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Provisional Peer Reviewed Toxicity Values for

Propylene glycol
(CASRN 57-55-6)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR PROPYLENE GLYCOL (CASRN 57-55-6)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).
2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.
3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically

updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

Propylene glycol (PG) is a Food and Drug Administration (FDA) approved food additive and is certified as Generally Recognized As Safe (GRAS) by the FDA (FDA, 1982). The 1997 HEAST lists subchronic and chronic oral RfD values of 3E+1 and 2E+1 mg/kg-day, respectively, for propylene glycol (U.S. EPA, 1997). The subchronic RfD was derived using a NOEL of 3 g/kg-day from a 20-week study in rats (Guerrant et al., 1947) and an uncertainty factor of 100. The chronic RfD was derived using a NOEL of 2.1 g/kg-day from a 2-year study in rats (Gaunt et al., 1972) and an uncertainty factor of 100. A 1987 HEED is listed as the source document for these derivations (U.S. EPA, 1987a), and this HEED is the only EPA report on propylene glycol in the CARA list (U.S. EPA, 1991, 1994a). No RfD is available for propylene glycol on IRIS (U.S. EPA, 2007) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2000). ATSDR (1997) did not derive any oral MRLs for propylene glycol, and no Environmental Health Criteria Document is available for the chemical (WHO, 2002). An acceptable daily dietary intake for propylene glycol of 25 mg/kg-day was derived by the FAO/WHO Joint Expert Committee on Food Additives in 1973 (WHO, 1974) using the NOEL from the study used to derive the chronic RfD in the HEED (U.S. EPA, 1987a).

No RfCs for propylene glycol are derived in the 1987 HEED, listed in the HEAST, or available on IRIS (U.S. EPA, 1987a, 1997, 2007). Based on information in the HEED, it is concluded in the IRIS summary for propylene glycol that available data are inadequate for the derivation of an inhalation RfC and that the verification status is currently not verifiable (U.S. EPA, 2007). ATSDR (1997) derived an MRL of 0.009 ppm for intermediate duration inhalation to propylene glycol using a LOAEL of 51 ppm (6 hours/day, 5 days/week) for nasal hemorrhaging in rats and an uncertainty factor of 1000 (Suber et al., 1989). No occupational

exposure limits have been recommended or promulgated by ACGIH (2001), NIOSH (2002) or OSHA (2002).

Propylene glycol is not listed in the HEAST cancer table (U.S. EPA, 1997) and no carcinogenicity assessment is available on IRIS (U.S. EPA, 2007) or in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2000). The carcinogenicity of propylene glycol has not been assessed by IARC (2002) or WHO (2002), or tested by NTP (2002).

Literature searches were conducted from 1986 thru 2007 for studies relevant to the derivation of provisional toxicity values for propylene glycol. Databases searched included: TOXLINE, MEDLINE, CANCERLIT, TSCATS, RTECS, CCRIS, DART, EMIC/ EMICBACK, HSDB, GENETOX and CCRIS.

REVIEW OF PERTINENT DATA

Human Studies

No pertinent data were located regarding health effects of propylene glycol in humans following oral or inhalation exposure. Propylene glycol is designated as a Generally Recognized As Safe (GRAS) food additive by the Food and Drug Administration (FDA), where it is approved for use as a direct food ingredient (FDA, 1982). Propylene glycol is widely used as an emulsifier, plasticizer, humectant, surfactant, solvent and antimicrobial agent in processed foods, as well as in some oral pharmaceutical and dermal cosmetic formulations (ATSDR, 1997). Dermal studies indicate that propylene glycol is a minimal local irritant in humans, except in cases of unusual sensitivity (ATSDR, 1997).

Animal Studies

Oral Systemic Toxicity

Information on the subchronic oral toxicity of propylene glycol is available from a number of dietary, drinking water and gavage studies in rats, rabbits, cats and dogs, as summarized below.

Short-Term and Subchronic Toxicity Studies

Groups of 5 male and 5 female young rats of unspecified strain were fed diets containing propylene glycol in concentrations of 0, 1, 3, 6, 10, 15, 20, 30, 40, 50 or 60% (10,000-600,000 ppm) for 20 weeks (Guerrant et al., 1947). Study endpoints included clinical condition, body weight, food consumption, water consumption and urine output, blood hemoglobin and glucose levels, and histopathology (scope of examinations not specified). Histopathological lesions were increased in incidence and severity at 100,000 ppm and higher dose levels, body weight gain was decreased at 300,000 ppm and higher, and mortality typically occurred after a few days of exposure at 400,000 ppm and higher. The pathological changes occurred particularly in the kidneys and included degeneration, interstitial hemorrhage and edema, glomerular nephritis, and

calcification of the cortex. No adverse effects were observed at 60,000 ppm or lower. Using a food consumption factor of 0.11 kg food/kg bw/day based on the average of male and female subchronic reference values for food consumption and body weight in F344 rats (U.S. EPA, 1987b), the 60,000 ppm NOAEL and 100,000 ppm LOAEL correspond to estimated doses of 6600 and 11,000 mg/kg-day, respectively.

Groups of 15 male and 15 female Charles River CD rats were fed 0 or 50,000 ppm of propylene glycol in the diet for 15 weeks (Gaunt et al., 1972). Renal concentration tests, hematological evaluations (hemoglobin content, packed cell volume, red cell and reticulocyte counts, and total and differential white cell counts), serum chemistry determinations (urea concentration and serum glutamic-oxaloacetic transaminase [SGOT] and serum glutamic-pyruvic transaminase [SGPT] activities), and organ weight measurements (brain, heart, liver, spleen, kidneys, adrenals, pituitary and gonads) showed no compound-related effects. Histopathology was not evaluated. Using a food factor of 0.11 kg food/kg bw/day based on the average of male and female subchronic reference values for food consumption and body weight in F344 rats (U.S. EPA, 1987b), the 50,000 ppm free-standing NOAEL corresponds to an estimated dose of 5500 mg/kg-day.

Groups of 10 Wistar male rats were exposed to drinking water containing 0, 5 or 10% (0, 50,000 or 100,000 ppm) of propylene glycol for 5 weeks (Vaille et al., 1971) following an initial one week observation period. Body weight and drinking water consumption were monitored throughout the study. Housing and environmental conditions were not reported. Based on drinking water consumption reported by the authors and the specific gravity of 1.036 for propylene glycol, the exposure levels corresponded to 0, 5.2 and 10 g/kg-day, respectively. At the end of the exposure period, the animals were sacrificed by exsanguination under ether anesthesia. Hematological endpoints examined included red blood cell (RBC) counts, white blood cell counts and blood sugar (glucose oxidase-peroxidase method). Histopathology was performed on major organs (liver, kidneys, heart, spleen, pancreas and adrenals). No gross or microscopic lesions were found in any of the organs examined. Decreased RBC count, increased liver weight, hyperglycemia and a decrease in blood urea were reported in both treatment groups. All of these effects were reported as being statistically significant ($p < 0.01$) across the three groups, although it was not clear whether pair-wise comparisons were made. The method of statistical analysis was not reported. Absolute liver weight was increased by 20% and 36% in the low and high dose groups, respectively. The increase in liver weight is not considered toxicologically significant in the absence of other indications of liver toxicity. Blood urea was decreased by 18% to 28%, but is not considered to be of toxicological significance. RBC counts were decreased by 7% and 10% in the low and high dose groups, respectively. Although minimal, the increase in RBC is consistent with hematological effects observed in other species (cats and dogs). Blood sugar was increased by 39% and 78% in the low and high dose groups, respectively. Together, the reduced RBC counts and hyperglycemia are considered to be adverse establishing a LOAEL of 5.2 g/kg-day (5200 mg/kg-day) in this study. A NOAEL was not identified.

In the same study (Vaille et al., 1971), groups of 7 male rabbits were exposed to drinking water containing 0 or 5% (50,000 ppm) propylene glycol for 8 weeks. Based on drinking water consumption and body weight data reported by the authors, the 5% PG exposure level

corresponded to a daily dose of 4.75 g/kg. Endpoints included only body weight and blood sugar. No effects on either endpoint were observed. A free-standing NOAEL of 4.75 g/kg (4750 mg/kg-day) was established for hyperglycemia in rabbits.

