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Provisional Peer Reviewed Toxicity Values for

Ethyl ether (Diethyl ether)
(CASRN 60-29-7)

Superfund Health Risk Technical Support Center
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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
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
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 ETHYL ETHER (DIETHYL ETHER) (CASRN 60-29-7)

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

Diethyl ether, also known as ethyl ether, ether, and ethoxyethane, is commonly used as a solvent and has been used as a general anesthetic. Diethyl ether is highly flammable. The empirical formula for diethyl ether is $C_4H_{10}O$ (Figure 1). Diethyl ether has a molecular weight of 74.12 and a boiling temperature of 34.6°C.

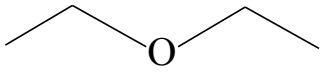

Figure 1. Diethyl Ether Structure

The U.S. Environmental Protection Agency's (U.S. EPA) Integrated Risk Information System (IRIS; U.S. EPA, 1990) lists a chronic RfD for diethyl ether of 0.2 mg/kg-day; this value is based on a NOAEL of 500 mg/kg-day for decreased body weight in male rats exposed to diethyl ether by gavage for 90 days (American Biogenics Corporation, 1988, unpublished report) (verification date: 11/15/89). A composite uncertainty factor (UF) of 3000 (10 to extrapolate from subchronic to chronic exposure, 10 for interspecies extrapolation, 10 to account for intraspecies variability, and a partial UF of 3 for database insufficiencies) was applied. IRIS (U.S. EPA, 1990) does not list a chronic RfC or cancer assessment for diethyl ether.

The Health Effects Assessment Summary Tables (HEAST; U.S. EPA, 1997) lists a subchronic RfD of 2.0 mg/kg-day based on the NOAEL of 500 mg/kg-day obtained from the 90-day gavage study in rats (American Biogenics Corporation, 1988, unpublished report) where the target organ was identified as the liver, and an UF of 300 was applied. The HEAST (U.S. EPA, 1997) also lists the chronic RfD of 0.2 mg/kg-day derived by IRIS (U.S. EPA, 1990), but it does not list subchronic or chronic RfCs or carcinogenic potency values. The Drinking Water Standards and Health Advisories list (U.S. EPA, 2006) does not report an RfD or carcinogenicity assessment for diethyl ether. The CARA list (U.S. EPA, 1991, 1994) includes a Health Effects Assessment (HEA; U.S. EPA, 1987) for diethyl ether that derived subchronic and

chronic RfDs but did not derive subchronic or chronic RfCs or carcinogenic potency, citing inadequate data. The subchronic and chronic RfDs derived in the HEA (U.S. EPA, 1987) were based on the NOAEL of 500 mg/kg-day reported by American Biogenics Corporation (1988, unpublished report); the subchronic RfD of 5.0 mg/kg-day for diethyl ether applied an UF of 100 (10 for interspecies extrapolation and 10 for intraspecies sensitivity), and the chronic RfD of 0.5 mg/kg-day applied an UF of 1000 (10 for interspecies extrapolation, 10 for intraspecies sensitivity, and 10 to extrapolate from subchronic to chronic exposure).

The American College of Governmental Industrial Hygienists (ACGIH, 2006) recommends a Threshold Limit Values (TLV)-Time Weighted Average (TWA) of 400 ppm (1200 mg/m^3) and a short-term exposure limit (STEL)/C of 500 ppm (1500 mg/m^3). The Occupational Safety and Health Administration (OSHA) (2007) permissible exposure limit (PEL) is also 400 ppm (1200 mg/m^3). The National Institute for Occupational Safety and Health (NIOSH) (2007) has not recommended an exposure limit for diethyl ether. NIOSH (2007) concluded that the documentation cited by OSHA was inadequate to support a PEL and that the OSHA PEL may not be protective of workers. Neither the Agency for Toxic Substances and Disease Registry (ATSDR) (2007) nor the World Health Organization (WHO) (2007) have reviewed the toxicity of diethyl ether. The International Agency for Research on Cancer (IARC) developed a monograph on volatile anesthetics (1976, 1987) that discussed health effects associated with working in the surgical operating-room environment, but it did not consider diethyl ether except as part of this group of chemicals. IARC (1976, 1987) concluded that although there may be an increased risk of cancer from exposure to volatile anesthetics, it is not possible to identify which agents are responsible; thus, IARC has concluded that volatile anesthetics are not classifiable as to their carcinogenicity to humans.

Literature searches for studies relevant to the derivation of provisional toxicity values for diethyl ether (CASRN 60-29-7) were conducted in MEDLINE, TOXLINE special, and DART/ETIC (1960's−June 2007); BIOSIS (2000−June 2007); TSCATS/TSCATS2, RTECS, CCRIS, HSDB, and GENETOX (not date limited); and Current Contents (January 2007−June 2007). The NTP status reports (NTP, 2007) were also searched to identify relevant data. An updated literature search (June 2007−November 2008) was conducted using PubMed.

REVIEW OF PERTINENT DATA

Human Studies

Diethyl ether has been widely used as an inhalation anesthetic agent since the mid-1800s, although it is no longer recommended for clinical use due to the development of more potent, less flammable agents. Thus, an extensive body of literature is available on acute inhalation exposure of humans to anesthetic concentrations of diethyl ether. Anesthesia is induced in humans by air concentrations of 100,000 to 150,000 ppm (300,000 to 450,000 mg/m^3), and it is maintained by a concentration of approximately 50,000 ppm (150,000 mg/m^3). The lowest anesthetic limit is 19,000 ppm (58,000 mg/m^3). Exposure to concentrations over 100,000 ppm (300,000 mg/m^3) has occasionally been fatal (Mehlman, 2000). Use of diethyl ether as an anesthetic has been associated with renal injury, and nephritis has been reported in rare cases

(Mehlman, 2000). Patients exposed to diethyl ether anesthesia have shown abnormal liver function characterized by transient increases in aspartate aminotransferase and alkaline phosphatase and reduced serum albumin (Mehlman, 2000).

Nelson et al. (1943) conducted a study to determine tolerability and "sensory limits" of human volunteers to subanesthetic concentrations of diethyl ether in a series of controlled acute exposures. Subjects (number or sex not reported) were exposed to 0, 100, or 200 ppm (0, 303, or 606 mg/m^3) diethyl ether for 3 to 5 minutes in a large inhalation chamber. After exposure, subjects were asked to classify the exposure as "no reaction, slightly irritating, or very irritating." The "majority" of subjects reported that 200 ppm, but not 100 ppm, was irritating to the nose (incidence and severity not reported) and reported that they could tolerate exposure to 100 ppm for an 8-hour working day. No additional details are reported.

