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Provisional Peer Reviewed Toxicity Values for

Ethylene Diamine (CASRN 107-15-3)

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
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 ETHYLENE DIAMINE (CASRN 107-15-3)

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 science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions or the EPA Headquarters Superfund Program sometimes request that a frequently used PPRTV be reassessed. 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.

This document has passed the STSC quality review and peer review evaluation indicating that the quality is consistent with the SOPs and standards of the STSC and is suitable for use by registered users of the PPRTV system.

INTRODUCTION

The 1997 HEAST (U.S. EPA, 1997) provides a subchronic and chronic oral RfD of 2E-1 and 2E-2 mg/kg-day, respectively, for ethylene diamine. These assessments were based on a NOAEL of 22.6 mg/kg-day (transformed from a value of 50 mg/kg-day of ethylene diamine dihydrochloride) for decreased heart weight and hematological changes in rats dosed orally for 3 months (Yang et al., 1983). The subchronic RfD included an uncertainty factor of 100 (10 for interspecies extrapolation and 10 to protect sensitive individuals) while the chronic RfD included an uncertainty factor of 1000 (10 for interspecies extrapolation, 10 to protect sensitive

individuals and 10 for extrapolation from subchronic to chronic exposure). The source document was a Health and Environmental Effects Document (HEED) for Ethylene Diamine (U.S. EPA, 1988). No RfD assessment for ethylene diamine is available on IRIS (U.S. EPA, 2006) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2000). The CARA list (U.S. EPA, 1991, 1994) did not identify additional documents. The Food and Drug Administration allows the use of ethylene diamine as a direct food additive; however, a tolerance of zero has been established for residues of ethylene diamine in milk (FDA, 2002a,b).

The HEAST does not report a subchronic or chronic RfC for ethylene diamine, although the HEED (U.S. EPA, 1988) derived a subchronic inhalation RfD of 1 mg/m³ based on a NOAEL (for depilation) of 145 mg/m³ in rats exposed for 30 days (Pozzani and Carpenter, 1954) and an uncertainty factor of 100. The HEED did not derive a chronic inhalation RfD. No RfC for ethylene diamine is available on IRIS (U.S. EPA, 2006). The OSHA (2002) PEL, ACGIH (2001) TLV and NIOSH (2002) REL are all 10 ppm (24.58 mg/m³), based on irritation, asthma and skin sensitization in humans.

No cancer assessment for ethylene diamine is available in the HEAST (U.S. EPA, 1997) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2000). There is a carcinogenicity assessment for this chemical on IRIS, last revised in 1996, that assigned ethylene diamine to Group D, *not classifiable as to human carcinogenicity*, based on no human data and inadequate animal data (U.S. EPA, 2006). IARC (2002) has not assessed the carcinogenicity of ethylene diamine.

The NTP (2002) status report, a chemical assessment by the WHO (1999) and a recent review (Cavender, 2001) were also consulted for relevant information. Literature searches were conducted from 1985 to March, 2002. The databases searched were: TOXLINE, MEDLINE, CANCERLIT, RTECS, GENETOX, HSDB, CCRIS, TSCATS, EMIC/EMICBACK and DART/ETICBACK.

REVIEW OF PERTINENT DATA

Human Studies

Ethylene diamine is a known sensitizer in humans (U.S. EPA, 1988). Sensitization has been shown to occur by inhalation and dermal exposure in workers (Aldrich et al., 1987; Lewinsohn and Ott, 1991; U.S. EPA, 1988), and by injection and oral exposure in asthma patients taking aminophylline (1:2 mixture of ethylene diamine and theophylline) as a bronchodilator (Hatfield, 1987).

