST OF TABLES

List of Figures

1.0 CHEMISTRY, USE, AND HUMAN EXPOSURE

1.1 Chemistry

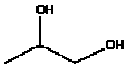
1.1.1 Nomenclature

The Chemical Abstracts Service Registration Number (CASRN) 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, alpha propylene glycol (*2*).

1.1.2 Formula and Molecular Mass

Figure 1-1. Formula and molecular weight (mw) of propylene glycol.

Chemical formula



$C_3H_8O_2$
mw: 76.095

1.1.3 Chemical and Physical Properties

Viscous, tasteless, colorless, ordorless hydroscopic liquid with a low vapor pressure.

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

Property	Value
Vapor Pressure	0.07 mm Hg @ 20C[b]
Melting Point	-59 C
Boiling Point	188.2 C
Specific Gravity	1.0361 g/cc @ 20C[b]
Solubility in Water	Soluble
Log K_{ow}	-0.912[b]
Stability	Stable
Reactivity	Can react with oxidizing agents

[a]HSDB (*2*), [b] ATSDR (*3*)

1.1.4 Technical Products and Impurities

According to HSDB (*2*), both industrial-grade and USP grade propylene glycol have at least 99.5% purity; impurities include chlorides (1-10 ppm max), iron (0.5-1.0 ppm max), heavy metals (5 ppm max), arsenic (3 ppm max), sulfate (0.006 wt% max) and water (0.2 wt% max)

Registered trade names for propylene glycol are PG-12 and Sirlene. Manufacturers of propylene glycol are or have included ARCO Chemical Co, Newtown Square, PA, Production site: Bayport, TX; Dow Chemical USA, Midland, MI, Production sites: Freeport, TX, Plaquemine, LA; Olin Chemical Corp, Stamford, CT Production site: Brandenburg, KY; Texaco Chemical Company, Houston, TX, Production site: Port Neches, TX; Eastman Chemical Co, Texas Eastman Div. Kingsport, TN, Production site: South Charleston, WV, Huntsman, Lyondell Chemical Company, Houston, TX; Huntsman Corporation, Salt Lake City, UT (*3*) (*4*).

1.2 Use and Human Exposure

1.2.1 Production Information

Propylene glycol is manufactured by direct hydrolysis of propylene oxide by water producing propylene glycol, dipropylene glycol (10%) and tripropylene glycol (1%) products which are separated by distillation (*2*).

The 1989-1995 U.S. production volumes for propylene glycol were approximately 1,000 million pounds per year; 174 million pounds were imported into the US in 1994 (*3*). According to HSDB (*2*):

> Approximately 41% of propylene glycol produced is used in production of unsaturated polyester resins, 29% is exported, 11% is used in foods, pharmaceuticals or cosmetics, 7% is used in semi-moist pet food, 4% is used as a humectant for tobacco, 4% is used in functional fluids, and 4% is for miscellaneous uses.

1.2.2 Use

The major use of propylene glycol is as an intermediate in the manufacture of unsaturated polyester resins (*2*). Propylene glycol is 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.

The following summary obtained from ATSDR (*3*) and 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, de-icing 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 (*5*) (*2*).

Propylene glycol has FDA approved use in food, tobacco and pharmaceutical products (*6*). It is considered to be GRAS (generally recognized as safe) for direct addition to foods (*6*). 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" (*6*).

Propylene glycol is an additive/softener in pet food products. However, due to the formation of Heinz bodies (denatured proteins, primarily hemoglobin) in the erythrocytes of cats, 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 (*7*).

Propylene glycol has been reported to be an effective antidote for ethylene glycol poisoning (*8*).

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 EPA (*9*) to monitor storm water runoff and to recycle de-icing 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 (*3*). 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. (*10*).

1.2.4 Human Exposure

General Population Exposure

The general population can be exposed to propylene glycol through dermal contact with consumer products such as cosmetic products, anti-freeze solutions, coolants, windshield de-icers, 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]**. No data were available for average daily intake in the United States. (Louekari et al. (*11*) [from Market Basket Study, Japan 1982]).

Because propylene glycol is readily soluble in water, drinking, bathing in, or showering with contaminated water is another potential exposure route. However, we were 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. (*12*), emissions of volatile organic compounds 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 were from 690 $\mu g/m^2$/hr 24 hrs after installation and 193 $\mu g/m^2$/hr at 168 hrs after installation. The other three carpet types did not have propylene glycol emissions.

Propylene glycol is used as a softener for cellulose wrapping film (*2*) (*5*). In order to estimate the human exposure of propylene glycol from cellulose packaging, Castle et al. (*5*) studied the migration of propylene glycol from experimental cellulose wraps. Samples were unwrapped at intervals and analyzed for their propylene glycol content. Food samples were extracted and measured by capillary gas chromatography with flame ionization detection. The results of wraps with propylene glycol were compared to packaging using triethylene glycol (Table 1-2); the authors concluded that "higher levels of migration occurred for propylene glycol than for triethylene glycol and the presence of a coating **[on the wrap]** reduced the migration of both softeners."

Table 1-2. Migration of propylene glycol from various types of cellulose wraps to various food types (from Castle et al. (*5*)).

Food type	Number of samples	Storage (days)	Range of triethylene glycol concentrations in food (mg/kg)	Range of propylene glycol Concentrations in food (mg/kg)
Boiled sweet	4	168 or 450	<10-172	10-272
Toffee	4	168 or 450	12-454	<10-1530
Madeira cake	4	21 or 28	<10-69	<10-365
Fruit cake	4	84 or 336	<10-17	<10-154
Meat pie	6	3 or 7	Not analyzed for triethylene glycol	<10-118

FDA estimated that the human daily dietary intake of propylene glycol to be a 'few mg per kg **[body weight]** per day' (*13*) **[No details were given on how exposures were estimated.]**. The average daily dietary intake of propylene glycol in Japan is estimated to be 43 mg/person/day **[0.7 mg/kg bw/day based on a 60 kg person]** (*11*). Human acceptable daily intake of propylene glycol is < 25 mg/kg body weight per day (*14*).

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 that results from heating or spraying processes (*2*).

Neither the Occupational Safety and Health Administration (OSHA) nor the American Conference of Governmental Industrial Hygienists (ACGIH) have established exposure limits for propylene glycol vapors. No 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 (*15*).

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 (*2*).

Laitinen et al. (*16*) examined exposure to ethylene and propylene glycol in Finish motor servicing workers. Ten male mechanics from 5 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 **[units for air concentrations are questionable]**. Urine samples were collected after the work shift and analyzed for ethylene glycol, oxalic acid, and propylene glycol. Urinary concentrations of ethylene glycol were significantly higher in mechanics than controls (7.3±4.7 vs. 1.7±0.7 mmol/mol creatinine, respectively). Levels of oxalic acid were also higher in mechanics, but statistical significance was not achieved (47±11 vs. 36±14 mmol/mol creatinine). Propylene glycol concentrations were not increased in urine from mechanics. The study authors noted that ethylene glycol excretion was higher in workers who conducted major engine repairs and were exposed to ethylene glycol for longer time periods. Because ethylene glycol was not detected in air, but was detected in the urine of workers, the study authors concluded that exposure occurred through dermal contact **[it is noted later that dermal absorption appears to be very low].**

In a study simulating concentrations of propylene glycol mist used in aviation emergency training, Wieslander et al. (*17*) concluded that short (one minute) exposure 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 fifteen minutes of exposure.

Propylene glycol is a component of de-icing solutions used at airports (Table 1-3). The antifreeze components in a de-icing solution vary with the manufacturer, usage, and environmental conditions. Performance criteria for deicing fluids are governed by specifications of the Aerospace Division of Society of Automotive Engineers (SAE) (*18*). Exposure to workers using de-icing solutions is by inhalation and dermal exposure.

Table 1-3. Composition of various Aircraft De-icing Fluids [a] (from Klecka et al. (*19*)).

Product	Type	Ethylene glycol	Propylene glycol	Diethylene glycol
Dow 146AR	I	94 %	-	-
Dow 1000PG	I	-	89 %	-
AEA-I[b]	I	-	15 %	80 %
Texaco WD-20	I	38 %	13 %	-
Dow Flightgard 2000	II	-	20 %	39 %

[a] The balance of the product is composed of water, proprietary additives, thickeners, neutralizers, rust inhibitors.
[b]Association of European Airlines type I de-icing fluid.

A Health Hazard Evaluation (HHE) on occupational exposure to propylene glycol during in aircraft de-icing operations was provided by NIOSH (*20*). Evaluation of de-icing procedures was conducted at the Denver International Airport (DIA) in March, 1996. De-icing fluids are low viscosity glycols used to remove ice or snow which would increase drag on the aircraft. At DIA,

United Airlines uses a 50% solution of propylene glycol in water, heated to 180° F for de-icing aircraft. Trucks with duel 800 gallon tanks, spray hoses, and booms are used. The amount of fluid used for de-icing each plane ranges from 50-200 gallons. Personal breathing zone air samples were collected from 6 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 liters per minute for six hours and analyzed by GC/MS for propylene glycol according to NIOSH Method 5523.

Table 1-4 Exposure to Airborne Propylene Glycol HETA 95-0069

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 the worker being accidentally sprayed with the de-icing fluid during sampling.

The seven workers had a range of exposures from 10-21 mg/M^3 with a mean of 15 mg/M^3 . The author concluded that "There was no hazard from overexposure to de-icing fluid. …Airborne exposure to propylene glycol was low and propylene glycol has low toxicity."

Strengths/Weaknesses:

Utility:

Propylene glycol and glycols are not bioaccumulative in organisms and rapidly biodegrade in the soil and in water (*19*). However, this process is oxygen-demanding and can deplete dissolved oxygen levels in water (*21*). The Clean Water Act requires airports to implement plans for de-icer management to control storm water contamination. Therefore, airports are required by EPA (*9*) to monitor propylene glycol storm water runoff and to scavenge and recycle de-icing solutions.

1.3 Utility of Data

1.4 Summary of Human Exposure Data

Propylene glycol is produced primarily for use as an intermediate in the manufacture of unsaturated polyester resins and in the production of plasticizers, 2-mthylpiperazine, 1,2-propylene diamine, hydroxylated polyester, polyester-type fluorescent resin matrix, and polyether polyols.

It is used as an antifreeze, de-icing solution, and as an additive to paints and coatings to improve freeze-thaw capability, and is used as a lubricant or heat-transfer fluid in situations where there may be food contact. Propylene glycol is approved by the FDA for use in food, tobacco and pharmaceutical products and has GRAS status for direct addition to foods. US production volumes for propylene glycol approximate 1,000 million pounds per year.

The general population can be exposed to propylene glycol by oral intake, dermal contact, and inhalation. No data were available for the US average daily intake of propylene glycol from food products or food packaging. However, in Japan, the estimated average daily intake of propylene glycol as a food additive was reported to be 43 mg per person. WHO food additive series (*14*) lists the acceptable human daily intake of propylene glycol at <25 mg/kg body weight per day. 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. Information on dietary exposure to propylene glycol is judged to be limited.

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 has been determined. NIOSH estimated that 1,748,454 people (1981-1983 NOES survey) are potentially exposed to propylene glycol in the workplace, primarily through contact with trade name products containing propylene glycol.

Two occupational exposure studies measuring propylene glycol were located. In a study by Laitine et al (*16*), motor servicing worker exposure to propylene glycol and ethylene glycol was measured. Urinary levels of ethylene glycol, but not propylene glycol, were detected. However, although air measurements were taken, the actual amount of propylene glycol and ethylene glycol that the workers were in contact with was not measured, so it is not possible to determine whether the lack of urinary levels of propylene glycol is due to a lack of exposure, or to its low dermal absorption.

A single Health Hazard Evaluation (HHE) report by NIOSH on occupational exposure to propylene glycol during aircraft de-icing operations was located (*20*). A 50% solution of propylene glycol in water, heated to 180° F was used in de-icing aircraft. Personal breathing zone air samples over a six hour period were collected on eight workers. Air samples were collected on XAD-7 OVS tubes at a flow rate of 0.5 liters per minute and analyzed by GC/MS according to NIOSH Method 5523. The seven workers had a range of exposures from 10-21 mg/m^3 with a mean of 15 mg/m^3.

The Panel judged the information in these reports inadequate to reach conclusions concerning occupational exposure to propylene glycol.

2.0 GENERAL TOXICOLOGY AND BIOLOGICAL EFFECTS

2.1 Toxicokinetics and Metabolism

The first step in the review of toxicokinetics and metabolism data for propylene glycol was an examination of authoritative reviews (*3*) (*4*) and an independent review (*22*). It was noted that the toxicokinetic sections in those reviews were somewhat brief, and a decision was made by CERHR to review relevant original studies. Because the studies identified no major issues (i.e., toxicokinetic differences between humans and animals) and because toxicity of propylene glycol in humans and animals appears to be limited to very high doses (see Section 2.2), the majority of studies in this Section were only briefly summarized.

2.1.1 Absorption

Human

Studies of the pharmacokinetics of propylene glycol in humans have occurred primarily in conjunction with on-going patient therapy where propylene glycol was administered as a vehicle for medications. Those studies provide limited qualitative information on absorption.

Oral. In 6-16 adults administered propylene glycol (20.7 g/dose three times daily or 41.4g/dose twice daily). These oral doses were given in conjunction with 100 mg phenytoin in 7.25 ml of alcohol USP, 6 microliters 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 in these studies was determined by the authors to be 3.8 and 4.1 hours. The average total body clearance was determined by the authors to be approximately 0.1 L/kghr, although there was a 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 (*23*).