U.S. EPA (1987a) and WHO (1974) summarized other subchronic oral toxicity studies in which no gross or microscopic pathologic effects were observed in rats that were exposed to propylene glycol in drinking water at concentrations of 1-10% (10,000-100,000 ppm) for durations of 100-234 days (Kesten et al., 1939; Seldenfeld and Hanzlik, 1932; Weatherby and Haag, 1938; Auerbach Associates, 1977). Using a drinking water factor of 0.16 L water/kg bw/day based on the average of male and female subchronic reference values for water consumption and body weight in F344 rats (U.S. EPA, 1987b), the 10,000-100,000 ppm NOAELs correspond to estimated doses of 1600-16,000 mg/kg-day.

Evaluation of the liver and kidneys showed no functional changes (galactose excretion, uric acid excretion, rose bengal test, phenolsulfonphthalein test) or histopathological lesions in 4 dogs that consumed 5% propylene glycol in the drinking water for 5-9 months (Van Winkle and Newman, 1941). The average daily intake of propylene glycol was 5.1 ml/kg-day (WHO, 1974), indicating that a NOAEL of 5300 mg/kg-day (after adjusting for the specific gravity of PG) was identified in dogs. No gross pathologic changes or adverse effects on growth rate were noted in groups of 1-3 rabbits that were treated by gavage in doses of 1, 2, 3, 4 or 8 ml/kg (1-8.3 g/kg-day) for 50 days (Braun and Cartland, 1936), indicating that the NOAEL was 8300 mg/kg-day. The primary reports of these studies were not available and additional information was not provided in the available summary (WHO, 1974).

A series of subchronic and shorter duration studies in cats (as summarized below) found that dietary exposure to propylene glycol induced Heinz body formation, decrease erythrocyte survival and other effects indicative of hemolytic action on red blood cells (Bauer et al., 1991, 1992; Christopher et al., 1989; Weiss et al., 1990). Heinz bodies are granules that form as a result of hemoglobin oxidation and denaturation and are bound to the interior surface of red blood cell membranes. Propylene glycol has been added to semimoist cat foods at concentrations of 6-13% as a preservative, humectant and texturizer, as well as a source of readily metabolizable carbohydrates.

In the Christopher et al. (1989) study, propylene glycol was fed to groups of 5 or 6 mixed-breed adult cats of both sexes in dietary concentrations of 12% for 5 weeks or 41% for 3 weeks (Christopher et al., 1989). Reported mean chemical intakes were 1.6 and 8 g/kg-day in the low and high concentration groups, respectively. Each cat served as its own control using data collected during a 3-week pre-exposure period. Hematological endpoints evaluated at 2-7-day intervals throughout study included total erythrocyte counts, total and differential white blood cell counts, hematocrit, hemoglobin, packed cell volume, mean corpuscular volume, mean cell hemoglobin, mean cell hemoglobin concentration, red cell distribution width, histograms (leukocytes, erythrocytes and platelets), reticulocyte counts, Heinz body indices (counts, turbidity index, erythrocyte reduced glutathione concentration), total precipitated hemoglobin, methemoglobin concentration, and erythrocyte survival (half-life measured using ^{14}C-cyanate hemoglobin). Bone marrow endpoints included differential counts, myeloid-to-erythroid (M:E) ratio, unit particles, cellularity and morphology. Other endpoints included clinical condition,

body weight and food intake, serum chemistry (alkaline phosphatase, alanine transaminase, γ-glutamyltransferase and amylase activities, and total bilirubin, total protein, albumin, BUN, electrolytes, glucose and creatinine concentrations), gross pathology, and histology of the liver and spleen.

Dose-dependent Heinz body formation and decreased erythrocyte survival were the main findings in the Christopher et al. (1989) study. Compared to pre-exposure values, mean percentage of erythrocytes with Heinz bodies increased ≈ 800% (from 3.0 to 28.0%) and ≈ 1300% (from 4.8 to 68.6%) by the end of the exposure period in the 1.6 and 8 g/kg-day groups, respectively. A dose-dependent increase in the size of the Heinz bodies and changes in RBC survival, packed cell volume (PCV), reticulocyte numbers, and iron pigment level in liver and spleen were also observed. RBC survival decreased approximately 19% and 60% in the 1.6 and 8 g/kg-day groups, respectively. Both the percentage increase in number of Heinz bodies and percentage decrease in RBC half-life were proportional to the percentage difference in propylene glycol concentration in the 12 and 41% diets. There were no significant changes in red blood cell count at either dose level or methemoglobin concentration at 1.6 g/kg-day. The methemoglobin level was 65% higher at 8 g/kg-day than in the low dose group but had no clinical manifestations. PVC was unaffected at 1.6 mg/kg-day but had a 21% decrease at 8 g/kg-day that was accompanied by reticulocytosis (both punctate and aggregate reticulocytes were increased) and marked bone marrow erythroid hyperplasia (indicated by an M:E ratio of ≈ 1:2 and increases in early erythroid precursors and erythroblasts). There was also a 58% decrease in erythrocyte reduced glutathione concentration at 8 g/kg-day (glutathione is an intracellular antioxidant involved in protecting hemoglobin sulfhydryl groups from oxidation, an early step in Heinz body formation). Histological increases in iron pigment in the liver and spleen that were dose-dependent and consistent with decreased RBC lifespan were observed at ≥1.6 g/kg-day. There was an unexplained 30% decrease in food consumption in the low dose group that was accompanied by a slight weight loss (cats gained a mean 0.78 ± 0.13 kg during the control period but lost 0.21 ± 0.14 kg during the exposure period); most cats in the high-dose group ingested the entire portion of treated food with no reported changes in weight gain. Non-hematological effects included reduced responsiveness to external stimuli and slight to moderate ataxia in 50% (3 of 6) of the cats at 8 g/kg-day. Due to the dose-dependent and hemolytic nature of the erythrocyte effects, the lowest dose of 1600 mg/kg-day is a LOAEL. A NOAEL was not identified.

In a following study, 9 adult cats were fed a diet containing 8.32% propylene glycol for 8 weeks (Weiss et al., 1990). Each cat served as its own control using data collected during an 8-week pre-exposure period. No food or chemical intake data were reported. Using a food factor of 0.02 kg food/kg bw based on dietary concentrations and intakes of propylene glycol in other cat studies by the same group of investigators (Bauer et al., 1991; Christopher et al., 1989), the estimated daily chemical intake is 1.7 g/kg-day. The cats were given a mean oral dose of acetaminophen of 23.4 mg/kg-day (5 cats) or 47.8 mg/kg-day (4 cats) pre-exposure and during the fourth week of exposure. Acetaminophen is a known oxidant that was administered to test whether exposure to propylene glycol increased the susceptibility of erythrocytes to oxidative damage. Complete blood counts, hemoglobin and methemoglobin levels, and numbers of Heinz bodies were evaluated. Other endpoints included clinical condition, body weight and most of the serum chemistry indices evaluated in the Christopher et al. (1989) study. Results of weekly

analyses for each cat while on the control diet were pooled for comparison with results for the cats while on propylene glycol diets. Propylene glycol-related effects included a slight (10%) decrease in mean body weight and a 446% increased number of Heinz bodies compared to pre-exposure values. Alterations in methemoglobin levels and various other hematological and serum chemistry indices were slight and remained within reference ranges. Effects of acetaminophen were evaluated 4 hours following treatment. Methemoglobin concentrations were significantly increased in both of the acetaminophen dose groups, indicating that erythrocyte susceptibility to oxidative stress was increased by the exposure to propylene glycol. Based on Heinz body induction and other indications of increased susceptibility of erythrocytes to oxidative damage by propylene glycol, the 1700 mg/kg-day dose is a LOAEL.