Limited information is available regarding subchronic and chronic inhalation exposure of humans to diethyl ether. Several epidemiological studies of operating theater personnel and dental assistants have evaluated the effects of exposure to multiple (mixed) inhalation anesthetics on adverse health effects and cancer incidences (American Society of Anesthesiologists *Ad Hoc* Committee on the Effect of Trace Anesthetics on the Health of Operating Room Personnel, 1974; Bruce et al., 1968, 1974; Cohen et al., 1980; Corbett et al., 1974; Doll and Peto, 1977; Lew, 1979; Linde et al., 1981; Lund, 1985; Spence et al., 1977; Vessey, 1978). Adverse health effects, including hepatic and renal disease and increased rates of spontaneous abortion and congenital abnormalities, were reported, but exposure to individual anesthetic agents was not examined. Thus, it is not possible to attribute adverse effects to exposure to a particular anesthetic agent. Increased cancer incidences were reported in many of these studies, but the site and type of cancer varied from study to study and the available studies did not examine exposure to individual anesthetics. A case-control study of multiple myeloma considered diethyl ether as a possible risk factor, but it did not find a significant increase in risk associated with exposure (Morris et al., 1986). In more recent studies, Wennborg et al. (2000) found a small decrease (not statistically significant) in average birth weight in infants born to mothers exposed to diethyl ether while working in academic research laboratories when compared to a control group (exposure levels or duration of exposure not reported), while Taskinen et al. (1994) found no increased risk of spontaneous abortion associated with diethyl ether exposure in female laboratory workers (exposure levels or duration of exposure not reported). In both studies, exposure to other solvents and chemicals occurred.

Adverse effects associated with diethyl ether exposure of workers in an explosives manufacturing plant were reported in a descriptive study by de Grosbois et al. (1986). Workers (n = 68) were classified as having moderate exposure (approximately 1200 mg/m^3; number of workers not reported) or high exposure (>1200 mg/m^3; number of workers not reported). Subjects in the unexposed control group (n = 74) were employed in a public health department or hospital and did not have exposure to "neurotoxic agents." Several deficiencies were noted in reporting of this study. These include the lack of information on the number of workers in each exposure group, demographic information on study participants, exposure duration of workers, methods used to measure air concentrations of diethyl ether, and incidence and severity data on symptoms. Workers in the low-exposure group reported eye irritation, persistent headache, dizziness, mood instability, and sleep disturbances during the workweek. Workers in the high-exposure group reported fatigue, difficulty concentrating, headache, dizziness, sexual

dysfunction, mood instability, and peripheral neuropathy (not further described). Data reporting was not sufficient to determine whether symptom frequency or severity increased with exposure concentration.

Other studies reporting health effects in workers with exposure to diethyl ether were on populations simultaneously exposed to other chemicals (Moen et al., 1990; Salandova et al., 1990; Mazitova, 1993). Moen et al. (1990) reported that seamen exposed to vapors of several industrial solvents and hydrocarbons (including diethyl ether, benzene, ethanol, ethylene, hexane, styrene, toluene, and xylene) on board chemical tanker ships showed a decrement in auditory memory and visual abstraction, compared with seamen who worked on dry cargo ships. Workers exposed to a mixture of ethanol and diethyl ether at levels greater than the occupational limits experienced narcosis and psychophysiologic alterations (Salandova et al., 1990). Mazitova (1993) reported adverse effects (characterized as a "high rate of functional diseases of the nervous system, locomotor system, female genital organs, and digestive system") in female workers involved in the production of diethyl ether and "smokeless powder."

Animal Studies

All the animal studies are summarized in Table 1.

Oral Exposure

The subchronic oral toxicity of diethyl ether was evaluated in a 90-day gavage study in rats (American Biogenics Corporation, 1988, unpublished report). A complete report of the study (e.g, all data tables and appendices, including magnitude of effect, results of statistical analyses, and data from individual animals) is not available; attempts to obtain the complete study report were unsuccessful. The summary provided below is based on an abbreviated report (American Biogenics Corporation, 1988, unpublished report). Groups of 30 male and 30 female Sprague-Dawley albino rats [Crl:CD(SD)BR-VAF+] were administered gavage doses of 0, 500, 2000, or 3500 mg/kg-day of diethyl ether in corn oil for 90 to 92 days. Initial body weight ranges were 184 to 244 g for males and 127 to 177 g for females. Animals were observed for mortality and morbidity twice daily and clinical signs once daily on test days 1−5 and 3 times daily on days 7−92. Body weights and food consumption were recorded weekly. At an interim sacrifice (Treatment Days 43−44) and at the end of the 90-day treatment period, blood was obtained from 9−10 rats/sex/group for hematology (red blood cell [RBC] count, white blood cell [WBC] count with differential, platelet count, hemoglobin, hematocrit) and clinical chemistry (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea nitrogen, creatinine, protein, globulin, bilirubin, chlolesterol, glucose, and electrolytes); urinalysis was also conducted (specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, and microscopic examination). Ophthalmic examination was performed on all animals prior to treatment and on all animals surviving to study termination. Necropsy was performed on all animals. Organ weights were obtained for the adrenals, brain, gonads, heart, liver, and spleen.

Histopathological examination was performed on comprehensive tissues from all animals from the control and high-dose groups and from all animals found dead or sacrificed moribund. Heart, kidneys, and liver were examined from all animals in the low- and mid-dose groups.