Workers have been exposed to ethylene diamine in chemical plants, the shellac and lacquer industry, and photography and chemistry laboratories (Baker et al., 1998; Boas-Traube et al., 1948; Dernehl, 1951; Lam and Chan-Yeung, 1980; Nakazawa and Matsui, 1990). The delay between initiation of exposures and onset of symptoms can range from several weeks to years. Symptoms were primarily respiratory (sneezing, nasal discharge, chest tightness, cough and bloody cough, wheezing, dyspnea, decreased pulmonary function and severe acute asthma) and dermal (allergic dermal manifestations, allergic coryza, pruritic skin eruptions and wheals). Some reports indicated that manifestation of these symptoms may be delayed by several hours after each exposure incident; all studies reported the uniform resolution of symptoms after cessation of exposure. Only one study reported that patients were examined for anaphylaxis, which was not detected (Nakazawa and Matsui, 1990).

Using company medical records, a retrospective study of 337 chemical factory workers exposed to a mixture of ethylene diamine and n-butyl amine for up to 8 years between 1974 to 1981 found that 38 had become sensitized, exhibiting symptoms of rhinitis, coughing and expiratory wheezing that subsided after removal from the work environment and reappeared after re-exposure (Aldrich et al., 1987). Dermal sensitivity was diagnosed in 2 of these 38 workers prior to diagnosis of respiratory sensitization. The incidence of sensitization was highest in jobs associated most strongly with exposure to ethylene diamine (e.g. coater machine operator). Air sampling between 1975 and 1981 for the coater machine workplace found that in 1975, 25% of measurements exceeded 1 ppm (2.458 mg/m³) and 4.5% exceeded 10 ppm (24.58 mg/m³); in 1976, 2.5% of measurements exceeded 1 ppm (2.458 mg/m³) and 0.1% exceeded 10 ppm (24.58 mg/m³); in 1980, 19.2% of measurements exceeded 1 ppm (2.458 mg/m³) and 4.8% exceeded 10 ppm (24.58 mg/m³). Measurements at other locations (in the years 1977-1979 and 1981) exceeded 1 ppm (2.458 mg/m³) less than 2% of the time and never exceeded 10 ppm (24.58 mg/m³). The study found a statistically significant relationship between smoking and a decreased latency for onset of respiratory symptoms. Exposure to n-butyl amine is a confounding factor in this study.

Similarly, review of medical surveillance records of a group of 197 plant workers involved in production of ethylene amines (including ethylene diamine) between 1947 and 1983 and an equal-sized group of workers without exposure to ethylene amines found 7 definite and 9 suspect cases of respiratory sensitization, and 23 definite and 17 suspect cases of skin sensitization, among the exposed workers (Lewinsohn and Ott, 1991). No significant differences in mortality or pulmonary function (forced vital capacity and forced expiratory volume) were found between the exposed and control groups. However, the authors note that employees were "carefully selected" for work with ethylene amines, so that a selection bias may have occurred, and exposure specifically to ethylene diamine was not studied.

A study of a group of 130 chemical factory workers exposed to amines and other chemicals found that 38 of the workers exhibited chemically-induced asthma, diagnosed by the

presence of recurrent dyspnea with wheezing breath and cough, an unequivocal association between workplace exposure to a specific agent and symptoms, and the lack of other possible explanations such as heart or lung disease (Hagmar et al., 1982). Of these, 2 current employees and one previous employee exhibited asthma induced by controlled exposure to ethylene diamine, and 13 current and 16 previous employees exhibited asthma induced by piperazine, the cyclic dimer of ethylene diamine. The characteristic clinical presentation was sore throat and hacking cough, followed by attacks of bronchoconstriction hours to months after further exposure, with attacks starting hours after the beginning of a shift and increasing in severity during the shift. Usually the asthmatics were “relatively free of symptoms” by the following morning.

Ethylene diamine is also directly irritating to the skin and respiratory tissues (U.S. EPA, 1988). Brief (5-10 seconds) inhalation exposure of human volunteers to 250 mg/m^3 was “inoffensive,” but 500 mg/m^3 caused a slight tingling sensation of the face and slight irritation of the nasal mucosa, and 1000 mg/m^3 was intolerable due to irritation of the nasal mucosa (Pozzani and Carpenter, 1954). Corrosive effects (edema, inflammation, erosion, bleeding, ulceration) on the skin, respiratory tissues, and gastrointestinal tract in two workers occupationally exposed without protection to high levels of mixtures containing ethylene diamine were attributed to the action of ethylene diamine (Marino et al., 1994; Su et al., 2000).