Strength/Weaknesses: This study provides data on the oral absorption of propylene glycol as well as on serum half-life, apparent volume of distribution and total body clearance after repeated oral doses. The results are in agreement with expectations from a highly water soluble small molecule: rapid absorption, distribution into total body water, relatively short half-life and rapid total body clearance. Only two high dose regimens (20.7 g, three times daily or 41.4 g, two times daily, both for a minimum of three days) were used, both of which were above metabolic saturation. Therefore, the half-life estimates are on the high side and total body clearance on the low side but not inconsistent with the IV experiment to be discussed later.

Utility (Adequacy) for CERHR Evaluation Process: Data are adequate to derive kinetic parameters but not to judge bioavailability.

Rectal. Absorption of propylene glycol through the rectum is 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 if assume a 70 kg bw]**. Propylene glycol and water (1:1) were used as solvents in the formulation of a rectal solution of paracetamol. 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 (*24*).

Strength/Weaknesses: This paper (*24*) reported C_{max} and t_{max} and their corrected values after curve fitting together with plasma levels, half-life and apparent volume of distribution and clearance after different doses of propylene glycol administered per rectum to 10 adults and 4 children. However, the children were 5-12 years of age so this paper provides no information on whether bioavailability might be greater than adults by a larger margin in very young children. The values are in the expected range providing confirmatory evidence for the reliability of kinetic parameters determined by Speth et al. (*25*). 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 reaching adult levels by the age of 5 years (*26*). The extent of oral absorption cannot be judged from these data but a visual inspection of plasma concentrations after IV infusion (*25*) and rectal administration (*24*) 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. However, the children were 5-12 years of age so this paper provides no information on whether bioavailability might be greater than adults by a larger margin in very young children.

Utility (Adequacy) for CERHR Evaluation Process: The study is useful to judge bioavailability indirectly.

Dermal. There is very 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 (*27*) 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. 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]** (*3*) (*28*). In an eight-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]** (*29*). 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 (*30*).

Strength/Weaknesses: The studies listed above show what is expected of a highly water-soluble substance, that dermal absorption of propylene glycol through the intact skin is very limited. It is equally clear, which is also expected, that once the stratum corneum is impaired (removed such as in burns or irritated), dermal absorption becomes a significant source of exposure.

Utility (Adequacy) for CERHR Evaluation Process: This study has minimal utility for drawing conclusions regarding propylene glycol penetration across human skin. A weakness of this study is the insensitive, non-quantitative method for assessing chemical uptake, and due to the extensive manipulation of the skin following the permeation period (excision which apparently produced bleeding) which may lead to losses of both skin and permeated chemical in handling the tissue. However, when combined with the single rat dermal penetration *in vitro* study (*31*) also showing no uptake, and given the difficulty water soluble molecules generally have in penetrating

the stratum corneum, one may conclude that the dermal absorption rate across intact skin is likely to be slow. Therefore, it can be expected that any dermal exposure to propylene glycol will result in systemic levels far below saturation of metabolic clearance.

Inhalation. Bau et al. (*32*) **[as reported in HSDB (*2*)]** reported that less than 5% of a technecium-labeled aerosol **[propylene glycol not directly measured]** containing 10% propylene glycol in deionized water was taken up by humans after inhalation for 1 hr in a mist tent. They measured the aerosol mass median diameter to be 4.8 to 5.4 micron, a size small enough that should have enabled penetration to the deep lung. Ninety percent of the dose was found in the nasopharnyx and it rapidly entered the stomach, with very little entering the lungs. Propylene glycol was not measured.

Strength/Weaknesses: Since propylene glycol was not directly measured, 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 kineticly related to intravenous 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 or not propylene glycol in a carrier medium could lead to significant exposure by inhalation. Bau et al. (*32*) 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 toxicologically of questionable relevance.

Animal

Animal studies demonstrate that propylene glycol is rapidly absorbed following oral exposure. ATSDR (*3*) reports the findings of a study by Christopher et al. (*33*), 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. (*34*), found that blood propylene glycol concentration (41.04 mM) reached its maximum level one hour after 4 New Zealand rabbits were administered 38.66 mmol/kg **[2.942 g/kg]** as a 28.4% aqueous solution by gavage. Morshed et al., (*35*) orally administered an aqueous solution of propylene glycol at 4.83-77.28 mmol/kg bw **[0.368-5.881 g/kg bw]** to six 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 (*36*) demonstrated peak blood levels of propylene glycol approximately two to three hours after oral dosing in dogs.

Strength/Weaknesses: The Christopher et al. (*33*) 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. (*35*) provided a more complete set of data indicating dose-dependent t_{max} for propylene glycol in the dose range of 0.4 to 5.9 g/kg. The authors did not calculate absorption half-lives nor was the extent of absorption determined. They concluded that gastrointestinal absorption occurred by a first order process because of the linear rise of plasma concentration at each of the 5 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., 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. (*37*) (*34*) papers and the Lehman and Newman (*36*) 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 (*38*) 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 (*39*).

Utility (Adequacy) for CERHR Evaluation Process: Available animal data are not very suitable for the quantitative evaluation 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.

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" (*3*). 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 (*40*). 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 (*40*). The appearance of propylene glycol seemed to be in three phases when in the presence of a skin penetration enhancer such as oleic acid (*31*). The first stage was the penetration of propylene glycol into the skin barrier, without any change of the dermal structure. The second stage was rapid distribution in and throughout the dermis, presumably accompanied by alteration of the dermal structure. In the third stage, propylene glycol was saturated in the dermis."

ATSDR (*3*) reported that hairless mouse skin over-estimates absorption of propylene glycol by human skin while shed snake skin under-estimates absorption. Therefore, the authors concluded that human skin should be used for absorption studies if possible.

2.1.2 Distribution

Apparent volumes of distribution calculated in human studies indicate that propylene glycol is uniformly distributed in total body water without a significant distribution to specific tissues. Following administration of propylene glycol, volumes of distribution were measured at 0.52

L/kg with oral dosing (*23*), 0.77-0.79 L/kg with rectal exposure (*24*), and ~0.55-0.94 L/kg with intravenous exposure (*25*).

Strength/Weaknesses: There are excellent data on the determination of the apparent volume of distribution of propylene glycol in both humans and animals (*23*) (*24*) (*25*), which demonstrate that it distributes into total body water. This is also in agreement with theoretical considerations that all low molecular weight glycols are associated with water molecules via hydrogen bonding and as such move with bulk flow of water driven by osmotic or hydrostatic pressure.

Utility (Adequacy) for CERHR Evaluation Process: It can be concluded with certainty that propylene glycol will distribute into the water compartment of the placenta and fetus.

2.1.3 Metabolism

In adult humans, the mean serum half-life of propylene glycol is approximately 2-4 hours (*25*). In the rat, metabolism is inhibited by pyrazole, indicating a role for ADH in this process (*35*) (*41*). 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 conjugate with glucuronic acid (*2*) (*22*). However, cats do not have the ability to produce the glucuronidated metabolite (*22*). Part of the propylene glycol dose is metabolized by the liver to lactic acid by ADH and further to pyruvic acid, carbon dioxide and water. The amount of propylene glycol eliminated by the kidneys has been estimated for humans at 45% (*42*), for dogs at 55-88% (*43*), and for rabbits at 24-14.2% (*44*). Alternate, stereo-specific reaction pathways have been described for the metabolism of propylene glycol and are described in detail below. Apart from pyruvate providing an energy source through oxidation in the Krebs cycle, lactic acid can also serve as an energy source as a precursor for glycogen synthesis. One study demonstrated increased gluconeogenesis in rats treated with propylene glycol (*45*). However, through excess production of lactic acid, large exposures to propylene glycol can produce a metabolic anion gap **[anion gap = (Na^+) – (Cl^- + total CO_2)]** and metabolic acidosis (*3*). Serum levels of >0.18 mg/L can result in toxicity (*42*).

Strength/Weaknesses: It is clear from the Yu et al. (*23*) paper that total body clearance of propylene glycol by the rabbit occurs by metabolic clearance and by renal excretion. Metabolic clearance accounts for 85.8 to 97.6% of total clearance. Because of its low hepatic extraction ratio (9 to 15%) liver blood flow will have very little impact on its clearance. Morshed et al. (*35*) provided evidence in the rat that the rate-determining step in the metabolic clearance of propylene glycol is the NAD-dependent alcohol dehydrogenase which was dose-dependently inhibited by pyrazole, leading to a dose-dependent increase in urinary excretion with the highest dose of propylene glycol and the highest dose of pyrazole causing two-thirds of the dose to be excreted in urine. There are no data in humans from which to assess the percentage fate of propylene glycol.

The Speth et al. (*25*) paper allows the conclusion that humans clear propylene glycol similar to rats and rabbits. However, saturation of metabolic clearance seems to occur at lower doses in humans than in rats and rabbits. **[The Speth et al. (*25*) 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.]** Table 2 of Speth et al. (*25*) 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 effected at about 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. (*25*) provide evidence of metabolic saturation in propylene glycol metabolism at doses in the range of 7 g/day as seen by lengthening t1/2 and AUC and C_{max} increasing in non-linear fashion. When this dose is converted to mmol/kg based upon the body weights reported for the 3 subjects

receiving this dose, the value is 1.6 mmole/kg which is considerably lower than the Km 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 (S.D.) 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 view is confirmed by Yu et al. (*23*) 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.

Renal excretion of the parent compound and its glucuronide as a small percentage of dose is plausible (*2*) (*22*). Propylene glycol through its major metabolic pathway becomes part of the 3 carbon pool (*46*). From here on it is all well-established biochemistry. Pyruvate can enter the Krebs cycle after decarboxylation and oxidation as acetyl CoA, which provides energy. Lactate, via phosphoenol pyruvate, can be detoxified into glucose and stored as glycogen as it has been demonstrated by Wittman et al. (*45*) for propylene glycol in rats. Doses of 0.5 to 2.0 g/kg of propylene glycol were administered to female rats and liver glycogen content and blood glucose determined 90 min after dosing. Liver glycogen content was nearly doubled and fasting blood glucose increased from 88 mg% to about 140 mg%. Lactic acidosis was not reported and 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.

The ATSDR document states on page 97 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 also human data as supportive evidence. Christopher et al. (*33*) showed in cats administered 12% (1.6 g/kg/day) or 41% (8.0 g/kg/day) propylene glycol in the diet (dry weight) for 22 days that there was a time-dependent increase in plasma lactate and in anion gap. Morshed et al. (*37*) (*34*) produced much 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 (*42*) demonstrated that repeated infusions of lorazepam dissolved in propylene glycol can lead to lactic acidosis with increased osmolar gap (21mOsm/L). Furthermore, increased blood glucose (296 mg/dL) and elevated pyruvate level (1.01 mg/dL) indicated that the same metabolic pathways of detoxification occur in humans as in animals. Glasgow et al. (*30*) reported earlier a good correlation between osmolality gap and serum propylene glycol concentrations in 10 infants. The half-life was reported as 19.3 hours (range 10.8 to 30.5 hours), which is about 10 times longer than in adults. Alcohol dehydrogenase activity is up to 10 times lower in infants (*26*) 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.

Utility (Adequacy) for CERHR Evaluation Process: The metabolism of propylene glycol is well understood and suitable data are available to interpret them for pregnancy and offspring as well.

Metabolism and Stereospecificity

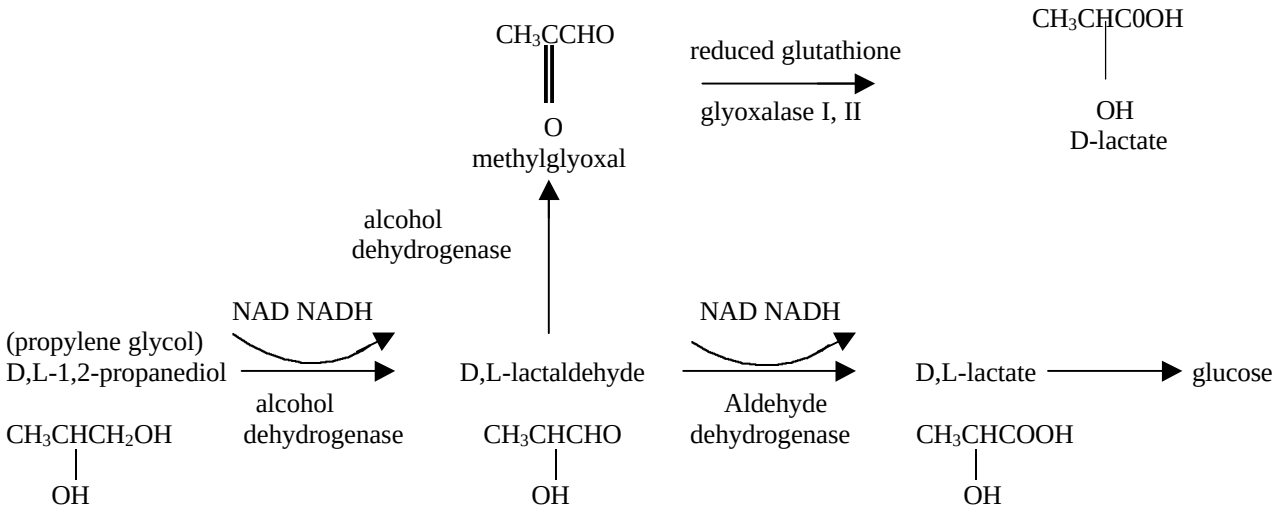
Synthesis of propylene glycol results in a 1:1 ratio of D and L stereoisomer forms. There is some, although incomplete, information in the literature about stereospecificity of the enzymes in the propylene glycol metabolic pathways (Figure 2-1). In what is considered to be the main pathway

of propylene glycol metabolism in mammals (*3*) (*33*), both the D and L forms of propylene glycol are oxidized by ADH to D,L-lactaldehyde, then to D, L-lactate by ALDH. L-Lactate contributes to glucose formation through gluconeogenic pathways (*33*). In the horse and rabbit, ADH will oxidize the L- form of propylene glycol and lactaldehyde more efficiently than the D- form (*47*). L-lactic acidosis has been observed in both humans and animals following exposure to propylene glycol (*33*) (*34*).