Table 1. Summary Table for Diethyl Ether

Species, Study Type	Exposure	Critical Effects	Comments	Reference
Oral Studies				
SD-Rat Subchronic	0, 500, 2000, or 3500 mg/kg-day for 90 days (gavage). 30 rats/sex/group	NOAEL: 500 mg/kg-day. LOAEL: 2000 mg/kg-day for decreased bw gain, clinical signs of anesthesia and a few deaths due to unknown causes; also adverse effects on hematological and clinical chemistry variables at 3500 mg/kg-day.	**Endpoints:** body weight, food consumption; comprehensive hematology, clinical chemistry, urinalyses, and histopathology. **This study is the basis for the IRIS RfD and is used to derive a subchronic p-RfD.**	American Biogenics Corporation, 1988, unpublished report
Inhalation Studies[b]				
Human Occupational	1200 mg/m^3 or >1200 mg/m^3 among 68 workers in an explosives factory	Respiratory tract irritation; neurological symptoms (headache, balance, dizziness fatigue, and hearing problems)	This study was designed to investigate exposure to ethanol and diethyl ether, alone and in combination. Endpoints based on self-assessment. Effects were noted at both concentrations. Info. reported here is based on abstract only (study is in French).	deGrosbois et al., 1986
Human Acute	100 or 200 ppm (303 or 606 mg/m^3) whole body exposure for 2−3 minutes	Upper respiratory tract irritation at 200 ppm (606 mg/m^3) but not 100 ppm (303 mg/m^3)	This study was conducted with volunteers. This study is frequently cited in criteria development documents and appears to be the basis for the European Economic Community TWA for diethyl ether.	Nelson et al., 1943
Subchronic Wistar rats, guinea pigs and rabbits	1908 ppm (5784 mg/m^3) 7 hours/day, 5 days/week for 7 weeks; (whole-body exposure); Rats: 10/sex/group Guinea pigs: 6 males and 5 females per group Rabbits: 2/sex/group	None NOAEL: 1908 ppm (daily average concentration of 1205 mg/m^3)	**Endpoints:** body weight, clinical appearance, respiratory pattern, behavior (including reflex responses to sound and light stimuli), hematology (hematocrit, hemoglobin, cell counts), SGOT, SGPT, organ weights (lung, heart, liver, kidney, spleen, testis); histopathology on liver only	Chenoweth et al., 1972

Table 1. Summary Table for Diethyl Ether

Species, Study Type	Exposure	Critical Effects	Comments	Reference
Subchronic SD-rats	0, 1000 (3031 mg/m^3), or 10,000 (30,310 mg/m^3) ppm, continuously for 35 days (whole-body) Rats: 8/sex/group	None NOAEL: 10,000 ppm (daily average concentration of 30,310 mg/m^3)	**Endpoints:** Body weight gain (at 7, 14, and 35 days), organ weights (heart, lung, kidney, liver, and spleen); hematology (control and high dose only); histopathology (nine major organs in limited numbers from each group)	Stevens et al., 1975
Subchronic ICR mice	0, 1000 (3031 mg/m^3), or 10,000 (30,310 mg/m^3) ppm, continuously for 35 days (whole-body) Mice: 24/sex/group	LOAEL: 1000 ppm (daily average 3031 mg/m^3)**:** increased absolute and relative liver wt (male mice only) At 10,000 ppm (daily average 30,310 mg/m^3): 100% mortality, moribund sacrifice; increased absolute and relative liver weights; and degenerative liver lesions.	**Endpoints:** Body weight gain (at 7, 14, and 35 days), organ weights (heart, lung, kidney, liver, and spleen); Histopathology (nine major organs in limited numbers from each group,except that all livers not destroyed by autolysis were examined) **This study is used as the basis for subchronic p-RfC.**	Stevens et al., 1975
Subchronic Hartley guinea pigs	0, 1000 (3031 mg/m^3) or 10,000 (30,310 mg/m^3) ppm, continuously for 35 days (whole-body) Guinea pigs: 8/sex/group	NOAEL: 1000 ppm (daily average 3031 mg/m^3) LOAEL: 10,000 ppm (daily average 30,310 mg/m^3): 100% mortality, moribund sacrifice; reduced bw.	**Endpoints:** Body weight gain (at 7, 14, and 35 days), organ weights (heart, lung, kidney, liver and spleen); histopathology (nine major organs in limited numbers from each group,except that all livers not destroyed by autolysis were examined)	Stevens et al., 1975
Reproductive Toxicity Rats	Unknown concentration	Fertility and sexual behavior was altered in comparison with controls exposed only to cotton ball without ether. Authors suggest that exposure during the critical neonatal phase of sexual brain development disrupts endocrine function which, in turn, disrupts mating and fertility later in life.	Endpoints: Testosterone levels, indices of mating, fertility and sexual behavior; sperm evaluation; evaluation of testes upon sacrifice; rats were killed at 90 days of age. Major limitation: no dose-response information	Arena and Pereira, 2002

Table 1. Summary Table for Diethyl Ether

Species, Study Type	Exposure	Critical Effects	Comments	Reference
Reproductive Toxicity Mice	0.32 or 1.6% (3200 or 16,000 ppm corresponding to 9700 or 49,000 mg/m^3) 4 hours/day , 5 days; examined 28 days after the last exposure.	No effects on sperm morphology at daily average concentrations of 1600 and 8100 mg/m^3	Study designed to study sperm morphology.	Land et al., 1981
Developmental Toxicity Mice, rats	65,000 ppm (197,000 mg/m^3) for mice, 73,000 ppm (212,300 mg/m^3) for rats for 1 hour in early or late embryogenesis	Increased incidence of generalized edema, skeletal variations; hepatocellular vacuolization (mice).	Sacrifice on day before birth; pups examined; effects on fetal growth unclear; mice appeared more sensitive than rats. This study is available only as an abstract.	Schwetz and Becker, 1970
Developmental Neurotoxicity Rats	Unknown concentration	Significantly reduced number of Purkinjie cells (cerebellum) in neonates of exposed dams in comparison with unexposed controls.	Neonates assessed 7, 24, 48, and 72 hours after birth	Garcia-Estrada et al., 1990

No mortalities were observed in the control and low-dose groups (American Biogenics Corporation, 1988, unpublished report). In the mid-dose and high-dose group, 4/60 (7%) and 15/60 (25%) mortalities, respectively, were observed; 9 of 15 deaths in the high-dose group occurred on Treatment Days 57−84. The cause of death was not reported. Body weights were significantly decreased compared to controls in males in the mid-dose group (Weeks 8−13) and males (Weeks 8−13) and females (Weeks 7−13) in the high-dose group. Food consumption in the high-dose group during Weeks 1−7 and Week 13 was significantly decreased compared to controls; food consumption in the low- and mid-dose groups was comparable to controls throughout the study. Data on the magnitude of these changes were not reported in American Biogenics Corporation (1988). Clinical signs observed in the high-dose groups, and to a lesser extent in the mid-dose group, were consistent with light anesthesia (lethargy, ataxia, irregular breathing, and salivation). American Biogenics Corporation (1988) reported that "few antemortem" clinical signs were observed in the control and low-dose group.

Changes in hematological parameters were observed in rats in the mid- and high-dose groups (American Biogenics Corporation, 1988, unpublished report). In the high-dose group, total WBC count was slightly decreased at the interim evaluation in males and at study termination in males and females. A dose-related increase in total RBC count was observed at the interim evaluation in males, with females reported as "demonstrating a clinically significant increase" (doses not reported); the study report did not indicate if increases were observed at study termination. Hemoglobin and hematocrit were increased at study termination in mid-dose (not statistically significant) and high-dose (statistically significant) males. Significant increases in alanine aminotransferase were observed in high-dose males and females at study termination, indicating a liver toxicity at this dose level. At the interim assessment, serum cholesterol was significantly increased in mid-dose and high-dose females. In males in the high-dose group, cholesterol was slightly increased at the interim evaluation (not statistically significant) and was significantly increased at study termination. Changes in other clinical chemistry parameters were not considered to be treatment-related. No treatment-related changes were observed on ophthalmologic examination.