Heinz body formation was further studied in groups of 7 adult cats that were fed diets containing 0, 6 or 12% propylene glycol for 12 weeks (Bauer et al., 1991). Average compound intake was reported to be 3.8 and 10.1 g/cat/day in the low and high dose groups, respectively. Using an estimated mean body weight of 2.8 kg based on reported data, the low and high intakes on a body weight basis were 1.4 and 3.6 g/kg-day. Hematological endpoints were evaluated every two weeks throughout the study and included total red and white blood cell counts, differential leukocyte counts, packed cell volume, mean cell volume, hemoglobin concentration, mean cell hemoglobin, mean cell hemoglobin concentration, red blood cell distribution, erythrocyte survival, and reticulocyte (punctate and aggregate) and Heinz body counts. Evaluations performed at the end of the study included bone marrow differential cell counts, gross pathology of all major organs, and histology of the bone marrow, liver and spleen. Significant and dose-dependent increases in Heinz bodies occurred within 2 weeks and persisted throughout the study. Heinz body production fluctuated from week 6 to the end of the study; but did not appear to be increasing, particularly in the last 4 weeks (Fig. 4 in Bauer et al., 1991). The average increase in Heinz body percentage from week 6 to week 12 was approximately 900% and 1600% in the 1.4 and 3.6 g/kg-day dose groups, respectively. Mean erythrocyte survival decreased approximately 30% (from 63.9 to 44.6 days) and 55% (from 63.9 to 28.7 days) in the 1.4 and 3.6 g/kg-day groups, respectively. Changes that were slight but statistically significant included reduced red blood cell counts at $\geq$1.4 g/kg-day and packed cell volume at 3.6 g/kg-day, and increased punctate reticulocyte counts, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration at 3.6 g/kg-day. There were no exposure related effects on food consumption or body weight or changes in the bone marrow or other tissues. Based on increased number of Heinz bodies and decreased erythrocyte survival, the 1.4 g/kg-day dose is a LOAEL.

Hematological effects were further evaluated in groups of 7 kittens (12-14 weeks old) that were fed diet containing 0, 6% or 12% propylene glycol for 13 weeks (Bauer et al., 1992). Reported compound intakes were 2.75 and 5.29 g/kg-day in the low and high concentration groups, respectively. These chemical intakes are higher than those in adult cats fed similar dietary concentrations of propylene glycol due to a higher food per kilogram body weight consumption by growing kittens. Endpoints included bimonthly evaluations of total RBC counts, total and differential white cell counts, hematocrit, hemoglobin concentration, mean corpuscular volume, mean cell hemoglobin, mean cell hemoglobin concentration, erythrocyte lifespan, and numbers of reticulocytes and Heinz bodies. Evaluations performed at the end of the study included bone marrow differential cell counts and M:E ratio, gross pathology, and

histopathology of the bone marrow, spleen and liver. There were no exposure-related changes in clinical condition, food consumption or body weight gain. A dose-related increase in Heinz bodies occurred at ≥2.75 g/kg-day within 2 weeks and persisted throughout the study; numbers were approximately 800% and 1700% higher than controls in the low and high dose groups, respectively. Other significant changes in the low and high dose groups included 44% and 63% reduced erythrocyte survival (mean lifespan) and approximately 50% and 65% increased punctate reticulocyte counts. The LOAEL for effects on red blood cells is 2.75 g/kg-day (2750 mg/kg-day.

Chronic Toxicity Studies

In addition to cat studies, information on the chronic oral toxicity of propylene glycol is available from dietary studies in rats and dogs (Gaunt et al., 1972; Hanzlik et al., 1939; Morris et al., 1942; Okumura et al., 1986; Weil et al., 1971). In the Hanzlik et al. (1939) study, groups of 5 young rats of unspecified strain and sex were fed diets in which propylene glycol was substituted for 25, 50, 75 or 100% of the carbohydrate for up to 24 months. Propylene glycol constituted 12.1, 24.2, 36.4 or 48.5% of the diet (121,000-485,000 ppm). Study endpoints appear to have been limited to clinical condition, body weight, food consumption and histopathology of visceral tissues. Effects included decreased weight gain at 121,000 ppm and higher, increased mortality at 364,000 ppm (the first death occurred after ≈ 20 weeks of exposure) and 485,000 ppm (100% mortality during the first month of exposure), and degenerative liver and kidney changes in the animals that died. Assessment of this study is complicated by imprecise and incomplete reporting. Assuming a food consumption factor of 0.05 kg food/kg bw/day for chronic exposure in rats, the 121,000 ppm LOAEL corresponds to an estimated dose of 6.05 g/kg-day (6,050 mg/kg-day). A NOAEL was not established.

Groups of 30 male and 30 female Charles River CD rats were fed diets containing 0, 6250, 12,500, 25,000 or 50,000 ppm of propylene glycol for 2 years (Gaunt et al., 1972). The mean daily compound intakes were reported to be 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. Endpoints included clinical condition, body weight, food intake, hematology (hemoglobin content, packed cell volume, red cell and reticulocyte counts, and total and differential white cell counts), urinary concentration (specific gravity, volume during water deprivation and load) and cell excretion in the control and two highest dose groups, and gross pathology, organ weights (10 organs) and histopathology (22 tissues and other tissues if grossly abnormal) on all animals that survived until the end of the exposure period. No exposure-related effects were found. A wide range of nonneoplastic histological abnormalities was observed in the kidneys, liver and lungs, but incidences of lesions were similar in treated and control animals and changes were consistent with those expected in aging rats. The NOAEL in this study is the highest dose of 2.1 g/kg-day (2100 mg/kg-day) in female rats.

In the Okumura et al. (1986) study, groups of 50 male and 50 female F-344 rats were fed diets containing 0, 2.5 or 5% (25,000 or 50,000 ppm) of propylene glycol for 2 years. No exposure-related changes in growth, food or water intake, appearance or behavior were reported. Hematological evaluations showed significant increases in average erythrocyte and leukocyte counts in males at ≥25,000 ppm, and mean corpuscular volume in females at ≥25,000 ppm.

There were no consistent or apparent changes in hematocrit, hemoglobin concentration, mean corpuscular hemoglobin or mean corpuscular hemoglobin concentration in either sex. Some serum chemistry changes were observed that included significantly increased cholesterol, lactate dehydrogenase and leucine aminopeptidase in males at ≥25,000 ppm, glutamyl transpeptidase in females at ≥25,000 ppm and males at 50,000 ppm, glutamic oxaloacetic transaminase and alkaline phosphatase in males at 50,000 ppm, and glutamic pyruvic transaminase in females at 50,000 ppm. The authors concluded that the alterations were suggestive of slight liver damage although it was noted that there was no evidence that results were outside normal ranges. Other endpoints, including histopathology, do not appear to have been investigated. Additional information on the design and results of this study was not obtained because the available report is in Japanese with only a study summary and tables in English. Considering that there were no indications that the alterations in this study were toxicologically significant, the NOAEL is the highest dose of 50,000 ppm. Using the standard food consumption factor of 0.05 kg food/kg bw/day for chronic exposure in rats, the 50,000 ppm NOAEL corresponds to an estimated propylene glycol intake dose of 2500 mg/kg-day.

Morris et al. (1942) conducted a poorly reported study in which groups of 6 male and 4 female albino rats were fed diets containing 0, 2.45 or 4.9% (24,000 or 49,000 ppm) of propylene glycol for 24 months. The corresponding daily chemical intakes were 0.9 and 1.8 ml/kg-day (0.9 and 1.9 g/kg-day). No effects on body weight gain, food consumption or survival were observed. Limited histological examinations (liver, kidney, lung, heart, spleen, lymph nodes, pancreas, stomach, intestine and adrenals) showed no lesions attributable to propylene glycol. Slight hepatic damage was noted, but it was not clear at which dose this was found, how many animals were involved, or what the nature of the damage was, and the study discussion only indicated that the group of animals receiving propylene glycol differed very slightly from the controls. The primary report of this study was not available and additional information was not provided in the available summaries (U.S. EPA, 1987a; WHO, 1974). Based on the available information, the highest NOAEL is 1900 mg/kg-day.