An alternate route of metabolism is thought to be the conversion of lactaldehyde to methylglyoxal by ADH and then to D-lactate by glyoxalase and reduced glutathione (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 instances where ketone levels are high, such as diabetes or starvation, methylglyoxal synthetase activity is induced, 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 (*33*). Therefore, in cases of ketosis, excess levels of D-lactate may be exacerbated by propylene glycol.

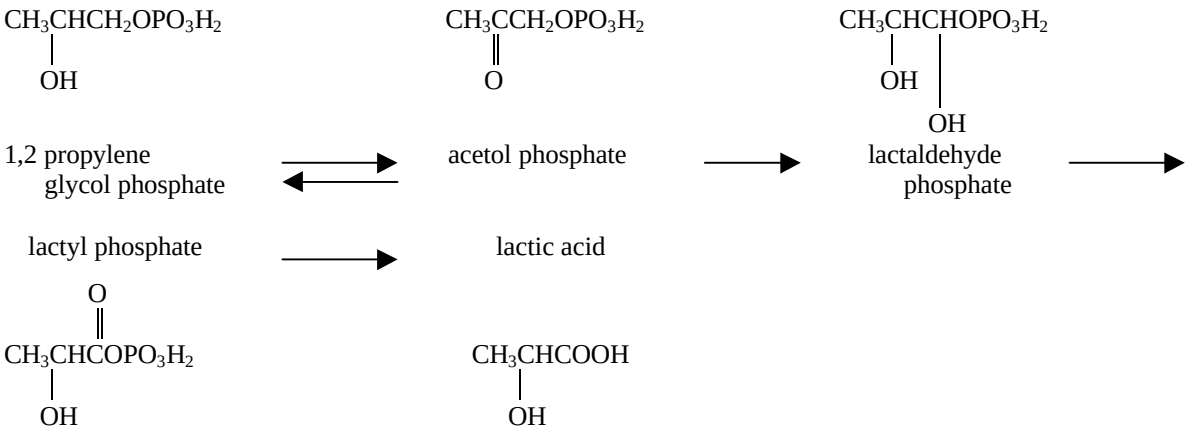
Figure 2-1. Propylene Glycol Metabolism in Mammals.



From Christopher et al. (*33*).

In a third possible metabolic pathway, propylene glycol can be phosphorylated, converted to acetol phosphate, lactaldehyde phosphate, lactyl phosphate and to lactic acid (see Figure 2-2) (*43*). 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 (*47*) (*46*).

Figure 2-2. Phosphorylated Propylene Glycol Metabolism in Mammals.



From Ruddick (*43*)

A limited number of studies were summarized in detail since they demonstrate evidence of *in vitro* stereospecificity of ADH (*47*), L-lactatemia in rabbits (*34*), and increased D-lactate formation in cats (*33*).

Stereospecificity of ADH was studied by Huff (*47*). *In vitro* studies of rabbit liver ADH Ks 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. Ks 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 is an issue with propylene glycol because the technical product contains the stereoisomers in a 1:1 ratio. Huff (*47*) determined the K_m values for oxidation of the D- and L-forms by alcohol dehydrogenase and found that L-propylene glycol is 5 to 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 animals (*33*) (*34*) and humans which was partially attributed to D-lactate being a poor substrate for gluconeogenesis, which is 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. However, here species differences were found in that rabbits converted the L-form more readily to lactic acid but rats and mice did it equally well with both forms (*47*) (*46*). Due to incomplete time point sampling and a lack of quantitative numbers regarding fluxes through the different pathways, it is not possible to piece together a complete picture of stereospecific metabolism of D,L-propylene glycol.

Toxicologically it is of no consequence whether L- or D-lactatemia develops because both cause lactic acidosis to the same extent. The longer half-life of D-lactate can be easily factored in via

the Michaelis-Menten equation into a physiologically based pharmacokinetic (PB-PK) model. The weakness of this approach is that D-lactate was shown to be efficiently utilized in man (*48*) 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-lactate 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 which 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. (*34*). Propylene glycol was administered to New Zealand White 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 body weight. Whole blood was withdrawn from the marginal ear vein after 24 hour fast and at 0.25, 1, and 3 h 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 calorimetrically, and the levels of lactate and pyruvate 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 maximum 1 h post-dosing. Treatment with propylene glycol significantly ($P<0.01$) increased the concentration of L-lactate, which plateaued 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 (*41*).

Table 2-1. Levels of propylene glycol and its metabolites in New Zealand White rabbits after oral propylene glycol (From Morshed et al. (*34*)**).**

Parameter	**Fast**	**0.25 h**	**1 h**	**3 h**
Propylene Glycol	00 (00)	30.23 ± 12.45*** (00)	41.04 ± 9.98*** (00)	36.55 ± 8.0*** (00)
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); ** $p<0.01$; *** $p<0.001$.

Strength/Weaknesses: The Morshed et al. (*34*) paper provides some useful information for the early phase of metabolism of propylene glycol in rabbits, although its usefulness specifically for the kinetics of propylene glycol is limited, primarily because of the 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 first order one compartment model would indicate a half-life of about 12 hours in the rabbit. It must be emphasized that neither assumption might be true, 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. (*34*) (*41*) paper is properly interpreted.]** The study in rats (*41*) 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 (saturation of alcohol dehydrogenase being the rate-determining step) compound with dose-dependent increase in its half-life.

The time course evaluated for propylene glycol-induced latactemia in rabbits was too short to allow for any conclusions regarding D- or L-lactate half-life in the study of Morshed, et al. (*34*). That study also contains contradictory data in that blood L-lactate concentrations peaked at the earliest time point (0.25 hr) and declined thereafter (see Table 2-1 above). However, the

propylene glycol concentration peaked at 1 hour and fell only slightly out to 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 the Morshed, et al. 1991 point out that the 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 as compared to 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: There is limited usefulness in the Morshed et al. (*41*) (*34*) data for reproductive and developmental considerations. What is clear form these papers is 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, 5-6 cats of each sex were fed a diet containing 12% propylene glycol (low dose, 1.60 g/kg/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/day) for 22 days (*33*). 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. Serum chemistries were performed on the samples. L- (+) lactate was determined enzymatically using L-lactate dehydrogenase and D-(-) lactate was determined on days 0, 10, 24 of the low dose diet and days 0, 6, 10, 24 of the high dose diet. Data was analyzed by analysis of variance 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 1.60 g/kg/d. Serum levels of D-lactate increased with days of propylene glycol ingestion and levels of L-lactate decreased in cats ingesting 1.6 g of propylene glycol/kg/d (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 one week following exposure, it is not known 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/d**

	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 (*33*)

Strength/Weaknesses: The Christopher et al. (*33*) paper is an important one 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/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. (*35*) 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: This is a useful study linking human data (*48*) with animal data regarding D-lactatemia.

Overall summary of metabolism: It appears that high, acute doses of propylene glycol can lead to L-lactic acidosis, which does not seem to be a major problem (until very high doses) because of the efficient conversion (detoxification) of L-lactate 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. By logical inference, 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, that 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. (*48*). 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 (*41*). The rate of L-lactate excretion and utilization were not reported in the human study (*48*).

Developmental Variations in Alcohol Dehydrogenase Activity

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

Placental Metabolic Capacity. Studies in humans and rodents suggest that the placenta has extremely limited capacity to metabolize propylene glycol. Pares et al. (*49*) 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 compared to the Class I ADH enzyme found in human liver. Zorzano and Herrera (*50*) 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 (*51*).

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

Sjoblom et al. (*51*) found that in Wistar rats ADH activity in liver was not detected at birth, was 3% of adult activity on pnd 20, and continued to increase with age to 65% and 82% of adult activity on pnd 21 and 47, respectively. Similar developmental patterns were noted for ALDH in rat liver.

Pikkarainen and Raiha (*26*) measured *in vitro* ADH activity in the livers of human fetuses, children, and adults (n=1–3/age group) using ethanol as a substrate. The ADH activity in 2-month old fetal livers was about 3–4% that of adults. In 4–5 month old fetuses, ADH activity was roughly 10% that of adults, and in infancy, activity was about 20% that of adults. ADH activity increased in children with age, and at 5 years of age reached a level that was within the ranges noted for adults. Great variation was noted in adult ADH activity.

Somewhat different results were reported subsequently by Smith et al. (*52*) 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 fetal liver that was 30% of adult values and in premature infants and children less than 1 year of age, activity was 50% of adult values. Individual enzyme activity was determined using starch gel electrophoresis with an in situ assay. A total of 222 liver samples were assayed, 56 from fetuses (9-22 weeks gestation), 37 from premature infants and infants less than 1 year of age, and 129 from adults 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. If one assumes that the enzyme most active in ethanol metabolism, ADH1B, also is most active in propylene glycol metabolism, then one would not predict significant fetal metabolism until later in gestational development (20-36 weeks).

Strength/Weaknesses: There are consistent data in both animals and humans showing that alcohol dehydrogenase is not significantly expressed perinatally. 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-Propylene glycols themselves are not the most toxic species, rather their metabolites D, L-lactate. Therefore a lack of in situ conversion in the fetus and low conversion rate in newborns would seem to decrease the toxicity of propylene glycol. Nevertheless, since lactate also distributes into total body water, the fetus will experience the mother's metabolic acidosis if present. In contrast, newborns and infants may be protected from metabolic acidosis after ingestion of propylene glycol. They may experience more severe CNS effects though, from lack of metabolism of the glycol.

Hepatic Metabolic Capacity in Humans Versus Rats Zorzano and Herrera (*53*) (*54*) 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).

Genetic Polymorphisms
Reviews by Agarwal (*55*), Bosron and Li (*56*), Pietruszko (*57*) and Burnell et al. (*58*) discussed genetic polymorphisms for ADH and ALDH in humans. Class I ADH, the primary ADH in human liver, is a dimer composed of randomly associated polypeptide units encoded by 3 loci (*ADH1A*, *ADH1B*, and *ADH1C*). Polymorphisms resulting in altered phenotypes are observed at the *ADH1B* (*ADH1B*2* and *ADH1B*3*) and *ADH1C* (*ADH1C*2*) loci. The *ADH1B*2* allele is estimated to occur in 15% of Caucasians of European descent, 85% of Asians, but less than 5% of African Americans. Fifteen percent of African Americans have the *ADH1B*3* allele, while this variant is essentially absent in other ethnic groups. Both the ADH1B*2 and ADH1B*3 enzymes have V_{max}, values for ethanol that are 100-fold higher than that exhibited by ADH1B*1. The ADH1B*2 and ADH1B*3 differ in that their affinities for ethanol are approximately 20- and 70-fold lower than ADH1B*1, respectively

There are two primary ALDH isoenzymes in human liver, ALDH2 (also referred to as E_2 , ALDHI, or $ALDH_2$) and ALDH1 (also referred to as an E_1 , ALDHII, or $ALDH_1$) (*55*) (*56*) (*57*). 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 by ADH and further 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% (*42*), for dogs at 55-88% (*43*), and for rabbits at 2.4-14.2% (*44*). Morshed et al. (*35*) provided evidence in the rat that increasing doses of propylene glycol increased elimination by the kidneys. With dosages of 19, 38, and 77.28 mmole/kg bw resulting in 2.3%, 7,%, and 17% 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.

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/hour; elimination half-life in those same subjects was about 4 hours (*23*). 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 (*24*). In six 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 (*25*).

Though limited, a small number of studies suggest that elimination in infants is slower than in adults. In an eight-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 (*29*). 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 (*30*).

Excretion of propylene glycol has been studied in patients with second and third degree burns over more than 20% of their total body surface (*28*). According to ATSDR (*3*), "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. The Speth et al. (*25*) is a very good study which provides all 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. (*24*) 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 (*25*). The data of Kollöffel et al. (*24*) 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. (*23*) estimated an elimination half-life of about 4 hrs. 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 are pointing in the same direction.

Prolonged half-lives of propylene glycol (*29*) (*30*) in the range of 10.8 to 30.5 hours in infants are entirely consistent with very low alcohol dehydrogenase activity perinatally (*26*).

The Kulick et al. (*28*) paper is not suitable for determination of elimination kinetics because only one time point was measured.

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

Animals

ATSDR (*3*) reports that, "Dose-dependent elimination 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.hr (0.63 g/kg/hour) was observed."

Yu and Sawchuk (*44*) studied the metabolism and elimination of propylene glycol after acute or chronic intravenous administration to NZW male rabbits. Rabbits were exposed acutely by IV injection to either 0.50,1.00, or 2.00 g/kg bw (3 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 to 6.3 mg/min/kg bw) over the course of 51 to 52 hours. Both Vmax and Km were lower in the case of prolonged exposure, but the Vmax/Km ratio was approximately three fold greater than under acute dosing. Plots of metabolic clearance from single rabbits dosed acutely vs continuously indicate higher metabolic clearance rates from continuous exposure**. [This raises the possibility of the induction of a second, low Km 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 (*43*) cited an earlier study by Lehman and Newman (*36*) 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]** two 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 lead 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 (*44*) paper on the urinary flow dependence of elimination as well as on the dose-dependence of metabolic clearance. This paper, in conjunction with the human data, provides a window of opportunity to construct a PB-PK model and to validate some of the assumptions routinely used in such models.

The Ruddick (*43*) and Lehman and Newman (*36*) papers are not suitable for quantitative kinetic evaluation.

