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Provisional Peer Reviewed Toxicity Values for

Diethylformamide
(CASRN 617-84-5)

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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 DIETHYLFORMAMIDE (CASRN 617-84-5)

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

The U.S. Environmental Protection Agency's (EPA) Integrated Risk Information System (IRIS) (U.S. EPA, 2007) does not list a chronic oral reference dose (RfD) for diethylformamide. Diethylformamide is not listed on the Drinking Water Standards and Health Advisories List (U.S. EPA, 2006). The Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997) lists both a subchronic and chronic RfD value of 1.1E-2 mg/kg-day for diethylformamide. This value is based on a free-standing NOAEL of 1.1 mg/kg-day in a 73-week rat study (Argus et al., 1965), derived in a Health and Environmental Effects Profile (HEEP) (U.S. EPA, 1986). Uncertainty factors of 10 each for interspecies extrapolation and protection of sensitive individuals were applied to the NOAEL to derive the RfD for subchronic and chronic exposure. The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994) includes no documents for diethylformamide other than the aforementioned HEEP (U.S. EPA, 1986). A toxicological review of diethylformamide is not available from the Agency for Toxic Substances and Disease Registry (ATSDR, 2007) or the World Health Organization (WHO, 2007).

No RfC is available for diethylformamide on IRIS (U.S. EPA, 2007) or in the HEAST (U.S. EPA, 1997). The American Conference of Governmental Industrial Hygienists (ACGIH, 2006), the Occupational Safety and Health Administration (OSHA, 2007), and the National Institute for Occupational Safety and Health (NIOSH, 2007) have not established occupational health standards for diethylformamide.

A carcinogenicity assessment for diethylformamide is not available in IRIS (U.S. EPA, 2007) or in the HEAST (U.S. EPA, 1997). The HEEP (U.S. EPA, 1986) assigned

diethylformamide to U.S. EPA weight-of-evidence Group D, not classifiable as to human carcinogenicity, based on inconclusive data from the study by Argus et al. (1965). Diethylformamide was not evaluated for carcinogenic potential by the International Agency for Research on Cancer (IARC, 2007) nor was it included in the National Toxicology Program's (NTP) 11th Report on Carcinogens (NTP, 2005).

Literature searches were conducted from the 1960's through May 2007 for studies relevant to the derivation of provisional toxicity values for diethylformamide. Databases searched included: MEDLINE (including PubMed cancer subset), TOXLINE (Special), BIOSIS, TSCATS/TSCATS 2, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS and Current Contents.

REVIEW OF PERTINENT DATA

Human Studies

No studies were located regarding the effects of subchronic or chronic exposure of humans to diethylformamide by oral or inhalation routes.

Animal Studies

Oral Exposure

Only one study was located that investigated the effects of long-term oral exposure to diethylformamide. This study by Argus et al. (1965) examined the effects of a number of protein-denaturing amides or amines that are structurally related to pro-carcinogenic nitrosamines, including diethylformamide. Specifically, diethylformamide was one of a five-chemical panel included in a chronic duration oral exposure study for the purpose of determining carcinogenic activity in rats (Argus et al., 1965). A group of 27 adult male Wistar rats weighing between 150 and 200 grams was exposed to diethylformamide (purity not reported) in water by gavage at a dose of 546 µg/rat, 5 days/week for 73 weeks. A group of nine untreated rats served as controls. Food and water were available *ad libitum*. Body weights were measured weekly; by the end of the experiment the rats weighed between 500 and 580 grams. Using the average of initial and final body weights provided in the report (175 g and 540 g, respectively), the average daily dose can be estimated as 1.1 mg/kg-day (daily gavage dose ÷ [average initial body weight + average final body weight]/2 × duration of exposure; 0.546 mg/day ÷ 0.358 kg × 5/7 days/week). All animals that died or were killed during the study underwent a complete necropsy. A list of specific tissues examined microscopically was not provided; however, it is apparent that the liver, kidneys, lungs, lymphatic tissue and spleen were examined for tumor localization and time-to-tumor. No statistical analysis of the results was conducted.

There was no discussion of the rate of mortality in the treated or control rats (Argus et al., 1965). Body weight was reported to have increased regularly in treated rats and no differences from controls were noted. As the study was designed to be a chronic duration cancer bioassay, it is not clear if Argus et al. (1965) investigated non-neoplastic lesions in association with diethylformamide treatment. No non-neoplastic effects were reported for diethylformamide;

however, some non-neoplastic histopathological observations were reported in the liver and/or kidneys of rats exposed chronically to other chemicals included in the testing panel (e.g., dioxane, diethylacetamide). Tumor localization and time-to-tumor were the only endpoints reported in rats exposed chronically to diethylformamide, suggesting the absence of any notable non-neoplastic lesions in the tissues examined. The only tumors observed were a lymphosarcoma in 1/27 diethylformamide-treated rats (after 92 days) and 1/9 control rats (after 399 days). It is possible that the early appearance of the lymphosarcoma in the treated animal was a compound-related effect, but the data are inconclusive. Based on the absence of any reported non-neoplastic lesions in rats, the dose of 1.1 mg/kg-day is a free-standing NOAEL for oral diethylformamide exposure.