Russian researchers have reported other symptoms in workers exposed to ethylene diamine, including vascular dysfunction, neurasthenic syndrome, narrowed peripheral vision, blood disorders (reticulocytosis, monocytosis and eosinophilia) and elevated levels total serum protein, γ -globulins and sialic acids (Assa, 1975; Valeeva, 1976; Valeeva et al., 1975, 1976, 1979). These findings have not been corroborated in other studies. Case studies of two workers occupationally exposed to high levels of mixtures containing ethylene diamine included reports of central nervous system effects (confusion, agitation, delirium, hallucinations, seizures, “deterioration of consciousness”), but these were attributed by the researchers to other chemicals present in the mixtures (Marino et al., 1994; Su et al., 2000). Parental employment in industries associated with ethylene diamine exposure was found not to be a risk factor for childhood brain tumors in a case-control study of 100 cases at an Ohio pediatrics hospital and 193 matched controls (Wilkins and Sinks, 1990).

Animal Studies

In order to avoid technical difficulties with corrosivity and reactivity, most oral studies of ethylene diamine toxicity in animals used salts, primarily ethylene diamine hydrochloride, rather than the parent compound. U.S. EPA (1988) concluded, based on the results of an acute oral toxicity study (Yang et al., 1983), that ethylene diamine and ethylene diamine hydrochloride were equitoxic when doses were corrected for molecular weight differences. U.S. EPA (1988)

also noted that the parent compound is expected to form the hydrochloride salt (ion) in the acidic environment of the stomach. Male Swiss Webster mice given an intravenous dose of 50 mg/kg, and oral gavage doses of 5, 50, 500 mg/kg of labeled ethylene diamine dihydrochloride (Leung, 2000) showed 87% bioavailability with a peak plasma concentration observed at about 1 hour after dosing. Ethylene diamine dihydroiodide is used in veterinary medicine as an iodine supplement (FDA, 2002c). However, studies of the toxicity of ethylene diamine dihydroiodide have focused exclusively on the effects of iodide in cattle (Andersson and Tornquist, 1983; Mangkoewidjojo et al., 1980; Schwink, 1981) and are, therefore, not useful for the assessment of ethylene diamine toxicity.

A subchronic study was conducted in which groups of 10 male and 10 female Fischer 344 rats were treated with ethylene diamine dihydrochloride in the diet for 3 months (Yang et al., 1983). Doses were 50, 260 or 1040 mg/kg-day in males (equivalent to 23, 117 or 470 mg/kg-day of ethylene diamine) and 50, 250 or 990 mg/kg-day in females (equivalent to 23, 113 or 447 mg/kg-day of ethylene diamine). Two groups of 10 males and 10 females each were used as controls. Body weight and food consumption were measured weekly; water consumption was measured monthly. Urinalysis was performed one week before sacrifice; blood chemistry and hematology were conducted at the end of the experiment. In addition to gross pathology, organ weights and histopathology of the brain, liver, kidneys, spleen, heart, adrenals and testes were performed.