2.2 General Toxicity

Recent reviews of studies of propylene glycol toxicity are available. The majority of information in this section is summarized from the reviews by ATSDR (*3*), by LaKind et al. (*22*), from the SIDS Initial Assessment Report for 11th SIAM (*4*), and USEPA Health and Environmental Effects Document on Propylene Glycol (*59*). No toxicity studies have been located on propylene glycol subsequent to the 2001 review. 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 (*43*) (*4*) (*3*). Symptoms of acute propylene glycol intoxication in animals are those of CNS depression or narcosis. Individuals with compromised hepatic or renal function would be less apt to clear propylene glycol and hence, be more susceptible to toxicity due to high blood levels (*60*) (*3*) (*2*). No system or organ has been established as a target for the acute oral lethal effects of propylene glycol (*61*) [see Section 2.1.6 Mechanism of Action]. Propylene glycol has GRAS status by the FDA for use as an indirect human food ingredient (*13*) and has FDA approved use in food, tobacco and pharmaceutical products (*6*).

Strength/Weaknesses: There is an adequate database to assess the toxicity of propylene glycol as documented in the listed documents (*59*) (*3*) (*22*) (*4*). Very high doses cause CNS, hematologic/hyperosmotic and perhaps cardiovascular effects as well as lactic acidosis. Animals lethally intoxicated undergo CNS depression, narcosis with eventual respiratory arrest. There are few apparent weaknesses like the statement that impaired renal function increases toxicity of propylene glycol. Very little of propylene glycol is cleared by the kidney, most of it is cleared by the liver. Therefore decreased renal clearance will have little effect on plasma levels and hence on toxicity.

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

2.2.1 Humans

Oral Exposure

A lethal oral dose of propylene glycol has not been reported for humans (*22*), 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 > 0.18 mg/L have resulted in toxicity (*42*). 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/day) resulted in seizures and CNS depression (*22*). In infants, mortality has occurred after repeated exposure to propylene glycol in medication; CNS depression and seizures have been reported after multiple oral doses (*30*) (*62*). Chronic ingestion of propylene glycol has resulted in lactic acidosis, stupor, and seizures in adults (*63*) (*42*) [see Sections 1.2.4 Human Exposure and 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.

Dermal Exposure

Contact dermatitis has been reported from propylene glycol exposure in a wide variety of topical preparations (*22*) and ingestion of propylene glycol in sensitized individuals has produced flares of dermatitis (*22*). 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 (*64*) (*65*). The nature of the skin reaction of propylene glycol-sensitive patients has been a matter of controversy (*66*) (*67*). In a study by Hannuksela and Forstrom, primary irritant reactions to the skin as well as 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, and not due to an allergic reaction (*65*).

Inhalation Exposure

In a study by Cohen and Crandall (*68*) [reviewed by LaKind et al. (*22*)], 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 (*17*) 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 one 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. A medical examination was performed both within 15 minutes before and after the exposure. It included an estimate of tear film stability breakup time, nasal patency by acoustic rhinometry, lung function by dynamic spirometry and a self-rated symptoms questionnaire. After one minute 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 one 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.

Parenteral Exposure

Hemolysis, central nervous system depression, hyperosmolality, and lactic acidosis have been reported after intravenous administration (*60*). Rapid intravenous 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 intravenous administration of drugs such as lorazepam, etomidate, phenytoin, diazepam, digoxin, hydralazine, esmolol, chlorodiazepoxide, multivitamins, nitroglycerin, pentobarbital sodium, phenobarbital sodium and trimethoprim-sulfamethoxazole. Therefore, patients, especially children and infants, receiving intravenous drugs can be at risk for propylene glycol toxicity (*22*) **[see Section 2.5 Potentially Sensitive Subpopulations]**.

Information on the dose of propylene glycol administered 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

Oral Exposure

LD_{50} oral toxicity values are listed in Table 2-3, below. A wide range of LD_{50} values has been reported for the rat. In a study by Morshed et al. (*37*), six male Wistar rats were dosed by gavage with saline or 2.942 g/kg/d 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.

Strength/Weaknesses: This study does not have strengths, only weaknesses. Controls gained 16.9 g during the first 10 days (1.69 g/d on average), 23.3 g during 20 days (1.17 g/d on average) and 40.15 g (1.34 on average during 30 days). Well-maintained rats do not display such variability in their weight gain pattern.

Utility (Adequacy) for CERHR Evaluation Process: None.

In a study by Weatherby and Haag (*69*) **[reviewed by OECD (*4*)]** 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 (*69*) 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>iIM.>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 0.111M.

Utility (Adequacy) for CERHR Evaluation Process: Very useful study for the characterization of acute toxicity, less so 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 to 21.00 g/kg) (*70*) **[reviewed in LaKind et al. (*22*); OECD (*4*)]**. Animals exhibited an increased respiratory rate, loss of equilibrium, depression, analgesia, coma, and died by 36 h after dosing. The minimum fatal dose was determined to be 18.9 g/kg (3 out of 9 deaths), with 100% mortality at a dose of 21 g/kg (4 out of 4 deaths).

Strength/Weaknesses: The Braun and Cartland (*70*) paper predates the Weatherby and Haag (*69*) 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: For the characterization of acute toxicity, this is a good paper, but not for assessing chronic toxicity.

Table 2-3. Propylene Glycol Oral Toxicity Values.

Species	LD_{50} (g/kg)	Reference
Rat	8-46	ATSDR (*3*)
Mouse	25-32	ATSDR (*3*)
Rabbit	18-20	ATSDR (*3*)
Dog	19	HSDB (*2*)
Guinea-Pig	18-20	ATSDR (*3*)
Human	>15 (estimated)	HSDB (*2*)

Chronic toxicity studies reflect that propylene glycol has a very low order of toxicity. In the following toxicity studies by Morris et al. (*71*) and Gaunt et al. (*72*), reproductive tissues were examined.

Albino rats (inbred strain, male and female, 20 rats/group) administered 0, 2.45% and 4.9% of propylene glycol in the diet (0, 1.23 and 2.45 g/kg/d, respectively) for two 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 testis **[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. **[No statistical analyses were performed and the histopathology of the liver is not described.]** (*71*) **[reviewed in LaKind et al. (*22*); OECD (*4*)]**.

Strength/Weaknesses: The Morris et al. (*71*) 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 (~3g/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 a slight liver damage was observed. The highest doses (1.7 to 2.1g/kg) used by Gaunt et al. (*72*) was close to the lower dose in this study and no liver effect was reported there. Therefore, the lower dose probably did not cause any liver damage. Failure to conduct statistical analyses weakens this study further.

Utility (Adequacy) for CERHR Evaluation Process: This study can only serve as a modest indicator that 3 g/kg propylene glycol chronically could perhaps cause slight liver injury.

In 2 year and 15 week toxicity studies in rats given propylene glycol in the diet (*72*), 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 15 week study was run concurrently with the 2 year study.

For the short-term study, groups of fifteen male and fifteen 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 thirty male and thirty 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 eight male and eight 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 specific gravity and urine volume were made 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 theses organs plus the following organs as well as any tissue which appeared abnormal was preserved in 10% buffered formalin: salivary gland, trachea, aorta, thymus, lymph nodes, pituitary, urinary bladder, colon, rectum, pancreas, uterus, and muscle **.**

For 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. (*72*) 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 and 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 kind of statistics were used. However, the reputation of BIBRA and of the authors of this paper provide credibility to the statement. It is unfortunate that a higher dose was not used because as conducted we did not learn anything about the chronic toxicity in rats, only about its 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 at least partially due to high rate of pulmonary infection.

Utility (Adequacy) for CERHR Evaluation Process: This study 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 (maternal NOAEL).

Propylene glycol administered in the drinking water of rats at doses >13.2 g/kg day for 140 days resulted in CNS depression and minor liver injury (reviewed by Mortensen (*65*); LaKind et al. (*22*)). In a 2-year drinking water study in rats (dosed up to1.834 g/kg/d), no renal pathology and very slight liver damage was found (*22*).

Strength/Weaknesses: The Seidenfeld and Hanzlik (*73*) paper predates all other publications thus far evaluated. It is enjoyable reading because of the 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 latter, more detailed experiments of Braun and Cartland (*70*) and Weatherby and Haag (*69*). Even though the style of the 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. (*71*) report. Thus, it can be concluded that slight hepatic injury could be expected in rats at a daily intake of 2 g/kg of propylene glycol.

Utility (Adequacy) for CERHR Evaluation Process: A useful study because now the Morris et al. (*71*) report can be viewed as confirmatory evidence for the slight liver damage to be 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 two 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 red blood cell 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 (*74*) and (*59*).

Strength/Weaknesses: Weil et al. (*74*) studied the toxicity of propylene glycol in beagle dogs fed in the diet at 2 and 5 g/kg/day for two years. A roughly isocaloric diet to the propylene glycol containing dextrose was fed to a positive control group. After appropriate statistical evaluation the conclusion arrived at was that 5 g/kg/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/day) was essentially identical to the rat NOAEL.

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

No effects were found on the kidneys in studies by VanWinkle and Newman (*75*) 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 (*22*).

Strength/Weaknesses: In these experiments (*75*) liver and kidney function of dogs provided drinking water containing 5% propylene glycol (5 .1 cm^3 = 5.3 g/kg body weight) was 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 to 33 mg%. Statistical analysis was not performed and if it had been, it certainly would have shown no difference. Hematology was not done.

Utility (Adequacy) for CERHR Evaluation Process: The studies of Van Winkle and Newman (*75*) might not be considered adequate by today's standards but they still provide useful data as confirmatory evidence for the NOAEL of 2 g/kg/day established by Weil et al. (*74*) in dogs.

Table 2-4. Summary of Toxicity of Propylene Glycol in Experimental Animals (data from OECD (*4*) and ATSDR (*3*)).

Species	Route	Dose/Duration	Findings (g/kg bw/d)	Study
Rat	Oral	1%-50% in drinking water for 140 d	NOAEL 13.2 (equiv to 10% in water)	Seidenfield and Hanzlik (*73*)
	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. (*72*)
	Inhalation	321 ppm for 90d	Enlarged goblet cells/thickened tracheal epithelium	Suber et al. (*76*)
	Inhalation	18 months [0.17–0.35 mg/L] continuous exposure	LOAEL 112 ppm (50% increase in body weight)	Robertson (*77*)
Rabbit	Dermal	0.52 g/one time	NOAEL 0.52 one time	Clark et al. (*78*)
	Inhalation	10% for 20 min or 120 min	Increased degenerated goblet cells @ 20 min&120 min	Konradova et al. (*79*)
Monkey	Inhalation	32-112 ppm. 13 months	LOAEL 112 ppm (increased hemoglobin)	Robertson (*77*)
Cat	Oral	0.080-4.24 g/kg/d in feed for 2-3 months	LOAEL 0.424 NOAEL 0.080 (Heinz body formation)	REVIEWED BY OECD (*4*)
	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. (*80*)
	Oral	1.6 g/kg/d for 5 wks or 8.0 g/kg/d for 22 d	Low dose, anion gap; high dose polyuria/polydypsia, ataxia, depression.	Christopher et al. (*33*)
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. (*74*)

Dermal exposure

Propylene glycol was tested on the clipped skin of New Zealand rabbits according to three protocols (the cosmetic protocol, the Association Francaise de Normalization protocol, and the Organization for Economic Cooperation and Development protocol), in all three tests propylene glycol was classified as a nonirritant (*22*).

Strength/Weaknesses: It is of dubious relevance to have negative results in rabbits regarding irritation when the irritation potential, although minimal, of propylene glycol has been established in man.

Utility (Adequacy) for CERHR Evaluation Process: None.

Inhalation exposure

ATSDR review (*3*) 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 (*79*). However, a subchronic exposure study in rats (*76*) did not support these findings. Rats exposed to 321 ppm over 90 days had thickened respiratory epithelium and enlarged goblet cells (*76*). 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 increased; there were no adverse effects noted on body weight or on the renal, respiratory, gastrointestinal, hepatic, and endocrine systems (*3*).

Strength/Weaknesses: Konradova et al. (*79*) 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 (*76*).

Utility (Adequacy) for CERHR Evaluation Process: None

The Suber et al. (*76*) 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 to 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 we know from Bau et al. (*32*) 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 which confirms the view arrived at for kinetic reasons that exposure by inhalation to propylene glycol will not be a significant toxicological problem.

Robertson et al. (*77*) examined 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 one 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 or not 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 has an unusual way to report data 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 is always important evidence.

Hematological effects

Results from animal studies indicate that intermediate and chronic exposure to propylene glycol may lead to hemolysis of red blood cells. 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 red blood cells were observed in male rats (*76*). In Rhesus monkeys, continuous exposure to concentrations of propylene glycol in air up to 112 ppm for 13 months caused increased hemoglobin counts compared to the control animals (*77*). After exposure of rats to 5% propylene glycol in the diet for 2 years, there were no hematological effects noted (*72*). However, Saini et al (*81*) **[reviewed by OECD, 2001]** found that a single oral dose given to female Wistar rats of either 0.73 or 2.94 g/kg, produced a reversible, statistically significant decrease in hemoglobin, packed cell volume, and red blood cell counts for 2 days. Electron microscopy revealed a rough red blood cell surface.

Cats exposed to oral administration of propylene glycol developed Heinz bodies in red blood cells and decreased red blood cell survival (*82*) (*80*) (*74*). Heinz bodies are composed of denatured proteins, primarily hemoglobin. Cats exposed orally to 1.2, 1.6, 2.4, and 3.6 g/kg of propylene glycol for 2, 5, or 17 weeks developed increased numbers of red blood cells with Heinz bodies. The cat is very sensitive to propylene glycol toxicity, with a 0.44 mg/kg/d dose reported to result in Heinz body formation in erythrocytes (reviewed by OECD (*4*)). This sensitivity is at concentrations that had been present in soft moist cat foods and lead FDA to remove propylene glycol from cat foods in 1996 (*7*).

Strength/Weaknesses: There are few and inconsistent changes in hematologic parameters in the Suber et al. (*76*) study. No inferences can be made for erythropoiesis.