Abnormal necropsy findings (lung discoloration, liver discoloration and/or enlargement, distension and/or discoloration of the stomach, and alterations of the smooth mucosa of the stomach) were observed in animals dying during the treatment period (American Biogenics Corporation, 1988, unpublished report). No treatment-related necropsy findings were observed in animals surviving to the end of treatment. Changes in absolute and relative organ weights were observed in males in the high-dose group and females in the mid- and high-dose groups. In males, absolute weights of the brain, heart, kidneys, liver, and spleen were significantly decreased, and relative weights of the brain, kidneys and testes were significantly increased inthe high-dose group. In females, relative liver weights were significantly increased in the mid-and high-dose groups. The changes in relative organ weights in both sexes in the high-dose group were probably secondary to decreased body weight gain in this group. No treatment-related histopathological changes were observed. Based on decreased body weight in male rats, the mid-dose of 2000 mg/kg-day is identified as the LOAEL in this study. Clinical signs of anesthesia were also apparently observed in this group, although it is unclear to what extent. There were a few deaths in this group as well, although it is unclear if they were treatment-related. The low dose of 500 mg/kg-day is the NOAEL.

Inhalation Exposure

Systemic Toxicity—Chenoweth et al. (1972) exposed (whole body) groups of 10 male and 10 female Wistar rats, 6 male and 5 female guinea pigs, and 2 male and 2 female rabbits to 2000 ppm diethyl ether for 7 hours/day, on a 5 days/week regimen, for 7 weeks. The mean measured concentration, and its associated standard deviation (SD), of diethyl ether was 1908 ± 208 ppm (5784 ± 631 mg/m^3). During exposures, animals were examined for clinical signs, including activity, symptoms of nasal and eye irritation, skin condition, respiratory distress, and reflex responses to sound and light. Body weights were measured three times weekly; food consumption was not measured. At study termination, blood was collected for hematology (cell counts, hematocrit, and hemoglobin concentration) and clinical chemistry (alanine aminotransferase and aspartate aminotransferase), organ-to-body weight ratios were determined (lung, heart, liver, spleen, kidney, and testes), and gross and histopathological examinations were conducted. Although the study report did not list the tissues examined microscopically, the upper respiratory tract was apparently not examined. No clinical signs of toxicity were observed during exposure. Body weight, hematology, and serum liver enzymes in exposed animals were not significantly different from controls. The organ-to-body weight ratio was significantly reduced for the heart (8.8% decrease; $p < 0.001$) and the liver (8.0% decrease; $p < 0.002$) in male rats, but not in female rats or in guinea pigs. A significant increase in the organ-to-body weight ratio of the testes (39.5% increase; $p < 0.002$) was observed in guinea pigs but not in rats. No histological changes were reported for these organs. Small changes in organ weights were observed in rabbits (statistical analysis not performed due to small number of animal subjects), but no histological effects were observed. Based on no adverse effects at the exposure concentration tested, a freestanding NOAEL is identified as 1908 ppm (daily average concentration[1] of 1205 mg/m^3); however, comprehensive endpoints, including histopathological examination of the upper respiratory tract, were not assessed.

Stevens et al. (1975) exposed (whole body) groups of young Sprague-Dawley rats (8/sex/group; 150−275 g), ICR mice (24/sex/group; 18−20 g) and Hartley guinea pigs (8/sex/group; 250−350 g) continuously to 0.1 or 1.0% (1000 or 10,000 ppm; 3031 or 30,310 mg/m^3) diethyl ether for up to 35 days. Each exposure group had its own matched chamber control (36/sex for rats, 16/sex for mice, and 3−4/sex for guinea pigs). Body weights were recorded on Days 7, 14, and 35 days of treatment. At the end of the treatment period, blood was collected from rats in the control and 10,000 ppm groups for hematology (RBC count and WBC count with differential and hematocrit); blood was not collected from mice or guinea pigs. For all animals, absolute and relative organ weights were determined for the heart, lung, kidney, liver, and spleen; histopathological examination was performed on selected organs (the heart, lungs, liver, kidney, spleen, skeletal muscle, jejunum, proximal femur, and brain). Histopathological examination of the upper respiratory tract was not performed. Due to high mortality in the 10,000 ppm group, mice and guinea pigs in that dose group and their matched controls were sacrificed after 20 days of exposure. Mortality incidence and cause of death were not reported. All rats survived the 35-day exposure period. Body weight gain in rats, mice, and guinea pigs showed considerable variation at all time points, and even between control groups

[1] mg/m^3 = ppm × MW / 24.45
Daily average $NOAEL_{[ADJ]}$ (mg/m^3) = E (mg/m^3) × D/24 × W/7
$NOAEL_{[ADJ]}$ = NOAEL adjusted for duration of experimental regimen
E = NOAEL (experimental exposure level in mg/m^3)
D = number of hours exposure/24 hours
W= number of days of exposure/7 days

(except for the control rats). The investigators concluded that the only treatment-related effect on body weight occurred in guinea pigs exposed to 10,000 ppm: control guinea pigs gained 24 g while guinea pigs treated with 10,000 ppm diethyl ether lost 21 g ($p < 0.05$). Hematology parameters in rats exposed to 10,000 ppm were similar to matched controls. Organ weight analysis revealed significant ($p < 0.01$) increases in absolute (26%) and relative liver weight (11%) in male mice exposed to 1000 ppm. In mice exposed to 10,000 ppm, for males, absolute liver weights increased by 76% and relative liver weights increased by 86%; for females, absolute liver weights increased by 59% and relative liver weights increased by 66%. Treatment-related effects on organ weights were not observed for rats or guinea pigs. Histopathologic examination revealed a significant increase in the incidence of degenerative lesions (not specified) of the liver only in mice exposed to 10,000 ppm diethyl ether. In mice treated with 10,000 ppm diethyl ether, degenerative lesions were observed in 4/20 mice, compared to 0/64 (pooled controls) mice in the matched control ($p < 0.01$, Fisher exact test conducted for this report). Degenerative hepatic lesions were not observed in mice exposed to 1000 ppm diethyl ether. No treatment-related histopathologic lesions were observed in other organs examined in mice or in any organs examined in rats or guinea pigs. Based on no treatment-related effects in rats at the highest concentration tested, a freestanding NOAEL of 10,000 ppm (daily average concentration of 30,310 mg/m^3) is identified. For guinea pigs, 1000 ppm (daily average concentration of 3031 mg/m^3) is identified as a NOAEL and 10,000 ppm (daily average concentration of 30310 mg/m^3) is considered a frank effect level (FEL) due to high mortality. For mice, the low concentration (1000 ppm or 3031 mg/m^3) is identified as a freestanding LOAEL based on increased liver weight in male mice as a precursor to more severe changes in the same organ such as liver weight and pathology in both males and females at the high concentration (10,000 ppm or 30,310 mg/m^3).