Intraperitoneal Exposures

The only other long-term study of diethylformamide toxicity located was performed by intraperitoneal injection. A group of 20 male Wistar rats were administered diethylformamide (60 mg/kg in saline) by intraperitoneal injection 5 days/week for 7 weeks (36 injections, average daily dose of 43 mg/kg-day) (Pham et al., 1971). There were 2 additional groups of 20 rats each that served as untreated and vehicle controls. The rats were observed for behavioral changes and body weight gain. Hematological, clinical chemistry and urinalysis parameters were assessed after 3 weeks and at study termination. Necropsy was performed at study termination, with histological examination of the heart, spleen, kidneys, adrenals, liver, lungs, pancreas, thyroid, testes and abdominal aorta. No mortality occurred. Body weight was progressively reduced throughout the study in treated rats. The only noteworthy changes in laboratory studies were an increase in leukocyte count (24 and 39 percent increase over control[1] levels at 3 and 7 weeks, respectively), a reduction in the level of gamma globulin (33 and 9 percent decrease as compared to control[1] levels at 3 and 7 weeks, respectively) but not other serum protein levels, and decreased urinary excretion of calcium, sodium and potassium in treated rats. No treatment-related histopathological lesions were observed.

Inhalation Exposure

No data regarding the toxicity of diethylformamide in animals following inhalation exposure were located.

Other Studies

Acute Studies

Pham et al. (1971) reported intraperitoneal LD_{50} values of 1200 mg/kg and 1300 mg/kg diethylformamide for rats and mice, respectively, observed for up to 30 days. Doses less than 500 mg/kg did not produce mortality in rats within 30 days. Acute intraperitoneal intoxication was characterized by decreased motor activity, immobility, prostration and reduced pain and righting reflexes for up to six hours following dosing. The animals generally remained sluggish

[1]Pham et al. (1971) does not indicate whether this is untreated or vehicle control.

thereafter. In rats and mice treated at lethal doses, lethargy was followed by a period of nervousness and convulsions within three hours of dosing. Histology demonstrated necrosis and polynuclear parenchymal cells in the liver of mice.

Amato et al. (1996) reported no notable signs of hepatotoxicity in the livers of male Sprague-Dawley rats examined following a single intraperitoneal injection of up to 2 g/kg in 0.9% saline. However, in this same study, male CD-1 mice treated with a single intraperitoneal injection of 1 g/kg or 2 g/kg demonstrated histological evidence of centrilobular vacuolization in their livers.

In Vitro Studies

In an *in vitro* study of the hemolytic behavior of human erythrocytes in aqueous diethylformamide solutions (0.0 to 100%), complete hemolysis was achieved after 45 minutes in solutions at 37 degrees Celsius containing as little as 0.5% diethylformamide (Cadwallader and Phillips, 1969). Cadwallader and Phillips (1969) identified the low pH of the diethylformamide solutions (2.5-4.0) as the apparent reason for total hemolysis at very low concentrations of diethylformamide.

Metabolism

Diethylformamide is oxidized by cytochrome P450 (CYP) isozymes to an intermediate N-(hydroxyethyl)ethylformamide (HEEF), which under basic conditions rapidly decomposes to monoethylformamide (MEF) and acetaldehyde. This metabolic oxidation catalyzed by CYP isozymes has been demonstrated in both human liver and rat liver microsomes (Amato et al., 1996, 2001). In control rat liver microsomes, diethylformamide is deethylated according to Michaelis-Menten kinetic parameters (Amato et al., 1996). Microsomes treated with selective CYP2E1 inducers demonstrated biphasic kinetics showing a low K_m (70 μM – 250 μM) with a V_{max} of about 0.2 nmol/min·mg of protein. In reconstituted systems, purified CYP2E1 and CYP2C11 showed that CYP2E1 partially accounted for the low K_m diethylformamide deethylase, whereas CYP2C11 might account for the high K_m deethylase. Human liver microsomes monophasically metabolize diethylformamide (Amato et al., 2001). In an experiment with a reconstituted system using *E. coli* membranes expressing different human recombinant CYPs (1A1, 1A2, 2B6, 2C10, 2E1 and 3A4), the CYP2E1 isoform showed the highest turnover. Diethylformamide deethylation was moderately affected by the CYP2E1, CYP2C10 and CYP3A4 isoforms. Amato et al. (2001) compared the kinetic constants obtained from human liver microsomes to those with a high affinity for the CYP2E1 enzyme in the rat liver microsomes (Amato et al., 1996), and found the K_m and V_{max} values were slightly lower in the rat microsomes. This finding may indicate a different metabolic pattern between the two species during diethylformamide transformation.