Utility (Adequacy) for CERHR Evaluation Process: None

Increased hemoglobin concentration can be a sign of enhanced destruction of erythrocytes but the Robertson et al. (*77*) study has a very large uncertainty attached to it as discussed earlier.

Utility (Adequacy) for CERHR Evaluation Process: Since the hemolytic capability of propylene glycol was demonstrated *in vitro* in human erythrocytes (*69*), these primate data could be viewed as a red flag but certainly not as proof.

Strength/Weaknesses: Saini et al. (*81*) reported hematologic effects of propylene glycol in rats administered single doses of 0.7 or 3 g/kg by gavage. There is sufficient experimental detail given to deem the results reliable. However, Gaunt et al. (*72*) did not find any hematologic effect after feeding about 2 g/kg/day for 2 years. It is very likely that the acute changes seen by Saini et al. (*81*) have been overcome by 2 years due to adaptation.

Utility (Adequacy) for CERHR Evaluation Process: This is a useful report confirming 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: Christopher et al. (*82*) reported D-lactic acidosis and Heinz body formation in cats administered daily 1.6 or 8g/kg propylene glycol for up to 35 days. Authors conclusively demonstrated a dose-dependent reduction of erythrocyte survival.

Utility (Adequacy) for CERHR Evaluation Process: Excellent study establishing a plausible mechanism for propylene glycol-induced hemolysis.

Strength/Weaknesses: Bauer et al. (*80*) in essence confirms the findings of Christopher et al. (*82*) and refines the dose response on Heinz body formation and erythrocyte survival.

Utility (Adequacy) for CERHR Evaluation Process: Important confirmatory evidence for the impairment of hematopoiesis by propylene glycol.

Strength/Weaknesses: This is a very reliable study in dogs (*74*) conducted by toxicologists of very high reputation. The only significant findings were at 5g/kg/day of propylene glycol after 2 years; hemoglobin, hematocrit and total erythrocyte count were lower, whereas poikilocytes and reticulocytes were increased. These results are compatible with increased hemolysis.

Utility (Adequacy) for CERHR Evaluation Process: Hemolysis potential of high doses of propylene glycol, which is a plausible effect, is firmly established in two species (cat, 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 (*3*).

2.3.2 Experimental systems

In Vitro

ATSDR (*3*) provided the following summary of *in vitro* genotoxicity studies of propylene glycol:

> "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. Genotoxicity of Propylene Glycol *in Vitro* (from ATSDR (*3*)).

Species (test system)	**End point**	**Results with activation**	**Results without activation**	**Reference**
Prokaryotic organisms: *S. typhimurium*	Gene mutation	Negative	Negative	Clark et al. (*78*)
S. typhimurium	Gene mutation	Negative	Negative	Pfeiffer and Dunkelberg (*83*)
Mammalian cells:				
Human fibroblasts	Chromosome aberrations	Negative	Negative	Tucker et al. (*84*)[a]
Chinese hamster cells	Chromosome aberrations	Negative	Negative	Tucker et al. (*84*)
Chinese hamster lung cells	DNA damage	Negative	Negative	Swenberg et al. (*85*)

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 (*83*). A modified Ames test used histidine-dependent Salmonella typhimurium strains TA98, TA100, TA1535, TA1537. Propylene glycol (98 % purity, diluted in water, test volume, 0.1 ml) was added to 2 ml distilled water and 0.1ml (10^8) bacteria. This mixture was added to 2 ml Topagar and poured into a Petri dish containing histidine-free agar, incubated 48 h, 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 studied mutagenicity of ethylene oxides, propylene oxide, of various halo-alcohols, and of several glycols. The test systems used were those normally used for Salmonella typhimurium strains TA98, T4100, TA 1535 and TA 1537 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, whereas the glycols were uniformly negative. There are no weaknesses apparent in these experiments.

Utility (Adequacy) for CERHR Evaluation Process: This is experimental confirmation of the expected and the plausible.

Propylene glycol was one of the chemicals evaluated by Swenberg et al. (*85*) 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 h, then the radioactivity was removed and the cells were incubated for 4-20 h in non-radioactive medium. Cells were then exposed to test chemicals for up to 4 h 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 h 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: Clastogencity of a large number of compounds was tested by an *in vitro*/alkaline DNA elution assay (*85*). This is just a footnote in a table containing a long list of chemicals. The complete lack of experimental detail regarding propylene glycol diminishes its value.

Utility (Adequacy) for CERHR Evaluation Process: Very little utility although it confirms the expected and the plausible.

Propylene glycol is listed as a chemical giving a negative results in the sister chromatid exchange assay using normal human fibroblast cells. The highest concentration tested was 0.1 molar (*84*). **[Details of this assay were not given in this publication.]**

Strength/Weaknesses: Sister chromatid exchange was tested with a huge number of chemicals as reviewed by Tucker et al. (*84*). Propylene glycol was found to be negative in this test system.

Utility (Adequacy) for CERHR Evaluation Process: It is enough 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 (*86*). 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 (DMSO solvent, 32 mg/ml maximum non-cytotoxic dose) and positive in the chromosomal aberration test (max. dose 32 mg/ml). A chemical is positive in the chromosomal aberration test if the total incidence of cells with aberrations is 10% or more. For propylene glycol in saline, 38% of cells had aberrations after 48 hrs and the incidence of polyploid cells was reported to be 1%. These results were not discussed further by the authors.

Strength/Weaknesses: A large number of food additives was screened for mutagenicity and clastogenicity (*86*). The Ames test was conducted in the usual *Salmonella typhimurium* strains and chromosomal aberrations 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: Not useful because the biological significance of these *in vitro* data are not clear.

FDA (*87*) submitted propylene glycol for mutagenic evaluation **[discussed in the *in vivo* section, below].** 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, 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* (*87*). There is sufficient experimental detail to satisfy any doubting mind that propylene glycol is neither mutagenic nor clastogenic.

Utility (Adequacy) for CERHR Evaluation Process: It confirms the expected and is plausible.

In Vivo

Propylene glycol was tested in the mouse micronucleus test along with 38 other food additives (*88*). The micronucleus test was conducted in 8 week old ddY mice (6/dose group). Animals were injected (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 (PCEs) per mouse were scored under 100x power and the number of micronucleated polychromatic erythrocytes (MNPCEs) 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 the 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.

Strength/Weaknesses: Propylene glycol was negative in the micronucleus test (*88*). A large dose range (2.5 to 15.0 g/kg) 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: *In vivo* confirmation for a lack of clastogenicity of propylene glycol.

Table 2-6. Results of the Micronucleus Test using Mouse Bone Marrow Cells (*88*).

Propylene glycol, saline, ip	MNPCEs (%)	PCEs (%)	Mortality	Trend Test
0 mg/kg	0.20+/-0.19	43.9+/-12.2	0/6	NS*
2500	0.20+/-0.18	53.6+/-9.2	0/6	
5000	0.17+/-0.10	52.8+/-6.3	0/6	
10000	Mortality	mortality	6/6	

*NS; non-significant

FDA submitted propylene glycol for mutagenic evaluation (*87*) 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 above.

In the host mediated assay (*in vivo*, mice) doses of propylene glycol at 30, 2500, 5000 mg/kg and negative control of saline, positive controls of 350 mg/kg ethyl methane sulfonate and 100 mg/kg dimethyl nitrosamine were tested. Acute studies (1 dose by gavage of chemical, followed by ip inoculation with *S. typhimurium* 30 min after dosing) produced no significant increases in mutation frequencies with Salmonella TA-1530 and with all levels of Salmonella G-46, except

the 5000 mg/kg level, which produced a weak/questionable positive. 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 min 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, if was concluded by the authors 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, 2500, 5000 mg/kg, and a negative control of saline, and a positive control of 0.3 mg/kg 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, 2500, 5000 mg/kg, and a negative control of saline, and a positive control of 0.3 mg/kg triethylene melamine were tested. Propylene glycol produced no significant increases in aberrations of the bone marrow cells when administered orally at these dosage levels.

Strength/Weaknesses: The Litton Biogenics, Inc. (*87*) report has already been discussed under the *in vitro* section. The 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: Everything that could be done in 1974 was done for propylene glycol. Therefore, its lack of genotoxicity was clear even then.

Table 2-7. *In Vivo* Genotoxicity Results (*87*).

Assay	Dose of Propylene Glycol	Endpoint	Result
Host mediated assay, mice	30, 2500, 5000 mg/kg	Increase in mutation frequencies – Salmonella TA-1530 and G-46, Saccharomyces D3	Negative
Dominant Lethal Assay, male rats treated	30, 2500, 5000 mg/kg	Increase in % dead implants in pregnant, untreated female	Negative
Cytogenetics studies, rats	30, 2500, 5000 mg/kg	Chromosome aberrations (bone marrow)	Negative

2.4 Carcinogenicity

2.4.1 Human Data

No human data on carcinogenicity in humans were identified

2.4.2 Experimental Animal Data

A very limited number of experimental studies on the carcinogenic potential of propylene glycol were identified.

Oral Exposure

ATSDR (*3*) cites a long-term dietary toxicity study in rats by Gaunt et al. (*72*) [see section 2.2, General Toxicity]. Rats were fed propylene glycol up to 5% (2500 mg/kg/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 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.]** 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 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. 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 for male rats and 1.7 g/kg for female rats **[highest dose used].**

Strength/Weaknesses: Gaunt et al. (*72*) 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.

Dermal Exposure

In skin painting studies Stenback and Shubik (*89*) 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, 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 by the authors, but not described in the text. The authors concluded that there was no increase in dermal tumors in female Swiss mice, or change in longevity after chronic treatment with propylene glycol.

Strength/Weaknesses: Stenback and Shubik (*89*) 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 dimethylbenzanthracene. 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 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 moderately useful study the outcome of which could have been predicted.

2.5 Potentially Sensitive Subpopulations

Data on sensitive subpopulations are primarily associated with individuals with compromised liver or kidney function. As discussed in the section on Toxicokinetics, 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 function of the liver or kidney would be at increased risk for developing propylene glycol toxicity (*42*). In patients with renal insufficiency, high propylene glycol levels have been associated with lactic acidosis (hyperlactemia) (*90*) (*91*). Propylene glycol has been found in the blood of alcoholics with cirrhosis of the liver, without detectable measurable blood alcohol levels (*92*).

Propylene glycol toxicity can be suspected in patients having an abnormal serum osmolal gap[1]. A review of the patient's medication history will often identify that propylene glycol was used as a vehicle in the medications administered. The following information has been taken primarily from data presented in clinical case studies; some examples of clinical cases of suspected propylene glycol toxicity are summarized in Table 2-8 below.

Oral and Intravenous Use

In some individuals with a chemical sensitivity to propylene glycol, oral or parenteral administration may exacerbate dermatitis (*93*).

Propylene glycol is used as a vehicle for intravenous administration of drugs such as lorazepam, etomidate, phenytoin, diazepam, digoxin, hydralazine, esmolol, chlordiazepoxide, multivitamins, nitroglycerin, pentobarbital sodium, phenobarbital sodium and trimethoprim-sulfamethoxazole

[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.

and is a vehicle for some intravenous vitamin preparations. Serum concentrations of propylene glycol received through intravenous medications have been shown to correlate with serum lactate concentrations (*94*). In children, seizures and respiratory depression have occurred after taking liquid medications containing propylene glycol (*95*) (*96*).

Infants
The decreased size of premature infants and an increased serum half-life **[see Toxicokinetics section]** for propylene glycol in premature infants (*29*) (*30*) would predispose them to a greater probability of toxic effects from over-administration of propylene glycol. Particular concern would be for very small infants and those receiving multiple intravenous 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 (*30*) (*62*), in one case associated with cardiorespiratory arrest (*62*).

In one report, propylene glycol was shown to have a longer (16.9 hr) half-life in a premature infant when compared with the half-life in adults (5 h) (*29*). Glasgow (*30*) measured the serum half-life in infants. Ten infants received 10 ml IV of daily multivitamin preparation once a day for five days. Four infants had a serum level >3.0 g/L propylene glycol. The range of serum values was 0.65-9.5 g/L. In the control group, propylene glycol was not detected in six infants; two other infants had propylene glycol serum levels of 0.7g/L. 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 h with a range of 10.8-30.5 hours.

Propylene glycol is commonly used as a vehicle in topical, oral, or injectable medications. Since it is a GRAS chemical (*13*), it is not required to be listed as an ingredient on the package insert. 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. At present, labeling is voluntary and specific inert ingredients may not be listed, or may be listed under a general term such as ‘emulsifier’ or ‘humectant’ (*97*).