Weil et al. (1971) fed groups of 5 male and 5 female beagle dogs diets that provided propylene glycol in doses of 0, 2.0 or 5.0 g/kg-day for 2 years. Endpoints included mortality, food and water intake, body and organ (liver, kidney and spleen) weights, hematology (total erythrocyte count, total and differential leukocyte counts, hemoglobin, hematocrit, erythrocyte fragility), blood chemistry (serum alkaline phosphatase, SGOT, SGPT, total bilirubin, glucose, bromsulphthalein retention and urea nitrogen), urine indices (volume, specific gravity, pH and microexamination), and liver chemistry (glycogen, triglycerides and total lipids, water content, and metabolic rate of liver slices). Comprehensive gross and histopathological evaluations were performed. No changes were observed in any of the study parameters at 2.0 g/kg-day. Hematological effects occurred at 5.0 g/kg-day that included significantly decreased hemoglobin, hematocrit and erythrocyte counts and increased numbers of reticulocytes, anisocytes and poikilocytes. The investigators concluded that these changes were indicative of some erythrocyte destruction with replacement from the bone marrow. A slight increase in total bilirubin was also reported at 5.0 g/kg-day. No exposure-related pathological changes were found in the bone marrow, spleen, liver or other tissues. The effects on red blood cells are consistent with those observed in subchronically exposed cats (Bauer et al., 1991, 1992;

Christopher et al., 1989; Weiss et al., 1990) and should be classified as adverse, indicating that this study identified a NOAEL and LOAEL of 2000 and 5000 mg/kg-day, respectively.

Inhalation Systemic Toxicity

Inhalation toxicity of propylene glycol was evaluated in groups of 19 male and 19 female Sprague-Dawley rats that were nose-only exposed to mean aerosol concentrations of 0.16, 1.01 or 2.18 mg/L (160, 1010 or 2180 mg/m^3) for 6 hours/day, 5 days/week for 90 days (Suber et al., 1989). A fourth (control) group was cage-exposed to room air. The MMADs (mass median aerodynamic diameter) of the aerosol were less than 2.22 and 1.96 μm for the medium and high concentration groups, respectively. The MMAD for the low concentration group was not obtainable, possibly due to evaporation caused by the large quantity of dilution air. No measurements were made of the particle size of the undiluted aerosol. The geometric standard deviations were 1.44 and 1.57 for the medium and high exposure groups, respectively. Endpoints included clinical signs, body weight, food consumption, respiratory physiology (respiratory rates and tidal volumes in 4 rats/group/sex on study days 7, 42 and 84), hematology (hematocrit, hemoglobin, red and white blood cell counts, mean red cell volume, mean red cell corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelets), serum chemistry (21 indices including urea nitrogen, total bilirubin and seven liver enzymes), organ weights (13 organs including lung, spleen, liver, kidneys and gonads), gross pathology and histopathology of the respiratory tract (nasal cavity, trachea, lungs and larynx). It is not indicated if histological examinations were performed on non-respiratory tissues.

Nasal hemorrhaging occurred in a high proportion of animals in all exposed groups ($\geq$160 mg/m^3) beginning during the second week of exposure and persisting throughout the study (Suber et al., 1989). The average incidences of nasal hemorrhaging in the control to high exposure groups from weeks 2-13 were <1, 64, 74 and 75% in males and <1, 14, 71 and 71% in females. The nasal hemorrhaging did not occur during non-exposure weekend periods and, in some cases, disappeared completely following a weekend recovery period. The initial two week delay in the appearance of nasal hemorrhaging suggested that propylene glycol acted as a dehydration agent on peripheral respiratory tissues. There were no significant changes in respiratory function (minute volume, tidal volume or respiratory rate) in any of the exposed groups (and respiratory rates within groups did not decrease as the animals became acclimatized to the nose-only exposure conditions), suggesting that the animals adapted to the exposures. No exposure-related gross pathological changes were observed in the respiratory tract although histological examinations showed thickening of the nasal cavity respiratory epithelium, with increases in the number of goblet cells and their mucin content, in both sexes at $\geq$1010 mg/m^3. There were no histological changes in the trachea, lungs or larynx. Ocular discharge also occurred in all exposed groups with trends similar to the nasal hemorrhaging, with females having generally less ocular discharge than males. Incidences of ocular discharge in the control to high exposure groups were 5, 16, 40 and 40% in males and 8, 14, 28 and 35% in females. Other effects included significantly decreased food consumption and body weight gain in females beginning on days 43-64 at $\geq$1010 mg/m^3 (weight gain was reduced 5-7% in the high exposure group), decreased white blood cell count and lymphocyte numbers in females at $\geq$1010 mg/m^3, decreased mean corpuscular hemoglobin concentration in females at 2180 mg/m^3, decreased serum sorbitol dehydrogenase in males at $\geq$1010 mg/m^3, decreased gamma-glutamyl

transferase in males at 2180 mg/m^3, and decreased absolute (but not relative) liver weight in males and kidney weight in both sexes at $\geq$1010 mg/m^3. Although nasal hemorrhaging occurred at all levels of exposure, it is considered to be a minimally adverse effect because it did not affect respiratory function, did not cause histopathological changes in the nasal cavity and other parts of the respiratory tract, and was reversible upon cessation of exposure. A LOAEL of 160 mg/m^3 was established in this study. The duration adjusted LOAEL ($LOAEL_{ADJ}$) is 28.6 mg/m^3 (160 mg/m^3 x 6/24 x 5/7). The $LOAEL_{HEC}$ of 8.6 mg/m^3 is obtained by multiplying the $LOAEL_{ADJ}$ by the RDDR of 0.3 for extrathoracic (ET) respiratory effects. The RDDR was calculated using the MMAD of 2.22 μm and geometric standard deviation (sigma g) of 1.44 reported for the middle dose level (1010 mg/m^3) in the Suber et al. (1989) study. These values are similar to those reported for the high dose level (2180 mg/m^3), and were used to calculate the RDDR, as the MMAD and sigma g for the critical dose level (160 mg/m^3) dose level were not obtained by the investigators.

A continuous exposure inhalation study was conducted in which a group of 20 male and female white rats (numbers of each sex not specified) was exposed to a supersaturated atmosphere containing calculated concentrations of 0.17-0.35 mg/L (170-350 mg/m^3) of propylene glycol vapor for up to 18 months (Robertson et al., 1947). A group of 10 unexposed animals was used as controls. The animals were allowed to breed during the study. An unexplainable ≈ 50% increase in body weight gain in the exposed males compared to controls during the first 12 months of the study was the only remarkable effect. Body weights of the females were not reported due to variations related to pregnancy and birth of young. There were no effects on general condition and conjunctival irritation was not observed. The treated rats bred as regularly and produced litters as large as did the controls. No differences were noted in general appearance and weight gain between pups of treated and control groups. Gross and histological examinations of the lungs, liver, kidneys and spleen, performed on 1-8 rats at monthly intervals from 3-18 months of exposure (38 and 39 total number of control and exposed rats, respectively), showed no exposure-related effects.

Groups of 14 or 15 rhesus monkeys of unspecified gender were continuously exposed to calculated propylene glycol vapor concentrations of 0.10-0.22 mg/L (100-220 mg/m^3, ≈ 60% saturated air) or 0.23-0.35 mg/L (230-350 mg/m^3, supersaturated air) for up to 13 months (Robertson et al., 1947). An unexposed control group contained 16 monkeys. The results in exposed monkeys were reported without specifying to which concentration they were exposed. Clinical condition, body weight, blood cell counts, hemoglobin level, urine concentrating ability (a measure of kidney function), microscopic appearance of the urine, and gross and histopathology (lungs, liver, kidneys, spleen, mesenteric lymph glands, adrenals, and sometimes stomach, intestines and testes) were evaluated in groups of 1-8 monkeys at monthly intervals from 1-13 months of exposure (16 and 29 total number of control and exposed monkeys, respectively). At autopsy, almost all of the control and exposed monkeys were infected with a parasitic nematode and a lung mite. Thirteen exposed and 10 control animals were sacrificed or died as a result of parasitism or infection, and comparison of the exposed and control groups was not possible beyond 5 months because of a small number of surviving control animals. No adverse effects attributable to propylene glycol exposure were observed.