Little additional information on the systemic toxicity of subchronic exposure to diethyl ether is identified. Kunz et al. (1966) reported that no significant changes in liver weight were observed in rats exposed to up to 3.5% (35,000 ppm; 106,100 mg/m^3) diethyl ether for 1 hour/day for 30 days (daily average 4421 mg/m^3). In rats exposed to anesthetic concentrations of diethyl ether, changes to the liver (enlargement of hepatic sinusoids, stasis, and perivascular edema) were observed (Rudin et al., 1999).

Developmental and Reproductive Toxicity—Several studies of developmental and reproductive effects of inhaled diethyl ether were located: Arena and Pereira (2002), Garcia-Estrada et al. (1990), Land et al. (1981), Lindskog (1958), and Schwetz and Becker (1970). Results of these studies suggest that inhalation exposure to high concentrations of diethyl ether might have adverse developmental effects, but the experimental procedures were not well reported and lacked the necessary details to derive LOAEL or NOAEL values for developmental effects. Lindskog (1958) observed decreased head growth and increased frequencies of skeletal variations, but no increase in malformations in pups from pregnant mice (strain not reported) subjected to diethyl ether anesthesia (concentration not reported) for 20 minutes on Gestational Days 9 to 12. Garcia-Estrada et al. (1990) found no effects on cephalic diameter, body weight, or viability (recorded 7, 24, and 48 hours after birth) in the offspring of 5 female rats exposed to diethyl ether (concentration not reported) twice for 10 minutes on Gestational Days 17 to 21. Histological examination found no delay in maturation of the cerebellum in diethyl ether-exposed offspring, although there was a significant decrease in the number of Purkinje cells of the cerebellum compared with unexposed controls.

Schwetz and Becker (1970) conducted a series of experiments in which pregnant rats and mice were exposed to 6.5−7.3% diethyl ether (65,000−73,000 ppm; 200,000−220,000 mg/m^3) for 1 hour at various times during organogenesis. In mice, exposure early in organogenesis produced hydronephrosis in 2/26 fetuses and an increase in resorptions, while exposure early or late in organogenesis produced an increase in skeletal anomalies, with no effect on fetal body weight or crown-rump length. In rats, there were no effects on resorptions or soft tissue or skeletal anomalies, but exposure early or late during organogenesis produced significant decreases in fetal body weight and length of long bones. This study is reported only as an abstract with limited experimental details. A follow-up report was not found in the searched literature.

Adverse effects on male fertility in adulthood were observed in rats exposed to anesthetic levels of diethyl ether as neonates (Arena and Periera, 2002). Immediately after birth, male rats (strain not reported) were exposed to diethyl ether (concentrations not measured) until anesthesia was observed. At adulthood, daily sperm production was decreased by approximately 30% ($p < 0.05$; data presented graphically) and the number of germ cells was reduced by approximately 30% in the testes ($p <0.05$; data presented graphically) and 20% in the caput/corpus epididymis ($p < 0.05$; data presented graphically) in comparison to unexposed controls. Testosterone levels and testicular and epididymal weights were not affected by diethyl ether exposure. Land et al. (1981) reported no effect on sperm morphology in mice exposed to 0.32 or 1.6% (3200 or 16,000 ppm; 9700 or 48,000 mg/m^3) diethyl ether on a 4 hours/day regimen, for 5 consecutive days (corresponding daily average concentrations of 1600 and 8100 mg/m^3), or examined 28 days after the last exposure.

Other Studies

Acute Studies

The behavioral and neuroendocrine effects of acute exposures to diethyl ether were examined in mice (Glowa, 1993). Male NIH mice (4−12 mice/exposure concentration) were exposed (whole body) to diethyl ether vapor at nominal concentrations of 0, 1000, 3000, 10,000, and 30,000 ppm (3031, 9094, 30,310, and 90,950 mg/m^3) for 5 or 30 minutes. Each mouse was treated with all exposure concentrations incrementally in each of 2 repeated exposure sessions; each exposure session was separated by at least 1 week, and within a session, each exposure was separated by an interval of 5 or 30 minutes. During exposure, mice were evaluated for operant behavior by responding to a 60-second fixed-interval (FI) reward schedule in which an award of milk was presented in response to a nose poke that followed a 60-second period of conditioning stimuli (green light in the presence of noise). The responses were quantified as a response rate (i.e., number of responses in an FI session divided by the time to complete the session). Exposures to diethyl ether at incremental concentrations of 1000 to 10,000 ppm resulted in concentration-dependent increases in response rates. The increase in responses was more pronounced in mice exposed for 30 minutes to each concentration. Exposure to 30,000 ppm (90,950 mg/m^3) resulted in decreased response rates, with complete extinguishment of the response in mice exposed for 30 minutes. Mice maintained righting reflexes following 30,000 ppm (90,950 mg/m^3) exposures, indicating that they were not anesthetized.

A separate group of mice were exposed for 5 or 30 minutes to single concentrations of diethyl ether (1000 to 30,000 ppm corresponding to 3031 to 90,950 mg/m^3) and were sacrificed immediately following exposure for measurement of plasma adrenocorticotrophic hormone (ACTH) and corticosterone concentrations (Glowa, 1993). Exposure to diethyl ether for

30 minutes resulted in concentration-dependent increases in plasma concentrations of ACTH and corticosterone (data presented graphically). The increase in plasma corticosterone ranged from approximately 350% of control in mice exposed to 3000 ppm to approximately 600% of control in mice exposed to 30,000 ppm diethyl ether. The increase in plasma corticosterone in mice exposed to 10,000 ppm (approximately 400% of control) was reported as statistically significant ($p = 0.0002$); however, statistical significance was not reported for the other exposure groups. Concentration-dependent increases were also observed in plasma ACTH in mice exposed to 3000 ppm (approximately 200% of control) or 30,000 ppm (approximately 400% of control); statistical significance was not reported. Plasma concentrations of corticosterone and ACTH in mice exposed to 1000 ppm diethyl ether for 30 minutes appeared similar to controls. The ACTH response was more pronounced in mice exposed to 10,000 ppm for 5 minutes (1244% of control) compared to mice exposed for 30 minutes (approximately 250% of control); whereas, the corticosterone response was less pronounced after the 5-minute exposure (approximately 150% of control) compared to the 30-minute exposure (approximately 400% of control). The statistical significance of increases in corticosterone or ACTH in mice exposed for 5 minutes was not reported.