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

Patient	Route	Findings
8 mo old male infant (Fligner (*29*))	Dermal; silver sulfadiazine therapy in propylene glycol for burns, 78% surface area, 10.6 g/L PG serum level	Cardiopulmonary arrest, respiratory acidosis, increased osmolal gap
3.4 kg infant, cardiac surgery, heart failure (Huggon (*62*))	IV, PG vehicle in enoximone and glyceryl trinitrate infusions	Hyperosmolality
Premature infants (MacDonald (*98*))	IV, propylene glycol as part of a daily multivitamin preparation, 3g/d PG (alternative product delivering 0.3g/d PG had no effect on other premature infants)	Seizures
Premature infant, 27 wk gestn.(Glasgow (*30*))	IV, propylene glycol as part of a daily multivitamin preparation, 9.3 g/L PG serum level	Serum hyperosmolality, acute renal failure
11 yr old boy, candidiasis-endocrinopathy syndrome with hypoparathyroidism (Arulanantham (*96*))	Oral, PG vehicle in dihydrotachysterol	Seizures
16 yr old boy, onset of seizures (Yorgin et al (*99*))	IV, PG vehicle in pentobarbital and phenobarbital	Exacerbation of seizures, reversible acute renal failure
39 yr old woman, history of seizures (Lolin (*100*))	Most likely ingestion, 4 g/L PG serum level	Status epilepticus, , metabolic acidosis, plasma hyperosmolality, respiratory depression
45 yr old man, , respiratory distress, on ventilator (Arbour (*63*))	IV, PG vehicle in lorazepam, 1.7 g/L PG serum level	Hyperosmolality, metabolic acidosis
58 yr old man, renal disease, chronic schizophrenia (Cate (*60*))	Most likely ingestion, 0.7 g/L PG serum level	Unconscious, lactic acidosis, azotemia
60 yr old man, respiratory distress, on ventilator (Arbour (*42*))	IV, PG vehicle in lorazepam, infused for 5 d at 2.5 g PG/hr	Hyperosmolality
70 yr old woman, complications with surgery (Bedichek & Kirschbaum (*101*))	IV, 479 g PG administered with etomidate and other medications over a 24 hr period	Seizures, status epilepticus

2.6 Summary

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 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 is 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 one hour of dosing and the average serum half-life was estimated to be from 1-4 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) (*24*). 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 (*26*). Glasgow et al. (*30*) reported an average half-life in 10 infants of 19.3 hours (range 10.8 to 30.5 hours), which is about 10 times longer than in adults. Alcohol dehydrogenase activity is up to 10 times lower in infants (*26*) 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 distribution of propylene glycol in humans and animals, demonstrating that it distributes into total body water. In human studies, volumes of distribution were measured at 0.52 L/kg with oral dosing (*23*), and 0.77-0.79 L/kg with rectal exposure (*24*), and approximately 0.55-0.94 L/kg with intravenous exposure (*25*). 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, with metabolic clearance accounting for >85% of total clearance. Renal excretion is a small percentage of the dose and excretion can be of propylene glycol or the glucuronidated form. Morshed et al (*35*) 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. (*25*) that humans clear propylene glycol similar 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. (*25*) and Yu et al. (*23*) 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 (S.D.). 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 (*45*).

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.

General Toxicity

Propylene glycol has very low systemic toxicity in experimental animals and very high doses are used to determine a toxic level (*3*) (*22*) (*4*). 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 infants after repeated exposure to propylene glycol in medication (see Potentially Susceptible 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, See Table 2-3) reported at very high oral doses.

In a 2 year study by Gaunt et al. (*72*) an average daily dose of 1.7 g/kg in male rats and 2.1 g/kg in female rats had no adverse effect on body weight gain, mortality, hematology, urinary cell excretion, renal function, serum chemistry and absolute and relative organ weights. Weil et al. (*74*) studied the toxicity of propylene glycol fed in the diet to dogs at 2 and 5 g/kg/d for two 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/d is a NOAEL for dogs and rats; 5g/kg/d is a LOAEL for dogs.

In a continuous inhalation study, Robertson et al. (*77*) examined chronic toxicity of propylene glycol (55-113 ppm) in Rhesus monkeys and rats for up to one 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 red blood cells. Cats exposed to oral administration of propylene glycol developed Heinz bodies in red blood cells and decreased red blood cell survival. Doses as low as 0.44 mg/kg/d have resulted in Heinz body formation in cat erythrocytes (*4*). In a study in dogs fed 5 g/kg/d for 2 years (*74*) evidence of red blood cell 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.

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.

Carcinogenicity

No human data on carcinogenicity in humans were identified.

Gaunt et al. (*72*) reported a 2 year bioassay where rats were fed up to 5% (2500 mg/kg/day) propylene glycol in their diet. No treatment-related neoplasms were noted. The Panel concluded that propylene glycol does not cause cancer at or near a toxic level administered in the diet.

Potentially Sensitive Subpopulations

There have been reports of propylene glycol toxicity in individuals with compromised liver or kidney function and in infants which 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. (*29*) reported at half-life of 16 h for a premature infant as compared to 5 h in adults. Glasgow (*30*) measured serum half-life in ten infants. Mean half-life of propylene glycol was calculated to be 19.3 h. with a range of 10.8-30.5 hr which is about 10 times longer than in adults. Alcohol dehydrogenase can be up to 10 times lower in infants, which would account for the prolonged half-life in infants.

Information is not available on the frequency of adverse events occurring as a result of propylene glycol intoxication. However, the few cases reviewed in this report and the request of the American Academy of Pediatrics speak to the need for propylene glycol to be included in the labeling when it is a part of the drug formulation.

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

No human data on developmental toxicity were identified.

3.2 Experimental Animal Data

3.2.1 Oral Exposure

Prenatal and Perinatal Toxicity Studies

FDA (*102*) conducted 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 (PG) as a water solution from gd. 6-15. Observation of the vaginal sperm plug was gd 0. Dose groups were 0, 16, 74.3, 345, and 1600 mg/kg/day. Aspirin at a dose of 150 mg/kg was used as a positive control. Body weights of the dams were recorded on gd 0, 6, 11, 15, 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 dose group survived to term. [No maternal deaths were reported in the other dose groups.] On gd 17 all dams were anesthetized and a Caesarean 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. **[Note that cartilage was not stained]**

The following tables (Tables 3-1 and 3-2) describe findings from the Food and Drug Research Laboratories, Inc. report (*102*).

Table 3-1. Mouse Maternal and Fetal Toxicity Data **[no statistical analyses reported].**

	Sham	Aspirin @ 150 mg/kg	16.0 mg/kg PG	74.3 mg/kg PG	345.0 mg/kg PG	1600.0 mg/kg PG
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						
Ave/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						
Ave/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
Ave. Fetus Wt., gms.	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

Table 3-2. Summary of Mouse Fetal Skeletal and Soft Tissue Findings.*

	Sham	Aspirin @ 150 mg/kg	16.0 mg/kg PG	74.3 mg/kg PG	345.0 mg/kg PG	1600.0 mg/kg PG
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

The following conclusion was reported by the study authors for mice :

"The administration of up to 1600 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."

Rats: Timed-mated Wistar albino rats (25/group) were dosed by oral intubation with propylene glycol as a water solution from gd 6-15. Observation of the vaginal sperm plug was gd 0. Dose groups were 0, 16, 74.3, 345, and 1600 mg/kg/day. Aspirin at a dose of 250 mg/kg was used as a positive control. Body weights of the dams were recorded on gd 0, 6, 11, 15, 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 Caesarean 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. **[Note that cartilage was not stained].** The following tables (Tables 3-3 and 3-4) are from the Food and Drug Research Laboratories, Inc. report **(*102*)**.

Table 3-3. Rat Maternal and Fetal Toxicity Data **[no statistical analyses reported].**

	Sham	Aspirin @ 250 mg/kg	16.0 mg/kg PG	74.3 mg/kg PG	345.0 mg/kg PG	1600.0 mg/kg PG
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						
Ave/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						
Ave/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
Ave. Fetus Wt., gms.	3.39	2.68	3.91	3.73	3.91	3.75
Dead Fetuses						
Total	--	--	--	--	--	--

Table 3-4 Summary of Rat Fetal Skeletal and Soft Tissue Findings*

	Sham	Aspirin @ 250 mg/kg	16.0 mg/kg PG	74.3 mg/kg PG	345.0 mg/kg PG	1600.0 mg/kg PG
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				
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

The following conclusion was reported by the study authors for rats:

"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."

Hamsters: Timed-mated outbred golden hamsters (25/group) were dosed by oral intubation with propylene glycol from gd 6 to gd 10. Observation of motile sperm in the vaginal smear was gd 0. Dose groups were 0, 15.5, 72, 334.5, 1550 mg/ kg/day. Aspirin at a dose of 250 mg/kg/day was used as a positive control. On gd 14, a Caesarean section was 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. **[Note that cartilage was not stained]**.

The following tables (Tables 3-5 and 3-6) contain information from the Food and Drug Research Laboratories, Inc. report (*102*).

Table 3-5. Hamster Maternal and Fetal Toxicity Data [no statistical analyses reported]

	Sham	Aspirin @ 250 mg/kg	15.5 mg/kg PG	72.0 mg/kg PG	334.4 mg/kg PG	1550.0 mg/kg PG
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						
Ave/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						
Ave/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
Ave. Fetus Wt., gms.	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	--	--	--	--	--	--

Table 3-6. Summary of Hamster Fetal Skeletal and Soft Tissue Findings.*

	Sham	Aspirin @ 250 mg/kg	15.5 mg/kg PG	72.0 mg/kg PG	334.4 mg/kg PG	1550.0 mg/kg PG
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						
Hydro-cephalus	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

The following conclusion was reported by the study authors for hamsters:

> "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."

Rabbits: Dutch-belted female rabbits were dosed by oral intubation with propylene glycol from gd 6 to gd 18. Dose groups were 0, 12.3, 57.1, 267, and 1230 mg/kg/day. 6-Aminonicotinamide (2.5 mg/kg) dosed on day 9 was a positive control. On gd 0, each doe received an injection of human chorionic gonadotropin (400 IU) and three hours later was artificially inseminated with diluted donor buck semen. On gd 29 a Caesarean 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. **[Note that cartilage was not stained]**

The following tables (Tables 3-7 and 3-8) contain information from the Food and Drug Research Laboratories, Inc. report (*102*).

Table 3-7. Rabbit Maternal and Fetal Toxicity Data **[no statistical analyses reported].**

	Sham	6-AN*@ 2.5 mg/kg	12.3 mg/kg PG	57.1 mg/kg PG	267 mg/kg PG	1230.0 mg/kg PG
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
Ave/dam	11.1	11.7	10.1	13.6	10.4	13.3
Live Litters						
Total #	11	10	9	11	12	13
Implantation Sites						
Ave/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						
Ave/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
Ave. Fetus Wt., gms.	42.3	32.5	36.4	39.9	42.9	39.0
Dead Fetuses						
Total	--	--	--	--	--	--

* 6-Aminonicotinamide, positive control

Table 3-8. Summary of Rabbit Fetal Skeletal and Soft Tissue Findings.**

	Sham	6-AN*@ 2.5 mg/kg	12.3 mg/kg PG	57.1 mg/kg PG	267 mg/kg PG	1230.0 mg/kg PG
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	
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				
Encephalo-cele		7/1				
Med Rotation of Hindlimbs		17/6				
Umbilical Hernia		1/1				
Scoliosis		1/1				
Harelip		2/2				

** Number of fetuses affected/Number of litters affected, * 6-Aminonicotinamide, positive control.

The following conclusion was reported by the study authors for rabbits:

> "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."

Based upon the conclusions of the study authors, the NOAEL levels for maternal and fetal toxicity of propylene glycol are presented in the following Table 3-9. **[The Expert Panel disagrees with these interpretations.]**

Table 3-9. NOAEL levels for maternal and fetal toxicity of propylene glycol

SPECIES	NOAEL (mg/kg/d)
Mice	1600
Rats	1600
Hamsters	1550
Rabbits	1230

Strengths/Weaknesses: In general, adequate numbers of animals (25 dams per treatment group) were employed in these studies. In most cases, average and percent summaries were provided without associated standard errors preventing an assessment of the statistical significance of differences reported. Differences between the negative control and dose groups were small and likely not statistically different but there were a few cases where it would have helped to be able to perform the formal analysis. 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 for rabbits. Results indicate that aspirin is only mildly teratogenic for mice and hamsters and strongly teratogenic for rats. 6-AN 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 are 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 3 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, larger numbers of wavy ribs and incomplete ossification of the vertebrae were observed at the same level as the positive control suggesting a PG effect. The incidences of these defects did not appear to be related to dose however. No PG effects were observed in rabbits.

Utility (adequacy) for CERHR evaluation process: These data 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 in helping 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 responsive to dose of the compound. The study suggests that the NOAEL level for mice, hamsters and rabbits is at least 1600, 1230, 1550 mg/kg/d, respectively, the levels given in Table 3-9. The appropriateness of the NOAEL level for rats (1600 mg/kg/d) given in Table 3-9 depends on the importance attributed to the rib and vertebrae malformations observed. The general lack of effect gives some measure of comfort, but one wonders if important observations that should have been made were not made. Due to the uncertainty of the quality of these data, the Panel judges these data as presented in this report insufficient to predict human health effects.

Kavlock et al. (*103*) employed an *in vivo* teratology screening procedure to evaluate propylene glycol along with 45 other chemicals. Timed-pregnant CD-1 mice (approx. 60 d 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/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 to be teratogenic in the literature. 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 pnd 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. The following neonatal values were recorded for pup survival and weight (Table 3-10):

Table 3-10. Pup survival and weight after treatment of pregnant CD-1 mice by gavage with propylene glycol (10 g/kg/d) from gd 8-12.

Compound	**PND 1 # live**	**PND 1 wt** (gm)	**PND 3 # live**	**PND 3 wt** (gm)
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 in the only 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 would 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

Prenatal toxicity

Chick eggs
In an early study by Gebhardt (*104*), propylene glycol was found to be teratogenic when injected into chick eggs. Eggs (ave wt 59 gms) 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 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 two 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 3-11 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 3-11. Teratogenic Effect of Propylene Glycol Injected into the Air Chamber of 4 Day Old Chick Embryos (*104*).

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 was performed in a non-mammalian species. An adequate number of embryos was 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 (*105*) further weakening their relevance.

Propylene glycol and dimethyl sulfoxide were compared with water as solvents for teratogens in chick embryos (*105*). 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 (*104*) had previously reported.

Strengths/Weaknesses: The study 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

Embryo culture

Kowalczyk et al. (*106*) 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 h 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 h in medium containing 6 to 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 h. The percentage of embryos cavitating and the blastocoel volume was recorded at 0, 4, 8, and 24 h after removal of the propylene glycol from the medium. All experiments were repeated at least 3 times (43 embryos/treatment group, ave.). 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 which was comparable with controls. Morulae cultured 24 h 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.