Genotoxicity

No data regarding the genotoxicity of diethylformamide were located.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR DIETHYLFORMAMIDE

Information relevant to the derivation of provisional RfDs (subchronic p-RfD and chronic p-RfD) for diethylformamide is very limited. Argus et al. (1965) reported no non-neoplastic effects in rats treated orally for 73 weeks with 1.1 mg/kg-day of diethylformamide. Pham et al. (1971) found some effects in rats treated with 43 mg/kg-day by intraperitoneal injection for 7 weeks, including reduced weight gain and a possible immunological effect indicated by increased leukocyte count and decreased serum gamma globulin, but did not clearly identify specific target organ effects of diethylformamide. Overt neurological effects and liver necrosis have been shown to occur following acute parenteral exposure to doses over 500 mg/kg (Pham et al., 1971).

The free-standing NOAEL of 1.1 mg/kg-day from Argus et al. (1965) can be used to derive provisional subchronic and chronic RfDs for diethylformamide. While a threshold for toxic effects of diethylformamide was not established by Argus et al. (1965), an adverse effect level of 43 mg/kg-day was identified from the Pham et al. (1971) injection study. However, the Pham et al. (1971) study employed a non-relevant route of exposure (intraperitoneal) to human health assessment.

The study by Argus et al. (1965) is deficient in that only one dose level was tested in one sex, a LOAEL was not identified and the control group was small and was not a vehicle control. However, the authors did administer diethylformamide for a sufficient portion of the life span of the treated rats and they did examine numerous endpoints (histopathology of liver, kidneys, lungs, lymphatic tissue and spleen). Thus, Argus et al. (1965) may serve as the principal study for the derivation of provisional chronic and subchronic oral RfD values. These provisional RfDs may be conservative because the threshold for toxic effects following oral exposure to diethylformamide may be considerably higher than the NOAEL defined by this study.

A **chronic p-RfD of 0.001 mg/kg-day** is derived by dividing the NOAEL of 1.1 mg/kg-day by an uncertainty factor of 1000, as shown below:

p-RfD = NOAEL / UF
= 1.1 mg/kg-day / 1000
= **0.001 mg/kg-day or 1E-3 mg/kg-day**

The uncertainty factor (UF) of 1000 is composed of the following:

- An UF of 10 was applied for interspecies extrapolation to account for potential pharmacodynamic and pharmacokinetic differences between rodents and humans.
- A default 10 fold UF for intraspecies differences was used to account for potentially susceptible individuals in the absence of quantitative information or information on the variability of response in humans.
- An UF of 10 was included for database insufficiencies due to the lack of supporting oral toxicity studies, including developmental studies and multi-generational reproduction studies.

A **subchronic p-RfD of 0.001 mg/kg-day** is derived by adopting the chronic RfD as the subchronic RfD, in the absence of relevant subchronic data.

Confidence in the principal study (Argus et al., 1965) is low because, despite investigation of endpoints over a significant portion of the lifespan of the species tested, only male rats were tested at one dose level and a LOAEL was not identified. In addition, the size of the control group was relatively small, the study did not include a vehicle control, and the purity of the diethylformamide tested was not reported. Confidence in the database is low because the dataset only includes a single oral study and the threshold for toxic effects following oral exposure was not identified. There is no information available on the potential of ingested diethylformamide to induce developmental, reproductive or neurological effects (a potential target organ as suggested by acute parenteral studies). Low confidence in the chronic and subchronic p-RfDs follows.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR DIETHYLFORMAMIDE

There are no inhalation studies available for use in developing subchronic and/or chronic provisional RfCs (p-RfC) for diethylformamide.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR DIETHYLFORMAMIDE

Weight-of-Evidence Descriptor

Studies evaluating the carcinogenic potential of oral or inhalation exposure to diethylformamide in humans were not identified in the available literature. Argus et al. (1965) observed no tumors except lymphosarcomas in 1/27 treated rats (after 92 days) and 1/9 control rats (after 399 days). It is possible that the early appearance of the lymphosarcoma in the treated animal was a compound-related effect, but the data are inconclusive. The study is inadequate as a cancer bioassay because group sizes were small, exposure duration was short (73 weeks), a single dose-level was tested that did not approach the maximum tolerated dose (MTD) and survival data were not reported. No genotoxicity data are available for diethylformamide. Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), inadequate information is available to assess the carcinogenic potential of diethylformamide.

Quantitative Estimates of Carcinogenic Risk

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

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