Exposure methodology is a major limitation in the Robertson et al. (1947) inhalation studies that precludes identification of reliable effect levels. Several different methods were used in generating the test atmosphere. In the first method, an atomizer was used to generate the test atmosphere. In the second method, liquid propylene glycol was dropped onto a hot plate, resulting in extensive decomposition of the compound in the vapor. In the third method, a vaporizer was used to supersaturate the test atmosphere, which created condensations of propylene glycol droplets, so that the animals were exposed to both an aerosol and a vapor. The study indicated that all three methods were used during the course of the rat and monkey experiments, but the amount of time each method was used and the precise concentration of propylene glycol generated by each method were not reported.

Reproductive and Developmental Toxicity

Reproductive toxicity of propylene glycol was evaluated in Swiss CD-l mice using the Reproductive Assessment by Continuous Breeding (RACB) protocol (Lamb et al., 1997; NTP, 1985). Propylene glycol was provided in drinking water at concentrations of 0, 1.0, 2.5 and 5.0%, which yielded reported chemical intake estimates of 0, 1.82, 4.80 and 10.1 g/kg-day, respectively. Twenty male and 20 female mice per exposure level were exposed during a l-week pre-cohabitation period and a subsequently for 14 weeks as breeding pairs. Offspring produced during the cohabitation period were examined, sexed, weighed, and killed to allow continuous mating of the parental generation. Males and females were separated at the end of the cohabitation period and the females were allowed to deliver and raise the last litter to weaning. Propylene glycol had no adverse effects on any measure of reproduction, including number of litters, litter size, pup weight and sex ratio. The study protocol did not call for necropsies on the F_0 mice in the absence of a fertility effect. Evaluation of the second generation, which was limited to the control and high-exposure groups, showed no effect on the reproductive capacity of the F_1 mice (no chemical-related effects on mating, fertility, or number, weight or viability of the F_2 offspring). Necropsies of the F_1 mice showed no effect on body or organ weights or serum total calcium levels in either sex, no change in sperm endpoints, and no change in estrous cycle parameters. In conclusion, this two-generation study found no effects on fertility and reproduction in mice at any dose level, indicating that the highest NOEL is 10.1 g/kg-day (10,100 mg/kg-day).

No adverse effects on fertility were observed in the Robertson et al. (1947) chronic inhalation toxicity study in rats. As summarized above, a group of 20 male and female rats were continuously exposed to a supersaturated atmosphere calculated to contain 0.17-0.35 mg/L (170-350 mg/m^3) of propylene glycol vapor for up to 18 months and allowed to breed during the study (Robertson et al., 1947). The exposed rats bred as regularly and produced litters as large as the control group.

A limited amount of information is available on the developmental toxicity of propylene glycol in mice and rabbits. In a teratology screening test, groups of 40 pregnant female Swiss mice were administered 0 or 10,000 mg/kg-day doses of propylene glycol by gavage on gestation days 8-12 (Chernoff and Kavlock, 1982; Kavlock et al., 1987). Evaluations were essentially limited to pregnancy percent, fetal viability (death and resorption percentages), and perinatal effects (survival and body weight on postnatal days 1 and 3). Uterine examinations for

implantation sites were performed on dams that had not given birth by postnatal day 3. No fetal teratological examinations were performed and postnatal necropsies were limited to pups that died. There were no effects of treatment on any of the studied endpoints, suggesting that 10,000 mg/kg-day may be a NOEL for developmental toxicity in mice.

A summary of the control group data from a rabbit developmental toxicity study in which propylene glycol was used as the vehicle for another chemical has been reported (Frieling et al., 2000). Eighteen female New Zealand white rabbits of the vehicle control group were administered 5 ml/kg-day (5.2 g/kg-day) of propylene glycol by gavage on days 8-18 of gestation. The dams were killed on gestation day 28 and subjected to uterine, ovarian and fetal examinations. Viable fetuses were examined for changes in the viscera (all fetuses), head (approximately half of the fetuses) and skeleton (remaining fetuses). Maternal effects were observed that included increased respiration rate, body weight stasis, markedly reduced food intake and three deaths. Comparison with laboratory background control data showed increased post-implantation loss (mainly embryonic resorptions), decreased fetal weight and markedly increased fetal abnormalities, including cardiovascular malformations, pelvic kidneys, digital defects and vertebral abnormalities (particularly of the caudal region). A second study was conducted to verify the above findings by using the same protocol but with propylene glycol from a different source (to control for possible contaminant effects) and a negative control group (Frieling et al., 2000). Fourteen rabbits were administered the same dose level of propylene glycol and 7 rabbits received an equivalent amount of distilled water as the negative control. The propylene glycol treated dams showed maternal and developmental effects similar to those in the first study, whereas findings in the water-treated rabbits were similar to the laboratory background control data. The available summary (abstract only) of the two studies provides no quantitative data (magnitude or incidence of effects) or additional relevant information on the results. The available information suggests that 5.2 g/kg-day is a FEL for maternal and developmental toxicity in rabbits. Without a full published report, however, the findings cannot be verified and the study cannot be used as the basis for a provisional RfD.

Carcinogenicity and Genotoxicity

There is no evidence that propylene glycol is carcinogenic in rats and dogs based on results of histological examinations in the chronic oral toxicity studies summarized above (Gaunt et al., 1972; Hanzlik et al., 1939; Morris et al., 1942; Okumura et al., 1986; Weil et al., 1971). In particular, no exposure-related neoplasms were observed in the well-designed study of Gaunt et al. (1972), in which groups of 30 male and 30 female Charles River CD rats were fed diets that provided mean daily propylene glycol doses of approximately 0.2, 0.4, 0.9 and 1.7 g/kg in males and 0.3, 0.5, 1.0 and 2.1 g/kg in females. The most numerous tumors included mammary fibroadenomas, pituitary adenomas and subcutaneous fibrosarcomas, but incidences were similar in treated and control groups and consistent with those expected in aging rats.

Propylene glycol was administered to rats and mice as a vehicle control in several carcinogenicity studies that were reviewed by Miller (1979) and summarized by U.S. EPA (1987a). These studies found no increase in tumor incidence following repeated exposure to propylene glycol by subcutaneous injection or topical application to the oral mucosa for $\geq$8 months. Twice weekly 0.02 ml (0.02 mg) dermal applications of 10, 50 or 100% solutions of

propylene glycol to shaved skin did not induce skin or other tumors in groups of 50 female Swiss mice after lifetime treatment (Stenback and Shubik, 1974).

The preponderance of available evidence indicates that propylene glycol is not genotoxic (ATSDR, 1997; U.S. EPA, 1987a). Negative results were obtained when propylene glycol was tested for genetic activity in *in vitro* microbial assays with *Salmonella typhimurium* (various strains) and *Saccharomyces cerevisiae* D4 with and without metabolic activation (Clark et al., 1979; Litton Bionetics Inc., 1976; Pfeiffer and Dunkelberg, 1980), genetic activity in mouse host-mediated assays with *Salmonella typhimurium* TA1530 (results were weakly or questionably positive with *S. typhimurium* G46 and equivocal with S*accharomyces cerevisiae* D3) (Litton Bionetics Inc., 1976), chromosomal aberrations in human fibroblasts and Chinese hamster cells *in vitro* (Abe and Sasaki, 1982; Swenberg et al., 1976), chromosomal damage in rat somatic cells *in vivo* (Litton Bionetics Inc., 1976), and dominant lethal mutations in rats (Litton Bionetics Inc., 1976).