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 cryopreservation of human oocytes. In an effort to optimize cryopreservation of oocytes, a number of studies examine methods to improve cryopreservation techniques (*107*) (*108*) (*109*) (*110*) (*111*) (*112*) (*113*) (*114*) **[not reviewed in this report].**

Studies by Damien et al. (*115*) 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 which will not adversely alter the development of the mouse pronuclear stage embryo and to determine the mechanism by which propylene glycol mediates embryo toxicity. Pronuclear mouse zygotes from superovulated B6D2Fi mice were evaluated. **[The number of zygotes and number of organ culture dishes per experiment were not reported.]** 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 (FDA) or Acridine Orange (AO) 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 3-12). 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 3-12. The effect of a 20 min exposure of propylene glycol (PG) on the percentage of zygotes showing FDA and AO fluorescence (*115*).

DYE	**0**	**1.5M PG**	**3.0M PG**	**6.0M PG**
FDA	100(52)	98(50)	81(53)*	5(64)*
AO	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:

Utility (adequacy) for CERHR evaluation process: These data are of little use to the Panel in the CERHR evaluative process.

Hydra Screening Assay
In an evaluation of the utility of the hydra prescreening developmental assay to predict experimental findings in laboratory animals (*116*), 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 700-1000 adult hydra are dissociated mechanically into component cells and randomly re-associated into small pellets (approx. 20 pellets) by gentle centrifugation. After 92 h 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 3-13)."

Table 3-13. Developmental Toxicity of Glycols and Glycol Ethers in Hydra (*116*).

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 study is the use of an invertebrate animal.

Utility (adequacy) for CERHR evaluation process: These data 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

3.4 Summary

Human Data

No human data on developmental toxicity were identified.

Experimental Animal Data

Prenatal developmental toxicity studies were conducted in mice, rats, hamsters and rabbits orally exposed to propylene glycol at the Food & Drug Research Laboratories, Inc. (*102*) under contract for the US Food and Drug Administration. NOAEL levels determined for maternal and fetal toxicity were at the maximum doses used: 1.6 g/kg/d for rats and mice, 1.55 g/kg/d for hamsters and 1.23 g/kg/d 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 Expert 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. (*103*). 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/d. 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 Expert Panel concluded that the available data are insufficient to evaluate the developmental toxicity of propylene glycol.

4.0 REPRODUCTIVE TOXICITY DATA

4.1 Human Data

There are no available data on the reproductive toxicity of propylene glycol in humans.

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 (*117*). 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 (3 males and 6 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" (see Table 4-1). **[Food consumption data are not provided.]** 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 5.4-7.8 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 10% and 20% propylene glycol dose groups) were continued through three additional generations. The animals from each of these groups were subdivided into 3 subgroups containing 3 males and 6 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). These data show that the percentage of females reproducing was 100% for the 0-30% propylene glycol dose groups and the average number of young born per litter ranged from 6.4-9.3 pups for the 0-30% propylene glycol dose groups. **[The authors did not comment on these data and failed to provide information on their statistical analyses.]**

Table 4-1. Composite Responses of Three Generations of Female Rats Produced on Propylene Glycol (PG) in the Diet (*117*).

% PG (w/ w)	# females	# females with litters	# litters born	# pups born	Ave wt of pups (gm)	# of pups weaned	Ave # of pups//dam	Ave # 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

Table 4-2. Composite Responses of Three Generations of Female Rats Produced on Propylene Glycol (PG) in the Diet (*117*).

% PG [a] /%PG [b]	# females	# females with litters	# litters born	# pups born	Ave wt of pups (gm)	# of pups weaned	Ave # of pups//dam	Ave # 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 3 generations
[b] Diet during 3 generation test period

Strengths/Weaknesses: The two rat studies cited above (*77*) and (*117*) were conducted over 50 years ago and 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: There has been one other multigeneration reproductive study on propylene glycol: "Propylene glycol: Reproduction and fertility assessment in CD-1 mice when administered in drinking water" (*118*).

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 (*118*) 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 animals 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]**.

Table 4-3. Summary of body weight and daily water consumption data (task 1).

Dose Group	Sex	# mice	Ave. Body Wt. (g) ±SE Day 0	Ave. Body Wt.(g) ±SE Day 14	% Change in Body Wt.	Ave. Daily Water Intake (g) ±SE Day 0-7	Ave. daily water intake (g) ±SE Day 7-14	Daily dose*
0%	M	8	35.4 ± 0.44	37.3 ± 0.39	+5	5.73 ± 0.24	5.31 ± 0.14	0
	F	8	25.8 ± 0.54	27.9± 0.80	+8	6.25 ± 0.29	6.19 ± 0.39	0
10%	M	8	34.8 ± 0.64	37.3 ± 0.85	+7	8.48 ± 0.37	9.18 ± 0.50	25.09
	F	8	25.2 ± 0.80	29.1 ± 0.95	+15	10.1 ± 0.56	9.57 ± 0.79	37.13

* (g/kg bw on day 7)

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 three 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, 10.1 gm/kg body weight/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 humanely 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 is 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 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 are 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 body weight). 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 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. (*119*).

Strengths/Weaknesses: The NTP multi-generational study (*118*) 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:

4.3 Utility of Data

4.4 Summary

Human Data

There are no available data on the reproductive toxicity of propylene glycol in humans.

Experimental Animal Data

The studies by Guerrant et al. (*117*) in rats were conducted over 50 years ago and prior to GLP protocols. Many experimental details were not provided and the results are judged by the Panel to be inconclusive.

In the NTP multi-generation study (*118*), 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 and 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, and 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 (one wk prior to cohabitation, 14 wks during cohabitation, and 3 wks after cohabitation) or in their progeny. These data are judged by the Panel to be relevant to consideration of human risk.

5.0 SUMMARIES, CONCLUSIONS, and CRITICAL DATA NEEDS

To be completed during the Expert Panel meeting.

5.1 Summary and Conclusions of Reproductive and Developmental Hazards

Start text here.

5.2 Summary of Human Exposure

5.3 Overall Conclusions

5.4 Critical Data Needs

6.0 REFERENCES

1. Chemfinder. Propylene glycol. 2002.
2. HSDB. Propylene glycol. National Library of Medicine; 2002.
3. ATSDR AfTSaDR--. Toxicological profile for ethylene glycol and propylene glycol. Atlanta, GA: ATSDR; 1997.
4. OECD. SIDS Initial assessment report for 11th SIAM. 2001.
5. Castle L, Cloke HR, Crews C, Gilbert J. The migration of propylene glycol, mono-, di-, and triethylene glycols from regenerated cellulose film into food. Z Lebensm Unters Forsch 1988;187:463-7.
6. FDA. Generally recognized as safe. Food and Drug Adminstration. Code of Federal Regulation 1982;21CFR184.1666.
7. FDA. GRAS status of propylene glycol; exclusion of use in cat food. Federal Register 1996;61.
8. Holman NW, Jr, Mundy RL, Teague RS. Alkyldiol antidotes to ethylene glycol toxicity in mice. Toxicology and Applied Pharmacology 1979;49:385-392.
9. USEPA. National pollutant discharge elimination system permit application regulations for storm discharges. 40 CFR Parts 122, 123, and 124. Federal Register 1990;55:47990.
10. Kolpin D, Furlong E, Meyer M, et al. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environ Sci Technol 2002;36:1202-1211.
11. Louekari K, Scott AO, Salminen S. Estimation of Food Additive Intake. Food additives, edited by Branen A.L., Davidson P.M. New York: Marcel Dekker, Inc., 1990.
12. Hodgson AT, Wooley JD, Daisey JM. Emissions of volatile organic compounds from new carpets measured in a large-scale environmental chamber. Air & Waste 1993;43:316-324.
13. FDA. Propylene glycol and propylene glycol monostearate. Food and Drug Administration. Federal Register 1977;42:30865-330866.
14. WHO. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. Geneva: World Health Organization; 1974.
15. (AIHA-WEEL) HoCG. American Industrial Hygiene Association (AIHA) Workplace Environmental Exposure Level (WEEL) Guide of 50 ppm has been determined. (Kirk-Othmer, 1992). 2002.
16. Laitinen J, Liesivuori J, Savolainen H. Exposure to glycols and their renal effects in motor servicing workers. Occup Med (Lond) 1995;45:259-62.
17. Wieslander G, Norback D, Lindgren T. Exeprimental exposure to propylene glycol mist in aviation emergency training: acute ocular and respiratory effects. Occup Environ Med 2001;58:649-655.
18. Corsi SR, Hall DW, Geis SW. Aircraft and runway deicers at General Mitchell International Airport, Milwaukee, Wisconsin, USA. 2. Toxicity of aircraft and runway deicers. Environ Toxicol Chem 2001;20:1483-90.
19. Klecka GM, Carpenter CL, Landenberger BD. Biodegradation of aircraft deicing fluids in soil at low temperatures. Ecotoxicol Environ Saf 1993;25:280-95.
20. NIOSH NIfOSH--. HETA 95-0069. Denver, CO: Denver Federal Center; 1997.
21. Sills RD, Blakeslee PA. The environmental impact of deicers in airport stormwater runoff. . Boca Raton, FL, 1992.
22. LaKind JS, McKenna EA, Hubner RP, Tardiff RG. A review of the comparative mammalian toxicity of ethylene glycol and propylene glycol. Crit Rev Toxicol 1999;29:331-65.
23. Yu DK, Elmquist WF, Sawchuk RJ. Pharmacokinetics of propylene glycol in humans during multiple dosing regimens. J Pharm Sci 1985;74:876-9.
24. Kolloffel WJ, Weekers LE, Goldhoorn PB. Pharmacokinetics of propylene glycol after rectal administration. Pharm World Sci 1996;18:109-13.
25. Speth PA, Vree TB, Neilen NF, et al. Propylene glycol pharmacokinetics and effects after intravenous infusion in humans. Ther Drug Monit 1987;9:255-8.
26. Pikkarainen PH, Raiha NCR. Development of alcohol dehydrogenase activity in the human liver. Pediatr Res 1967;1:165-8.

27. MacKee GM, Sulzeberger MB, Herrmann F, Baer RL. Histologic studies on percutaneous penetration with special reference to the effect of vehicles. Journal of ingestigative dermatology 1945;6:43-61.
28. Kulick MI, Wong R, Okarma TB, Falces E, Berkowitz RL. Prospective study of side effects associated with the use of silver sulfadiazine in severely burned patients. Ann Plast Surg 1985;14:407-19.
29. Fligner CL, Jack R, Twiggs GA, Raisys VA. Hyperosmolality induced by propylene glycol. A complication of silver sulfadiazine therapy. JAMA 1985;253:1606-9.
30. Glasgow AM, Boeckx RL, Miller MK, MacDonald MG, August GP, Goodman SI. Hyperosmolality in small infants due to propylene glycol. Pediatrics 1983;72:353-5.
31. Takeuchi Y, Yasukawa H, Yamaoka Y, et al. Behavior of propylene glycol (PG) in dermis after treatment of rat intact skin surfacwe with fatty acids, fatty amines or azone dissolved in PG. Biol. Pharm. Bull. 1995;18:304-309.
32. Bau SK, Aspin N, Wood DE, Levison H. The measurement of fluid deposition in humans following mist tent therapy. Pediatrics 1971;48:605-12.
33. Christopher MM, Eckfeldt JH, Eaton JW. Propylene glycol ingestion causes D-lactic acidosis. Lab Invest 1990;62:114-8.
34. Morshed KM, L'Helgoualch A, Nagpaul JP, Amma MK, Desjeux JF. The role of propylene glycol metabolism in lactatemia in the rabbit. Biochem Med Metab Biol 1991a;46:145-51.
35. Morshed KM, Nagpaul JP, Majumdar S, Amma MK. Kinetics of propylene glycol elimination and metabolism in rat. Biochem Med Metab Biol 1988;39:90-7.
36. Lehman AJ, Newman HW. Propylene glycol: Rate of metabolism absorption, and excretion, with a method for estimation in body fluids. San Francisco, CA: Department of Pharmacology and Division of Neuropsychiatry, Department of Medicine, Stanford University School of Medicine; 1937.
37. Morshed KM, Desjeux JF, Nagpaul JP, Majumdar S, Amma MK. The effect of propane-diols on the intestinal uptake of nutrients and brush border membrane enzymes in the rat. Biochem Med Metab Biol 1991;45:161-70.
38. Heilmair R, Lenke W, Lohr D. Toxicokinetics of diethylene glycol (DEG) in the rat. Arch Toxicol 1993;67:655-666.
39. Lenk W, Lohr W, Sonnenbichler J. Pharmacokinetics and biotransformation of diethylene glycol and ethylene glycol in the rat. Xenobiotica 1989;19:961-979.
40. Takeuchi Y, Yasukawa H, Yamaoka Y, et al. Effects of oleic acid/propylene glycol on rat abdominal stratum corneum: Lipid extraction and appearance or propylene glycol in the dermis measured by Fourier Transform Infrared/Attenuated Total Reflectance (FT-IR/ATR) spectroscopy. Chem. Pharm. Bull. 1993;41:1434-1437.
41. Morshed KM, Nagpaul JP, Majumdar S, Amma MK. Kinetics of oral propylene glycol-induced acute hyperlactatemia. Biochem Med Metab Biol 1989;42:87-94.
42. Arbour R, Esparis B. Osmolar gap metabolic acidosis in a 60-year-old man treated for hypoxemic respiratory failure. Chest 2000;118:545-6.
43. Ruddick JA. Toxicology, metabolism, and biochemistry of 1,2-propanediol. Toxicol Appl Pharmacol 1972;21:102-11.
44. Yu DK, Sawchuk RJ. Pharmacokinetics of propylene glycol in the rabbit. J Pharmacokinet Biopharm 1987;15:453-71.
45. Wittman JS, 3rd, Bawin RR. Stimulation of gluconeogenesis by propylene glycol in the fasting rat. Life Sci 1974;15:515-24.
46. Miller ON, Bazzano G. Propanediol metabolism and its relation to lactic acid metabolism. Ann N Y Acad Sci 1965;119:957-73.
47. Huff E. The metabolism of 1,2-propanediol. Bethesda, MD: National Institute of Allergy and Infetious Diseases, NIH; 1961.
48. Oh MS, Uribarri J, Alveranga D, Lazar I, Bazilinski N, Carroll HJ. Metabolic utilization and renal handling of D-lactate in men. Metabolism 1985;34:621-5.
49. Pares X, Farres J, Vallee BL. Organ specific alcohol metabolism: placental chi-ADH. Biochem Biophys Res Commun 1984;119:1047-55.