The Glowa (1993) study provides evidence for effects of inhalation exposures to diethyl ether on operant behavior at concentrations below that which produces anesthesia and for effects of diethyl ether on the hypothalamic-pituitary-adrenal (HPA) axis. The behavioral and corticosterone responses were more pronounced with increasing exposure duration (5 or 30 minutes); however, the ACTH response was more pronounced at the shorter exposure duration, suggesting that the ACTH response preceded (and stimulated) the corticosterone response. The HPA axis response may reflect a more general stress response to diethyl ether inhalation exposure. Statistical analyses of behavioral outcomes and most endocrine outcomes were not consistently reported, making it difficult to define NOAEL and LOAEL values from this study. However, behavioral and neuroendocrine effects were apparent in animals exposed to 3000 ppm (9094 mg/m^3) and exposure to 10,000 ppm (30,310 mg/m^3) diethyl ether resulted in a statistically significant increase in plasma corticosterone concentrations.

Additional data on the toxicity of acute exposure to inhaled diethyl ether are restricted to high-level exposures intended to elucidate mechanisms of action. Several studies demonstrated that exposure of rats to anesthetic levels of diethyl ether induced some hepatic enzymes and inhibited others. Ether deethylase activity was induced in rats anesthetized briefly several times for 3 days (Brady et al., 1988). Diethyl ether (*in vivo* or *in vitro*) competitively inhibites N-nitrosodimethylamine demethylase activity (Keefer et al., 1985; Tan et al., 1987) and inhibites alcohol dehydrogenase by an undetermined mechanism (Hobara et al., 1985). Diethyl ether anesthesia inhibites conjugation and biliary excretion of bilirubin (Gourley et al., 1985) and diflunisal, probably by inhibiting hepatic UDP-glucuronosyltransferase activity (Watt and Dickinson, 1990). Diethyl ether anesthesia of fasted rats has also increased biochemical indicators of lipid peroxidation in the tissues and decreased total liver and kidney cytochrome P-450 levels (Liu et al., 1991).

Other acute exposure inhalation experiments evaluated the effects of brief periods of diethyl ether anesthesia in rats on several biochemical endpoints in the brain, pituitary, and blood, and on the morphology of Type I hair cells in the vestibular apparatus. Kung et al. (1985) showed that diethyl ether anesthesia increased brain uptake, particularly in the hippocampus, of two radio-labeled brain imaging agents, di-B-(morpholinoethyl)-selenide and N,N,N'-trimethyl-

N'-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine, probably due to changing regional cerebral blood flow. Diethyl ether anesthesia increased plasma levels of norepinephrine in intact and adrenalectomized rats, possibly as a result of increased release from noradrenergic nerve endings (Brown and Fisher, 1986). Ramirez-Gonzalez et al. (1991) determined that diethyl ether anesthesia increases the level of endogenous β-endorphin-like reactivity in the blood. However, levels in the neurointermediate lobe of the pituitary, the probable source of reactivity in the blood, and levels in the brainstem were not altered significantly. Zierer (1991) observed that diethyl ether anesthesia decreases the levels of oxytocin, oxytocin-neurophysin, and its predominant metabolite, vasopressin-neurophysin in the pituitary. Diethyl ether anesthesia increases gamma-aminobutyric acid levels in the hypothalamus, cingulate cortex, and frontal cortex, but has no effect on gamma-aminobutyric acid levels in the pituitary (Manev and Pericic, 1985; Acosta et al., 1990). In an *in vitro* study of synaptosomes from whole mouse brains, Daniel and Harris (1988) showed that diethyl ether increases intracellular levels of Ca^{++} in the resting cell. A brief period of anesthesia with diethyl ether can induce morphologic changes in the hair cells in the vestibular apparatus of guinea pigs (Scarfone et al., 1991).

Genotoxicity Studies

Diethyl ether has been assayed for genotoxicity in a number of *in vitro* tests with mostly negative results. The occasional positive result may be explained by the age of the diethyl ether sample used. Diethyl ether commonly contains ether peroxides formed by reaction with free radicals (Chen et al., 1993). These compounds have significant mutagenic activity (Chen et al., 1993) and could induce a false positive result. For example, Fluck et al. (1976) found that an old sample of ether produced a positive reaction in a DNA repair test with *Escherichia coli* while a fresh sample did not. In other studies, results were negative in a DNA repair test with *E. coli* and in the Ames test with *Salmonella typhimurium* strains TA1535, TA100, TA1538, TA98, TA1537, or TA97 (De Flora et al., 1984; Wagner et al., 1992; Waskell, 1978). A *Tradescantia* micronucleus test also gave negative results (Ma et al., 1984), but a c-tumor test in Allium (Önfelt et al., 1986) was positive. Diethyl ether did not produce an increase in sister chromatid exchange in Chinese hamster ovary (CHO) cells (White et al., 1979).

DERIVATION OF A PROVISIONAL SUBCHRONIC ORAL p-RfD FOR DIETHYL ETHER

No data on oral exposure of humans to diethyl ether are identified. The only study evaluating the effects of subchronic or chronic oral exposure of animals to diethyl ether is a 90-day gavage study in rats (American Biogenics Corporation, 1988, unpublished report) that identifies a NOAEL and LOAEL of 500 and 2000 mg/kg-day, respectively, for decreased body weight in male rats; this NOAEL is used as the point of departure to derive the chronic RfD of 0.2 mg/kg-day listed on IRIS (U.S. EPA, 1990). In addition to decreased body weight, other effects observed primarily in rats administered 3500 mg/kg-day diethyl ether include 25% mortality, clinical signs of anesthesia, hematological effects, and organ weight changes consistent with decreased body weight. Mortality was 7% (4 of 60 animals) in the 2000 mg/kg-day group. On necropsy, treatment-related effects (lung, liver, and stomach) were only observed in animals dying during treatment; no treatment-related histopathological changes were observed in animals surviving to the end of treatment. Thus, decreased body weight in male rats was selected as the critical effect for derivation of the subchronic p-RfD. Because

group means or individual body weight data are not provided in the available study report, benchmark dose (BMD) analysis of body weights is not conducted. Therefore, a NOAEL/LOAEL approach is employed to derive the subchronic p-RfD.