Toxicokinetics and Mode of Action

The mechanism by which propylene glycol induces erythrocyte injury is suspected to involve oxidation of external sulfhydryl groups on the hemoglobin molecule, which results in structural changes in the hemoglobin, collapse of the molecule and aggregation into Heinz bodies (Bauer et al., 1991, 1992; Christopher et al., 1989; Weiss et al., 1990). Cat hemoglobin is unique in that is contains eight reactive sulfhydryl groups per molecule, which is more than the two to four normally found in other mammalian species, including rat, mouse, rabbit, dog, sheep, horse, ox and human (Taketa et al., 1967). Cats may be more susceptible to Heinz body formation than humans due to their higher number of reactive sulfhydryl groups per hemoglobin molecule (Christopher et al., 1989; Weiss et al., 1990), although some findings suggest that cat hemoglobin is less readily oxidized than human hemoglobin (Harvey and Kaneko, 1977). Heinz body induction is a toxicological concern because it can lead to increased RBC destruction and possible anemia. However, there were no biologically significant changes in RBC counts, hematocrit and hemoglobin values in the cat studies, indicating that hemolysis was not clinically evident. The lack of overt hemolysis and anemia in the presence of elevated numbers of Heinz body-laden erythrocytes is likely due to the unique nonsinusal histology of the cat spleen and is not expected to be the case in other species (Bauer et al., 1991; Blue and Weiss, 1981; Weiss et al., 1990). Cats have large pores in the splenic pulp venules which allow Heinz body-containing RBCs to flow unimpeded without deforming, thus preventing the cat spleen from effectively sequestering affected erythrocytes and allowing them to circulate for prolonged periods. In other species, affected erythrocytes are sequestered in the spleen and Heinz bodies are mechanically removed from the cells ("pitted out") as they pass through small interendothelial slits, a process that damages membrane components and contributes to hemolysis. The inability of cat spleen to remove intracellular granules from circulating erythrocytes is shown by the finding that Heinz bodies disappear from blood no faster in cats with spleens than in splenectomized cats (Jain, 1973). Consequently, the lack of observed Heinz bodies in other species is likely attributable to rapid removal of affected RBCs by the spleen, rather than resistance to Heinz body formation, and even a low numbers of Heinz bodies may cause overt hemolysis in species other than the cat (Bauer et al., 1991; Weiss et al., 1990). The major elimination pathway for PG in most mammals (including humans) is via glucuronidation in the liver and subsequent elimination in

the urine. LaKind et al. (1999) conclude that "…while cats were the most sensitive species tested, they are not viewed as being the most predictive of human response to PG." Unmetabolized PG is poorly removed from the blood stream by the kidneys. Cats are deficient in their ability to glucuronidate, resulting in an accumulation of circulating PG, with a corresponding increase in Heinz body formation and eventual RBC damage.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR PROPYLENE GLYCOL

Table 1 presents a summary of the oral-exposure toxicity studies for PG, listed in groups by type of study, generally in order of most sensitive to least sensitive. Some studies described previously are not listed explicitly because, either the LOAELs are higher than the representative LOAEL listed for the respective species, duration and endpoint (i.e.., the short-term cat hematological studies), or because LOAELs were not established and the NOAELs were reported as a range in the secondary references only for a group of studies (i.e., subchronic rat drinking water studies).

Referring to Table 1, hematological changes were found in cats (Bauer et al., 1991, 1992; Christopher et al., 1989; Weiss et al., 1990), dogs (Weil et al., 1971) and rats (Vaille et al., 1971). Kidney pathology was observed in rats exposed to 11 g/kg-day in the diet for 20 weeks (Guerrant et al., 1947). A two-generation reproduction study found no effects on fertility or reproduction in mice exposed to dietary doses as high as 10 g/kg-day (Lamb et al., 1997; NTP, 1985). Developmental toxicity, but with concurrent maternal toxicity, as shown by increased post-implantation loss, decreased fetal weight and increased fetal abnormalities, including cardiovascular malformations, pelvic kidneys, digital defects, and vertebral abnormalities, was reported in an abstract of a study of rabbits that were exposed by gavage to 5.2 g/kg-day for 10 days during gestation (Frieling et al., 2000). As this study was not published subsequently and details were lacking, it is not considered further as the basis for a p-RfD.

The data suggest that hematological endpoints comprise the critical effect. These effect levels are most appropriately classified as minimally adverse due to the lack of overt hemolysis and anemia, with the lowest LOAEL at 1400 mg/kg-day in adult cats (Bauer et al., 1991). Red blood cell counts were significantly reduced in rats that were exposed to 5.2 g/kg-day for 5 weeks (Vaille et al., 1971), although there were no adverse changes in red cell counts, hemoglobin levels, packed cell volume and/or reticulocyte counts in rats exposed to $\geq$5.5 g/kg-day for 15-20

Table 1. Summary of Oral-Exposure Studies for Propylene Glycol

Species	Duration (vehicle)	NOAEL[a]	LOAEL[a]	Effects	Citation
Short-term and Subchronic Studies					
Cat	12 weeks (food)	none	1,400 – 1,700	hematological	Bauer et al., 1991; others
Rat	5 weeks (water)	none	5,200	hematological, hyperglycemia	Vaille et al., 1971
Rat	20 weeks (food)	6,600	11,000	kidney pathology	Guerrant et al., 1947
Rabbit	8 weeks (water)	5,000	none	hyperglycemia	Vaille et al., 1971
Dog	5 – 9 mo (water)	5,300	none	none	Van Winkle & Newman, 1941
Rat	15 weeks (food)	5,500	none	none	Gaunt et al., 1972
Rabbit	50 days (gavage)	8,300	none	none	Braun & Cartland., 1936
Rat	subchronic (water)	1,600 – 16,000	none	none	Auerbach Associates, 1977; other
Chronic Studies					
Rat	2 years (food)	none	6,050	decreased weight gain	Hanzlick et al., 1939
Rat	2 years (food)	1,900	none	none	Morris et al., 1942
Dog	2 years (food)	2,000	5,000	hematological	Weil et al., 1971
Rat	2 years (food)	2,100	none	none	Gaunt et al., 1972
Rat	2 years (food)	2,500	none	none	Okumura et al., 1986
Developmental and Reproduction Studies					
Rabbit	GD 8-18[b] (gavage)	none	5,200	developmental/maternal toxicity/mortality	Frieling et al., 2000 (abstract only)
Mouse	GD 8-12 [b] (gavage)	10,000	none	none	Kavlock et al., 1987
Mouse	2-g repro [c] (food)	10,100	none	none	Lamb et al., 1997

[a] mg/kg-day
[b] developmental study; exposure on days of gestation indicated
[c] 2-generation reproduction study; equivalent to developmental and subchronic exposure

weeks or ≥2.1 g/kg-day for 2 years (Gaunt et al., 1972; Guerrant et al., 1947; Okumura et al., 1986). Dogs that were fed 5 g/kg-day for 2 years had changes suggestive of hemolytic anemia (decreased erythrocyte counts, hematocrit and hemoglobin values with increased anisocyte, poikilocyte and reticulocyte counts), although no effects were seen at 2.0 g/kg-day (Weil et al., 1971). Doses as low as 1.4 g/kg-day (for 12 weeks), induced Heinz bodies and related hematologic alterations in cats (Bauer et al., 1991; Christopher et al., 1989; Weiss et al., 1990). Because of the differences in hemoglobin structure and PG metabolism between cats and humans, resulting in a higher sensitivtiy to hematological effects in cats, the cat studies are not considered further as the basis for a p-RfD. As both the cat hematological LOAEL and the rabbit developmental toxicity LOAEL (Frieling et al., 2000) are rejected for consideration, the next lowest LOAEL is 5200 mg/kg-day (5 week exposure) for reduced RBC counts and hyperglycemia in rats (Vaille et al., 1971). RBC counts were decreased by 7% and 10% in the low and high dose (10 g/kg-day) groups, respectively. Although minimal, the result is consistent with hematological effects observed in other cats and dogs. Blood sugar was increased by 39% and 78% in the low and high dose groups, respectively. Together, the reduced RBC counts and hyperglycemia are considered to be adverse, but minimally so, establishing a minimal LOAEL of 5200 mg/kg-day. A NOAEL was not identified.