50. Zorzano A, Herrera E. Differences in the kinetic properties and sensitivity to inhibitors of human placental, erythrocyte, and major hepatic aldehyde dehydrogenase isoenzymes. Biochem Pharmacol 1990a;39:873-8.
51. Sjoblom M, Pilstrom L, Morland J. Activity of alcohol dehydrogenase and acetaldehyde dehydrogenases in the liver and placenta during the development of the rat. Enzyme 1978;23:108-15.
52. Smith M, Hopkinson DA, Harris H. Developmental changes and polymorphism in human alcohol dehydrogenase. Ann Hum Genet 1971;34:251-71.
53. Zorzano A, Herrera E. Differences in kinetic characteristics and in sensitivity to inhibitors between human and rat liver alcohol dehydrogenase and aldehyde dehydrogenase. Gen Pharmacol 1990b;21:697-702.
54. Zorzano A, Herrera E. In vivo ethanol elimination in man, monkey and rat: a lack of relationship between the ethanol metabolism and the hepatic activities of alcohol and aldehyde dehydrogenases. Life Sci 1990c;46:223-30.
55. Agarwal DP. Genetic polymorphisms of alcohol metabolizing enzymes. Pathol Biol (Paris) 2001;49:703-9.
56. Bosron WF, Li TK. Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases, and their relationship to alcohol metabolism and alcoholism. Hepatology 1986;6:502-10.
57. Pietruszko R. Alcohol and aldehyde dehydrogenase isozymes from mammalian liver-their structural and functional differences. Isozymes Curr Top Biol Med Res 1980;4:107-30.
58. Burnell JC, Li TK, Bosron WF. Purification and steady-state kinetic characterization of human liver β3β3 alcohol dehydrogenase. Biochemistry 1989;28:6810-6815.
59. USEPA. Research and development: health and environmental effects document for propylene glycol. Washington, DC: Office of Solid Waste and Emergency Response; 1987.
60. Cate JC, Hedrick R. Propylene glycol intoxication and lactic acidosis. N Engl J Med 1980;303.
61. Doull Ca. Casarett and Doull's toxicology: the basic science of poisons. Fifth Edition.: McGraw-Hill; 1996.
62. Huggon I, James I, Macrae D. Hyperosmolality related to propylene glycol in an infant treated with enoximone infusion. BMJ 1990;301:19-20.
63. Arbour RB. Propylene glycol toxicity related to high-dose lorazepam infusion: case report and discussion. Am J Crit Care 1999;8:499-506.
64. Shanahan R. The dermatopharmacologic activity of propylene glycol in selected cosmetic formulations. Graduate Division - College of Pharmacy and Allied Health Professions. Jamaica, NY: St. John's University, 1977.
65. Mortensen B, Nordic chemicals g. Health effects of selected chemicals 2. Propylene glycol. TA:Nord PG 1993:29.
66. Hannuksela M, Forstrom L. Reactions to peroral propylene glycol. Contact Dermatitis 1978;4:41-5.
67. Catanzaro JM, Smith JG. Propylene glycol dermatitis. Journal of the American Academy of Dermatology 1991;24:90-95.
68. Cohen BM, Crandall C. Physiologic benefits of "thermo fog" as a bronchodilator vehicle: Acute ventilation responses of 93 patients. The American Journal of the Medical Sciences 1964;247:57-61.
69. Weatherby JH, Haag HB. Toxicity of propylene glycol. Journal of the American Pharmaceutical Association 1938;27:466-471.
70. Braun HA, Cartland GF. The toxicity of propylene glycol. Journal of the American Pharmaceutical Association 1936;25:746-749.
71. Morris HJ, Nelson AA, Calvery HO. Observations on the chronic toxicities of propyplene glycol, ethylene glycol, diethyl glycol, ethylene glycol mono-ethyl-ether, and diethylene glycol mono-ethyl-ether. Washington, DC: Food and Drug Administration; 1942.
72. Gaunt IF, Carpanini FM, Grasso P, Lansdown AB. Long-term toxicity of propylene glycol in rats. Food Cosmet Toxicol 1972;10:151-62.
73. Seidenfeld MA, Hanzlik PJ. The general properties, actions and toxicity of propylene glycol. 1931.
74. Weil CS, Woodside S, Smyth HF. Results of feeding proplene glycol in the diet to dogs for two years. Food Cosmet Toixicol 1971;9.

75. Van Winkle W, Newman HW. Further results of continued administration of propylene glycol. Food Research 1941;6:509-515.
76. Suber RL, Deskin R, Nikiforov I, Fouillet X, Coggins CR. Subchronic nose-only inhalation study of propylene glycol in Sprague-Dawley rats. Food Chem Toxicol 1989;27:573-83.
77. Robertson OH, Loosli CG, Puck TT, Wise H, Lemon HM, Lester WJ. Tests for the chronic toxicity of propylene glycol and triethylene glycol on monkeys and rats by vapor inhalation and oral administration. J Pharmacol Exper Therap 1947;91:52-76.
78. Clark CR, Marshall TC, Merickel BS, Sanchez A, Brownstein DG, Hobbs CH. Toxicological assessment of heat transfer fluids proposed for use in solar energy applications. Toxicol Appl Pharmacol 1979;51:529-35.
79. Konradova V, Vavrova V, Janota J. Effect of the inhalation of a surface tension-reducing substance (propylene glycol) on the ultrastructure of epithelium of the respiratory passages in rabbits. Folia Morphol (Praha) 1978;26:28-34.
80. Bauer MC, Weiss DJ, Perman V. Hematologic alterations in adult cats fed 6 or 12% propylene glycol. Am J Vet Res 1992;53:69-72.
81. Saini M, Amma MK, Dash S, Nagpaul JP. Hematological alterations in propylene glycol-dosed female rats are minimal. Vet Hum Toxicol 1996;38:81-5.
82. Christopher MM, Perman V, White JG, Eaton JW. Propylene glycol-induced Heinz body formation and D-lactic acidosis in cats. Prog Clin Biol Res 1989;319:69-87; discussion 88-92.
83. Pfeiffer EH, Dunkelberg H. Mutagenicity of ethylene oxide and propylene oxide and of the glycols and halohydrins formed from them during the fumigation of foodstuffs. Food Cosmet Toxicol 1980;18:115-8.
84. Tucker JD, Auletta A, Cimino MC, et al. Sister-Chromatid Exchange: Second Report of the Gene-Tox Program. Mutation Research 1993;297:101-180.
85. Swenberg JA, Petzold GL, Harbach PR. In vitro DNA damage/alkaline elution assay for predicting carcinogenic potential. Biochem Biophys Res Commun 1976;72:732-8.
86. Ishidate MJ, Sofuni T, Yoshikawa K, et al. Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol 1984;22:623-636.
87. Litton Bionetics I. Mutagenic evaluation of compound FDA 71-56, propylene glycol. Kensington, MD: US Department of Commerce National Technical Information Service; 1974.
88. Hayashi M, Kishi M, Sofuni T, Ishidate M, Jr. Micronucleus Tests in Mice on 39 Food Additives and Eight Miscellaneous Chemicals. Food and Chemical Toxicology 1988;26:487-500.
89. Stenback F, Shubik P. Lack of toxicity and carcinogenicity of some commonly used cutaneous agents. Toxicol. Appl. Pharmacol. 1974;30:7-13.
90. Demey H, Daelemans R, De Broe ME, Bossaert L. Propylene glycol intoxication due to intravenous nitroglycerin. The Lancet 1984;June 16, 1984:1360.
91. Demey HE, Daelemans RA, Verpooten GA, et al. Propylene glycol-induced side effects during intravenous nitroglycerin therapy. Intensive Care Med 1988;14:221-226.
92. Casazza JP, Frietas J, Stambuk D, Morgan MY, Veech RL. The measurement of 1,2-propanediol, D, L-2,3-butanediol and meso-2,3-butanediol in controls and alcoholic cirrhotics. Alcohol Alcohol 1987;Suppl:607-9.
93. Trancik RJ, Maibach HI. Propylene glycol: irritation or sensitization? Contact Dermatitis 1982;8:185-9.
94. Kelner MJ, Bailey DN. Propylene glycol as a cause of lactic acidosis. J Anal Toxicol 1985;9:40-2.
95. Martin G, Finberg L. Propylene glycol: a potentially toxic vehicle in liquid dosage form. J Pediatr 1970;77:877-8.
96. Arulanantham K, Genel M. Central nervous system toxicity associated with ingestion of propylene glycol. J Pediatr 1978;93:515-6.
97. Pruitt. American Academy of Pediatrics. "Inactive" ingredients in pharmaceutical products. Committee on Drugs. Pediatrics 1985;76:635-643.
98. MacDonald MG, Getson PR, Glasgow AM, Miller MK, Boeckx RL, Johnson EL. Propylene glycol: Increased incidence of seizures in low birth weight infants. Pediatrics 1987;79:622-625.
99. Yorgin P, Theodorou A, Al-Uzri A, Davenport K, Boyer-Hassen L, Johnson M. Propylene glycol-induced proximal renal tubular cell injury. American Journal of Kidney Diseases 1997;30:134-139.

100. Lolin Y, Francis DA, Flanagan RJ, Little P, Lascelles PT. Cerebral depression due to propylene glycol in a patient with chronic epilepsy--the value of the plasma osmolal gap in diagnosis. Postgrad Med J 1988;64:610-3.
101. Bedichek E, Kirschbaum B. A case of propylene glycol toxic reaction associated with etomidate infusion. Arch Intern Med 1991;151:2297-2298.
102. Food and Drug Research Laboratories I. Teratologic evaluation of FDA 71-56 (propylene glycol) in mice, rats, hamsters and rabbits. 1973.
103. Kavlock RJ, Short RD, Jr., Chernoff N. Further evaluation of an in vivo teratology screen. Teratog Carcinog Mutagen 1987;7:7-16.
104. Gebhardt DO. The teratogenic action of propylene glycol (propanediol-1,2) and propanediol-1,3 in the chick embryo. Teratology 1968;1:153-61.
105. Landauer W, Salam N. Aspects of dimethyl sulfoxide as solvent for teratogens. Dev Biol 1972;28:35-46.
106. Kowalczyk CL, Stachecki JJ, Schultz JF, Leach RE, Armant DR. Effects of alcohols on murine preimplantation development: relationship to relative membrane disordering potency. Alcohol Clin Exp Res 1996;20:566-71.
107. Paynter SJ, O'Neil L, Fuller BJ, Shaw RW. Membrane permeability of human oocytes in the presence of the cryoprotectant propane-1,2-diol. Fertil Steril 2001;75:532-8.
108. Gook DA, Edgar DH, Stern C. The effects of cryopreservation regimens on the morphology of human ovarian tissue. Mol Cell Endocrinol 2000;169:99-103.
109. Gook DA, Edgar DH, Stern C. Effect of cooling rate and dehydration regimen on the histological appearance of human ovarian cortex following cryopreservation in 1, 2-propanediol. Hum Reprod 1999;14:2061-8.
110. Gook DA, Schiewe MC, Osborn SM, Asch RH, Jansen RP, Johnston WI. Intracytoplasmic sperm injection and embryo development of human oocytes cryopreserved using 1,2-propanediol. Hum Reprod 1995;10:2637-41.
111. Gook DA, Osborn SM, Johnston WI. Parthenogenetic activation of human oocytes following cryopreservation using 1,2-propanediol. Hum Reprod 1995;10:654-8.
112. Tucker M, Wright G, Morton P, Shanguo L, Massey J, Kort H. Preliminary experience with human oocyte cryopreservation using 1,2-propanediol and sucrose. Hum Reprod 1996;11:1513-5.
113. Tucker MJ, Morton PC, Wright G, Sweitzer CL, Massey JB. Clinical application of human egg cryopreservation. Hum Reprod 1998;13:3156-9.
114. Bruyas JF, Martins-Ferreira C, Fieni F, Tainturier D. The effect of propanediol on the morphology of fresh and frozen equine embryos. Equine Vet J Suppl 1997:80-4.
115. Damien M, Luciano AA, Peluso JJ. Propanediol-induced alterations in membrane integrity, metabolism and developmental potential of mouse zygotes. Hum Reprod 1989;4:969-74.
116. Johnson EM, Gabel BE, Larson J. Developmental toxicity and structure/activity correlates of glycols and glycol ethers. Environ Health Perspect 1984;57:135-9.
117. Guerrant N, Whitlock G, Wolff M, Dutcher R. Response of rats to diets containing varying amounts of glycerol and propylene glycol. Bull Nat Formul Comm 1947;15:205-229.
118. NTP. Propylene glycol: reproduction and fertility assessment in CD-1 mice when administered in drinking water. Cincinnati, OH: National Toxicology Program; 1985.
119. Morrissey RE, Lamb JCt, Morris RW, Chapin RE, Gulati DK, Heindel JJ. Results and evaluations of 48 continuous breeding reproduction studies conducted in mice. Fundam Appl Toxicol 1989;13:747-77.