The **subchronic p-RfD of 0.5 mg/kg-day** for diethyl ether, based on the NOAEL of 500 mg/kg-day for decreased body weight in male rats (American Biogenics Corporation, 1988, unpublished report), is derived as follows:

Subchronic p-RfD = NOAEL ÷ UF
= 500 mg/kg-day[2] ÷ 1000
= **0.5 mg/kg-day**

The UF of 1000 is composed of the following:

- A 10-fold UF for intraspecies differences is applied to account for potentially susceptible individuals in the absence of quantitative information or information on the variability of response in humans.
- A 10-fold UF is applied to account for potential toxicodynamic and toxicokinetic differences between rats and humans.
- A UF of 10 is applied for database insufficiencies. The database lacks oral toxicity data on another species, and lacks oral developmental and 2-generation reproduction studies. Limited inhalation studies, though, do suggest that diethyl ether may have adverse developmental effects at very high exposure concentrations.

Confidence in the key study is low-to-medium. It employs an adequate number of animals, evaluates comprehensive toxicological endpoints, and identifies both NOAEL and LOAEL values. However, the full report is not available, so quantitative data are lacking for many endpoints. Confidence in the database is low-to-medium due to the lack of oral data in a second species and the lack of oral studies on reproductive and developmental toxicity. Studies of reproductive and developmental toxicity by inhalation exposure suggest that diethyl ether may have adverse developmental effects but only at very high exposure concentrations. However, the reliability of these studies is uncertain because of limited information available on experimental details. Overall confidence in the subchronic p-RfD for diethyl ether is low-to-medium.

DERIVATION OF A PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION p-RfCs FOR DIETHYL ETHER

The available data on inhalation exposure of humans to subanesthetic concentrations of diethyl ether include several studies in hospital and dental workers exposed to multiple inhalation anesthetics (American Society of Anesthesiologists *Ad Hoc* Committee on the Effect of Trace Anesthetics on the Health of Operating Room Personnel, 1974; Bruce et al., 1968, 1974; Cohen et al., 1980; Corbett et al., 1974; Doll and Peto, 1977; Lew, 1979; Linde et al., 1981; Lund, 1985; Morris et al., 1986; Spence et al., 1977; Taskinen et al., 1994; Vessey, 1978; Wennborg et al., 2000), a few poorly reported studies of exposure of industrial workers (de Grosbois et al., 1986; Mazitova, 1993; Moen et al., 1990; Salandova et al., 1990), and an acute

[2]Because the original dosing was daily gavage for 90 days, no duration adjustment is necessary.

tolerability study (Nelson et al., 1943). Although studies in anesthesiology workers report numerous adverse health effects, including hepatic and renal disease and increased rates of spontaneous abortion and congenital abnormalities, exposures encompassed multiple anesthetic agents; thus, effects could not be attributed to diethyl ether exclusively. With the exception of the descriptive study on workers in an explosives factory by de Grosbois et al. (1986), studies of industrial workers were conducted on study populations simultaneously exposed to other chemicals. The descriptive study by de Grosbois et al. (1986) reports several adverse effects associated with exposure to diethyl ether: eye irritation, persistent headache, dizziness, mood instability, sleep disturbances, fatigue, difficulty concentrating, sexual dysfunction, and peripheral neuropathy (not further clarified). However, the study report lacks the necessary information for derivation of a p-RfC: exposure duration, population demographics, and detailed descriptions of study methods and incidence data. The acute tolerability study by Nelson et al. (1943) reported that exposure to 200 ppm (606 mg/m^3), but not 100 ppm (303 mg/m^3), diethyl ether for 3−5 minutes was irritating to the nose (incidence data were not reported). Thus, available data in humans are considered inadequate to serve as the basis for deriving a subchronic or chronic p-RfC.

Studies on the effects of subchronic inhalation exposure of animals to subanesthetic concentrations of diethyl ether were conducted by Chenoweth et al. (1972) and Stevens et al. (1975). Chenoweth et al. (1972) exposed rats, guinea pigs, and rabbits to 1908 ppm diethyl ether for 7 weeks. No adverse effects were associated with exposure, yielding a freestanding NOAEL of 1908 ppm (daily average 1205 mg/m^3). Stevens et al. (1975) exposed rats, mice, and guinea pigs to 1000 or 10,000 ppm diethyl ether for up to 35 days. In rats, a freestanding NOAEL of 10,000 ppm (daily average 30,310 mg/m^3) is identified. In guinea pigs, a NOAEL of 1000 ppm (daily average 3031 mg/m^3) is identified; high mortality of guinea pigs was observed at the 10,000 ppm (daily average 30,310 mg/m^3) concentration. For mice, the most sensitive response is observed in the liver: increased liver weight occurred in the male mice treated with the low diethyl ether concentration (1000 ppm or daily average 3031 mg/m^3), while more significantly increased liver weight and degenerative liver lesions, as well as increased mortality, occurred in both male and female mice treated with the high diethyl ether concentration (10,000 ppm or daily average 30,310 mg/m^3). Therefore, the low concentration (1000 ppm or daily average 3031 mg/m^3) is identified as a freestanding LOAEL based on increased liver weight as the first sign in a continuum of hepatic-related responses to diethyl ether. This LOAEL is considered an appropriate point of departure (POD) for deriving the subchronic p-RfCs.

Both the Chenoweth et al. (1972) and Stevens et al. (1975) studies evaluated comprehensive toxicological endpoints (body weight, clinical appearance, behavior response, hematology (in some species), organ weight and histopathology (in some species), and serum chemistry (Chenoweth et al. [1972] study only) in multiple animal species. Although histopathological examination did not show adverse response in the lungs, the upper respiratory tract tissues, which was suggested as a sensitive tissue to irritant effect in a human study (Nelson et al., 1943), was not assessed in either Chenoweth et al. (1972) or Stevens et al. (1975) study. Nevertheless, in a similar paper by Collins et al. (1978), 30-week inhalation exposure to a similar compound, dimethyl ether, did not result in any upper airway, lung, or liver histopathological changes. The exposure did result in decreased liver weights in male rats exposed to 2000 and 20,000 ppm of the chemical, but no similar response occurred in the female rats. Due to the similarities of the study designs and compounds, it is reasonable to assume that systemic effects (i.e., changes in the liver weights and degenerative liver lesions) can occur due

to diethyl ether exposure in the absence of portal-of-entry effects (i.e., upper airway irritation/histopathological changes). Thus, the uncertainty due to the lack of histopathological examination of the upper respiratory tract is addressed in the database UF.