A **subchronic p-RfD of 20 (2E+1) mg/kg-day** is derived by applying to the rat 5-week LOAEL of 5200 mg/kg-day for reduced RBC counts and hyperglycemia (Vaille et al., 1971) an uncertainty factor of 300, which includes factors of 3 for estimating a NOAEL from a minimal LOAEL (UF_L), 10 for interspecies extrapolation (UF_A) and 10 to account for variation in human sensitivity (UF_H). Multiple chronic and subchronic studies in several species are available. In addition, developmental and two-generation reproductive toxicity studies are available, therefore, a database uncertainty factor was not considered necessary.

subchronic p-RfD = LOAEL / UF
= 5200 mg/kg-day / 300
= 17 mg/kg-day (rounded to 20 or 2E+1 mg/kg-day)

The **chronic p-RfD of 20 (2E+1) mg/kg-day** is derived identically, as the shorter-term rat study defines the lowest LOAEL. A subchronic-to-chronic uncertainty factor (UF_S) is not needed, as the chronic studies in rats and dogs do not show adverse effects at lower doses.

p-RfD = LOAEL / UF
= 5200 mg/kg-day / 300
= 17 mg/kg-day (rounded to 20 or 2E+1 mg/kg-day)

Confidence in the principal study is low. Higher study confidence is precluded by the small numbers of animals tested, lack of a NOAEL, and minimal data reporting. Confidence in the database is medium because, although there are no indications of health effects in humans in the range of the p-RfD and there are a lot of animal studies, many of them are very old and poorly reported, and information on developmental toxicity is minimally reported. On the other hand, the critical hematological effects do not appear to become more severe after chronic exposure. The one (abstract) report of developmental and severe maternal toxicity at the same LOAEL established for the point of departure (POD) was not validated by subsequent

publication in the peer-reviewed literature and is discounted. Overall confidence in the subchronic p-RfD values is medium, as the strengths in the database, particularly the support for the long-term NOAEL and LOAEL, somewhat outweigh the low confidence in the principal study. An RfD based on the chronic data would be virtually the same, as the NOAELs of 2000 mg/kg-day or 2100 mg/kg-day in the Weil et al. (1971) dog study and the Gaunt et al. (1978) rat study, respectively, would most likely serve as the POD.

Due to its GRAS status, the general population is exposed to propylene glycol primarily through ingestion of food and pharmaceutical products containing the compound (ATSDR, 1997; FDA, 1982). Available information on the average daily dietary intake of propylene glycol is minimal and limited to data from Japan, where the compound is used as a food additive stabilizer and intake was estimated to be 43 mg/day per person (Louekari et al., 1990). This intake corresponds to 0.8 mg/kg-day, assuming a body weight of 55 kg for this population, and is well below the subchronic and chronic p-RfDs. The U.S. FDA has estimated that the human per capita dietary consumption of propylene glycol is 14 mg/kg-day, which is somewhat extreme, as it is based on the very conservative assumption that 10% of the U.S. population consumes the entire amount of the chemical used annually (CRC, 1997).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR PROPYLENE GLYCOL

Although two inhalation toxicity studies are available for consideration (Robertson et al., 1947; Suber et al., 1989), the mode of exposure is not relevant for any anticipated human exposure scenario. In both studies, animals were exposed to an aerosol generated by extreme physical means that would not occur at a contaminated site. Furthermore, the potential for generation of PG vapors is negligible, as the vapor pressure for PG is very low (0.07 mm Hg at ambient temperatures) and PG undergoes rapid photochemical oxidation in air with an estimated half-life of less than a day (ATSDR, 1997). The nasal irritation observed at 160 mg/m^3 (the lowest exposure level) in the Suber et al. (1989) study is most likely a non-specific particulate effect and should not be attributed to PG, per se. No effects were reported by Robertson et al. (1947). Because of these factors, derivation of a p-RfC for propylene glycol is not feasible.

DERIVATION OF A PROVISIONAL CARCINOGENICITY ASSESSMENT FOR PROPYLENE GLYCOL

There is no evidence that propylene glycol is carcinogenic based on results of histological examinations in four rat and one dog chronic oral toxicity studies (Gaunt et al., 1972; Hanzlik et al., 1939; Morris et al., 1942; Okumura et al., 1986; Weil et al., 1971). The most adequately designed of the rat studies is that of Gaunt et al. (1972), in which no propylene glycol-related neoplastic changes were observed in groups of 30 rats of each sex that were exposed to four dietary dose levels of compound ranging from 0.2-2.1 g/kg for 2 years. The dog study found no exposure-related histopathological effects in groups of 5 animals of each sex that were exposed to dietary doses of 2.0 or 5.0 g/kg-day for 2 years (Weil et al., 1971). Also, there is no evidence that propylene glycol was tumorigenic in rats and mice that were repeatedly

exposed by subcutaneous injection or topical application to the oral mucosa for ≥8 months (Miller, 1979; U.S. EPA, 1987a). Additionally, twice weekly dermal applications of propylene glycol for life did not induce skin or other tumors in mice (Stenback and Shubik, 1974). Genotoxicity assays of propylene glycol have yielded predominantly negative responses in bacteria, yeast and mammalian cells (ATSDR, 1997; U.S. EPA, 1987a). Under the U.S. EPA (2005) cancer guidelines, propylene glycol is not likely to be carcinogenic to humans.

REFERENCES

Abe, S. and M. Sasaki. 1982. SCE as an index of mutagenesis and/or carcinogenesis. Chapter 24. In: Sister chromatid exchange. Prog. Top. Cytogenet. 2: 461-514. (Cited in ATSDR, 1997)

ACGIH (American Conference of Governmental Industrial Hygienists). 2001. Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. ACGIH, Cincinnati, OH.

ATSDR (Agency for Toxic Substances and Disease Registry). 1997. Toxicological Profile for Ethylene Glycol and Propylene Glycol. PB/98/101108/AS.

Auerbach Associates. 1977. Propylene Glycol. Philadelphia, PA. p. 90. NTIS PB-280477.

Bauer, M.C., D.J. Weiss and V. Perman. 1991. Hematologic alterations in adult cats fed 6 or 12% propylene glycol. Am. J. Vet. Res. 53(1): 69-72.

Bauer, M.C., D.J. Weiss and V. Perman. 1992. Hematological alterations in kittens induced by 6 and 12% dietary propylene glycol. Vet. Hum. Toxicol. 34(2): 127-131.

Blue, J. and L. Weiss. 1981. Vascular pathways in nonsinusal red pulp - an electron microscope study of the cat spleen. Am. J. Anat. 161: 135-168.

Braun, H.A. and G.F. Cartland. 1936. The toxicity of propylene glycol. J. Am. Pharmacol. Assoc. 25: 746-749.

Chernoff, N. and R.J. Kavlock. 1982. An *in vivo* teratology screen utilizing pregnant mice. J. Toxicol. Environ. Health. 10: 541-550.

Christopher, M.M., V. Perman and J.W. Eaton. 1989. Contribution of propylene glycol-induced Heniz body formation to anemia in cats. JAVMA. 194(8): 1045-1056.

Clark, C.R., T.C. Marshall, B.S. Merickel et al. 1979. Toxicological assessment of heat transfer fluids proposed for use in solar energy applications. Toxicol. Appl. Pharmacol. 5: 529-535. (Cited in ATSDR, 1997)

CRC (Chemical Rubber Company). 1997. PAFA (Priority-Based Assessment of Food Additives) database. U.S. FDA, Center for Food Safety and Applied Nutrition. In: Food Additives. Toxicology, Regulations, and Properties. Clydesdale, F.M., Ed. CRC Press, Inc., Boca Raton, FL.

FDA (Food and Drug Administration). 1982. GRAS status of propylene glycol and propylene glycol monostearate. Federal Register. June 25. 47: 27810-27813.

Frieling, W.J.A.M., E. Heijink, S.A. Tesh et al. 2000. Embryo-foetal developmental toxicity of propylene glycol in NZW rabbits. Reprod. Toxicol. 14(6): 562-563.

Gaunt, I.F., F.M.B. Carpanini, P. Grasso and A.B.G. Lansdown. 1972. Long-term toxicity of propylene glycol in rats. Food Cosmet. Toxicol. 10: 151-162.