Acute exposure of mice to inhaled diethyl ether produces alterations in operant conditioning behavior and stimulated the hypothalamic-pituitary-adrenal (HPA) axis at subanesthetic concentrations (1000 to 30,000 ppm; 3031 to 90,950 mg/m^3) (Glowa, 1993). Because statistical analyses of behavioral outcomes and most endocrine outcomes are not reported, NOAEL and LOAEL values could not be defined, although behavioral and neuroendocrine effects were apparent in animals exposed to 3000 ppm (9094 mg/m^3). Due to the short exposure duration (5 or 30 minutes), this study is not considered suitable as a critical study for derivation of the p-RfCs. However, findings of this study indicate that acute inhalation exposures in mice, below anesthetizing levels, may be sufficiently stressful to stimulate the HPA or may act directly on the HPA. These observations raise some concerns regarding the lack of data on comprehensive evaluation of behavioral endpoints in subchronic inhalation studies.

Other information on acute effects of diethyl ether is obtained from studies evaluating high concentrations (e.g., anesthetic levels) (Kung et al., 1985; Manev and Pericic, 1985; Brown and Fisher, 1986; Daniel and Harris, 1988; Acosta et al., 1990; Ramirez-Gonzalez et al., 1991; Zierer, 1991; Scarfone et al., 1991). Results indicate that brief exposures to high levels can have significant effects on hepatic enzyme activities, several biochemical endpoints in the central nervous system (CNS), and on the morphology of Type I hair cells in the vestibular apparatus. The latter two observations, coupled with the fact that the major known effect of exposure to diethyl ether vapor is narcosis, suggest that subtle alterations on neurological function might be a critical effect of prolonged exposure to ethyl ether.

The 35-day mouse study (Stevens et al., 1975) is considered the principal study for deriving subchronic p-RfC. In this study, mice treated with the high concentration of diethyl ether were sacrificed after 20 days of treatment (before the termination of Day 35) due to high mortality; complete concentration-response data on liver weight changes at the termination of the study was not available which precludes the use of benchmark concentration modeling. Therefore, a NOAEL/LOAEL approach is employed to derive the subchronic p-RfC. The LOAEL of 3031 mg/m^3 in male mice for increased liver weight is used as the POD. Because animals were continuously exposed to diethyl ether in this study, this concentration is equivalent to the daily average concentration; therefore, no duration adjustment is necessary (i.e., $LOAEL_{[ADJ]}$ = 3031 mg/m^3).

Since diethyl ether is a lipophilic organic compound that caused liver toxicity in mice, the human equivalent concentration ($LOAEL_{[HEC]}$) based on the $LOAEL_{[ADJ]}$ is calculated for a systemic effect (liver toxicity) for category III vapor by multiplying the $LOAEL_{[ADJ]}$ by a ratio of the blood:gas (air) partition coefficients of diethyl ether between the laboratory animal species and the human $[(Hb/g)_A/(Hb/g)_H]$. Due to limited information available about the blood:air partition coefficients of diethyl ether in mice and humans, a comparable partition coefficients in these two species were assumed. Therefore, the $LOAEL_{[HEC]}$ of 3301 mg/m^3 is calculated as follows:

$$LOAEL_{[HEC]} = LOAEL_{[ADJ]} \times [(Hb/g)_A/(Hb/g)_H]$$
$$= 3031 \text{ mg/m}^3 \times 1.0$$
$$= 3301 \text{ mg/m}^3$$

The **subchronic p-RfC of 3 mg/m^3** for diethyl ether, based on the $LOAEL_{[HEC]}$ of 3031 mg/m^3 in male mice (Stevens et al., 1975), is derived as follows:

$$\textbf{Subchronic p-RfC} = LOAEL_{[HEC]} \div UF$$
$$= 3031 \text{ mg/m}^3 \div 1000$$
$$= \textbf{3 mg/m}^3$$

The composite uncertainty factor of 1000 is composed of the following:

- A 10-fold UF for intraspecies differences is used to account for potentially susceptible human individuals in the absence of information on the variability of response in humans.
- A partial UF of 3 is applied for interspecies extrapolation to account for potential toxicodynamic differences between mice and humans. Converting the mouse data to human equivalent concentrations by the dosimetric adjustment accounts for toxicokinetic differences between rats and humans; thus, it is not necessary to use a default UF of 10 for interspecies extrapolation.
- A partial UF of 3 is applied for use of a LOAEL for an effect of potential minimal biological significance. The LOAEL is derived based on increases in mouse liver weight in treated males (Stevens et al., 1975). This most sensitive response is observed only in the male mice, while more significantly increased liver weight and degenerative liver lesions are observed in both male and female mice treated with the higher concentration (30,310 mg/m^3) of diethyl ether. Therefore, the increased liver weight in male mice at the low concentration (3031 mg/m^3) is considered a potential early sign of hepatic response to diethyl ether.
- A UF of 10 is included for database uncertainties to address uncertainties surrounding the lack of upper airway histopathological examination in the Stevens et al. (1975) study and the lack of systemic neurobehavioral tests in longer duration inhalation exposures. In addition, the lack of quality data on developmental and reproductive studies also warranted a full default database UF.

Confidence in the critical study is medium. The critical study had several shortcomings: only two treated groups are included with a large concentration spacing (10-fold), and histopathologic examination did not include the upper airway tissues. However, the result from the critical study (on mice) is supported by other similar studies in different animal species (rat, guinea pig, and rabbit). Confidence in the database is low due to lack of comprehensive neurobehavioral, developmental, and reproductive studies by the inhalation route. The overall confidence in the subchronic p-RfC is low to medium.

The chronic p-RfC is not developed due to lack of sufficient data and potential significant uncertainties in the overall database for developing a chronic p-RfC value.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR DIETHYL ETHER

Weight-of-Evidence Descriptor

Studies evaluating the carcinogenic potential of oral or inhalation exposure to diethyl ether in humans or animals are not found in the available literature. Results of *in vitro* genotoxicity tests were mostly negative; occasional positive results were possibly related to the presence of ether peroxides that form in older samples of diethyl ether (Chen et al., 1993). Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), inadequate information is available to assess the carcinogenic potential of diethyl ether.

Quantitative Estimates of Carcinogenic Risk

Derivation of quantitative estimates of cancer risk for diethyl ether is precluded by the lack of suitable data.

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