Guerrant, N.B., G.P. Whitlock, M.L. Wolff and R.A. Dutcher. 1947. Response of rats to diets containing varying amounts of gllycerol and propylene glycol. Bull. Natl. Formulary Comm. 15: 205-229.

Hanzlik, P.J., W.H. Newman, W. Van Winkle, Jr. et al. 1939. Toxicity, fate and excretion of propylene glycol and some other glycols. J. Pharmacol. Exp. Ther. 67: 101-113.

Harvey, J.W. and J.J. Kaneko. 1977. Mammalian erythrocyte metabolism and oxidant drugs. Toxicol. Appl. Pharmacol. 42: 253-261.

IARC (International Agency for Research on Cancer). 2002. IARC Monographs Search. Examined January 2002. Online.
http://193.51.164.11/cgi/iHound/Chem/iH_Chem_Frames.html

Jain, N.C. 1973. Studies on the occurrence and persistance of heinz bodies in erythrocytes of the cat. Folia Haematol. (Leipzig). 99: 28-38.

Kavlock, R.J., R.D. Short and N. Chernoff. 1987. Further evaluation of an in vivo teratology screen. Teratogen. Carcinogen. Mutagen. 7: 7-16.

Kesten, H.D., M.G. Mulinos and L. Pomerantz. 1939. Pathologic effects of certain glycols and related compounds. Arch. Pathol. 27: 447-465.

LaKind, J.S., E.A. McKenna, R.P. Hubner and R.G. Tardiff. 1999. A review of the comparative mammalian toxicity of ethylene glycol and propylene glycol. Crit Rev Toxicol 29: 331-365.

Lamb, J.C., D.K. Gulati, L.H. Barnes and M. Welch. 1997. Propylene glycol. Environ. Health Perspect. 105(1): 231-232.

Litton Bionetics. 1976. Mutagenic Evaluation of Compound FDA 71-56. 00057-55-6, Propylene Glycol. Food and Drug Administration, Washington, DC. NTIS PB-257868.

Louekari, K., A.O. Scott and S. Salminen. 1990. Estimation of food additive intakes. In: A.L. Branen, P. Davidson and S. Salminen, Ed. Food Science Technology. Vol. 35: Food additives, p. 9-32. Marcel Dekker, Inc., New York, NY. (Cited in ATSDR, 1997)

Miller, L.M. 1979. Investigation of Selected Potential Environmental Contaminants: Ethylene Glycol, Propylene Glycols and Butylene Glycols. Franklin Research Center, Philadelphia, PA. 270 p. EPA-560/11-79-006.

Morris, J.H., A.A. Nelson and H.O. Calvery. 1942. Observations on the chronic toxicities of propylene glycol, eethylene glycol, diethylene glycol, ethylene glycol mono-ethyl-ether, diethylene glycol mono ethyl-ether. J. Pharmacol. Exp. Ther. 74: 266-273.

NIOSH (National Institute for Occupational Safety and Health). 2002. NIOSH Pocket Guide to Chemical Hazards. Examined January 2002. Online. http://www.cdc.gov/niosh/npg/npg.html

NTP (National Toxicology Program). 1985. Propylene glycol: Reproduction and fertility assessment in CD-l mice when administered in drinking water. Final report. NTP-84-FACB-038.

NTP (National Toxicology Program). 2002. Management Status Report. Examined January 2002. Online.
http://ntp-server.niehs.nih.gov/cgi/iH_Indexes/ALL_SRCH/iH_ALL_SRCH_Frames.html

Okumura, M., S. Yamada, K. Hayakawa and M. Ito. 1986. Hematological effect in F344 rats after long term administration of propylene glycol. Aichi-ken Eisei Kenkyusho Ho. 36: 87-93.

OSHA (Occupational Safety and Health Administration). 2002. Regulations for air contaminants (Standards 29 CFR 1910.1000 and 1915.1000). Online.
http://www.osha-slc.gov/OshStd_data/1910_1000_TABLE_Z-2.html and
http://www.osha-slc.gov/OshStd_data/1915_1000.html

Pfeiffer, E.H. and H. Dunkelberg. 1980. Mutagenicity of ethylene oxide and propylene oxide and of the glycols and halohydrins formed from them during the fumigation of foodstuffs. Food Cosmet. Toxicol. 18: 115-118. (Cited in ATSDR, 1997)

Robertson, O.H., C.G. Loosli, T.T. Puck et al. 1947. Tests for chronic toxicity of propylene glycol and oral administration. J. Pharmacol. Exp. Ther. 91: 52-75.

Seldenfeld, M.A. and P.J. Hanzlik. 1932. The general properties, action and toxicity of propylene glycol. J. Pharmacol. Exp. Ther. 44: 109-121.

Stenback, F. and P. Shubik. 1974. Lack of toxicity and carcinogenicity of some commonly used cutaneous agents. Toxicol. Appl. Pharmacol. 30: 7-13.

Suber, R.L., R. Deskin, I. Nikiforov et al. 1989. Subchronic nose-only inhalation study of propylene glycol in Sprague-Dawley rats. Food. Chem. Toxicol. 27(9): 573-583.

Swenberg, J.A., G.L. Petzold and P.R. Harbach. 1976. In vitro DNA damage/alkaline elution assay for predicting carcinogenic potential. Biochem. Biophys. Res. Comm. 72(2): 732-738. (Cited in ATSDR, 1997)

Taketa, F., M.R. Smits, F.J. DiBona and J.L. Lessard. 1967. Studies on cat hemoglobin and hybrids with human hemoglobin A. Biochemistry. 6: 3809-3816.

U.S. EPA. 1987a. Health and Environmental Effects Document for Propylene Glycol. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

U.S. EPA. 1987b. Recommendations for and Documentation of Biological Values for Use in Risk Assessment. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC. EPA/600/6-87/008. NTIS PB 88-179874.

U.S. EPA. 1991. Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. April.

U.S. EPA. 1994a. Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. December.

U.S. EPA. 1994b. Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. Office of Research and Development, National Center for Environmental Assessment, Washington, DC. October. EPA/600/8-90/066F.

U.S. EPA. 1997. Health Effects Assessment Summary Tables (HEAST). FY-1997 Update. Prepared by the Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC. July. EPA/540/R-97/036. NTIS PB 97-921199.

U.S. EPA. 2000. Drinking Water Standards and Health Advisories. Summer 2000. Office of Water, Washington, DC. Online. http://www.epa.gov/ost/drinking/standards/

U.S. EPA. 2005. Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum, Washington, DC. EPA/630/P-03/001F. Federal Register 70(66):17765-17817. Online. http://www.epa.gov/raf

U.S. EPA. 2007. Integrated Risk Information System (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Online. http://www.epa.gov/iris/

Vaille, C., C. Debray, C. Roze et al. 1971. (Hyperglycemic action of propylene glycol.) Ann. Pharm. Fr. 29: 577-582. (Fre) (Cited in WHO, 1974)

Van Winkle, W., Jr. and H.W. Newman. 1941. Further results of continued administration of propylene glycol. Food Res. 6: 509-516.

Weatherby, H.J. and H.B. Haag. 1938. Toxicity of propylene glycol. J. Am. Pharm. Assoc. 27: 466-471.

Weil, C.S., M.D. Woodside, H.F. Smyth and C.P. Carpenter. 1971. Results of feeding propylene glycol in the diet to dogs for two years. Food Cosmet. Toxicol. 9: 479-490.

Weiss, D.J., C.B. McClay, M.M. Christopher et al. 1990. Effects of propylene glycol-containing diets on acetaminophen-induced methemoglobinemia in cats. JAVMA. 196(11): 1816-1819.

WHO (World Health Organization). 1974. 1,2-Propylene Glycol. In: WHO Food Additive Series No. 5, Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series, 1974, No. 539; FAO Nutrition Meetings Report Series, 1974, No. 53. WHO, Geneva. Online. http://www.inchem.org/documents/jecfa/jecmono/v05je90.htm

WHO (World Health Organization). 2002. Online catalogs for the Environmental Health Criteria Series. Examined January 2002. Online. http://www.who.int/dsa/cat98/chemtox8.htm#