No mortalities occurred (Yang et al., 1983). Food consumption was significantly and markedly reduced in females at 447 mg/kg-day; there was also a small increase in food consumption in females at 23 mg/kg-day. Water consumption was significantly reduced in all treated female groups in a dose-related manner. Body weight gain was significantly reduced in both males and females at the high dose. The biological relevance of statistically significant changes in female absolute heart weight were unclear: an increase was seen at 23 mg/kg-day and dose-related decreases were seen at 113 and 447 mg/kg-day. Other statistically significant organ weight changes (liver, adrenal, brain, kidney, spleen, testes) were seen only at the high-dose and may have been secondary to decreased weight gain. Serum alkaline phosphatase was significantly increased in males at the low and high dose and in females at the high-dose. Dose-related statistically significant increases in serum alanine aminotransferase were seen in males at the mid- and high-dose (15% and 25% increases, respectively); a similar significant increase was seen in females only at the high-dose (34%). In high-dose males and females, statistically significant increases in serum aspartate aminotransferase and decreases in serum glucose were observed. Statistically significant hematological changes were seen only at the high-dose (decreased red blood cell counts for both males and females, decreased hematocrit in females). In both males and females, urine pH and urine triple phosphate were significantly decreased at the high-dose; the authors note that these effects are consistent with the medical and veterinary use of ethylene diamine hydrochloride to acidify urine.

No treatment-related gross lesions were reported (Yang et al., 1983). Histological analyses observed hepatocellular pleomorphisms in 7/10 females (statistically significant) and 2/10 males (nonsignificant) at the high-dose level, but not in control or other treatment groups. These lesions consisted of increased cell and nuclei size, increased variation in nuclei size and shape, increased frequency of multinucleate hepatocytes and occasional hepatocyte degeneration. The increased incidence of mild tracheitis in male rats at 470 mg/kg-day was statistically significant (0/10, 1/10, 1/10, 7/10). The effects seen at the low-dose (10% increase in food consumption, 7% decrease in water consumption and a 7% increase in absolute heart weight in females) are not clearly adverse or dose-related; therefore, 23 mg/kg-day of ethylene diamine is a NOAEL for this study. Similarly, effects seen in females at 113 mg/kg-day are of unclear relevance (7% decrease in water consumption and a 7% decrease in absolute heart weight in females). Multiple endpoints (serum chemistry and histopathology) indicated that the liver was a target-organ of ethylene diamine in this study. The most sensitive indication of hepatotoxicity was an increase in serum alanine aminotransferase in male rats treated with 117 mg/kg-day of ethylene diamine, making this dose a LOAEL.

Unpublished NTP data regarding another subchronic study in F344 rats, performed at Battelle Columbus Laboratories, was described (Hermansky et al., 1999), but no information regarding this study was identified in the NTP status report, the NTP 1998 Annual Plan or the NTP 2001 Annual Plan (NTP, 2002). Reportedly, rats received gavage doses of the dihydrochloride salt equivalent to 0, 100, 200, 400, 600 or 800 mg/kg-day of ethylene diamine 5 days per week for 13 weeks. Ocular lesions were seen at 100 and 200 mg/kg-day; kidney and uterine lesions were observed at 600 and 800 mg/kg-day. No evidence of hepatocellular pleomorphism was reported. Hermansky et al. (1999) speculated that gavage administration resulted in rapid absorption in the stomach, leading to decreased initial hepatic exposure via the portal venous system and increased peak blood levels, resulting in the differences in health effects observed between the unpublished 13 week study and their 2-year study.

A 2-year chronic bioassay in Fischer 344 rats did not observe evidence of carcinogenicity (Hermansky et al., 1999). Groups of 100 male rats received 20, 100 or 350 mg/kg-day of ethylene diamine hydrochloride (equivalent to 9, 45 or 158 mg/kg-day of ethylene diamine) in the diet. Groups of 100 female rats received 20, 100 or 360 mg/kg-day of ethylene diamine hydrochloride (equivalent to 9, 45 or 162 mg/kg-day of ethylene diamine) in the diet. Two control groups of 100 animals/sex were used. Interim sacrifices of 10-20 rats/sex/group were conducted at 6, 12 and 18 months. Body weight, food and water consumption were measured. Clinical chemistry, hematology and urinalysis were performed at each interim and the terminal sacrifice. For all animals, gross necropsy, organ weights (brain, liver, kidneys, spleen, heart, adrenals and testes), and histopathology of 40 tissues and all gross lesions were performed.

Survival was higher than 75% in all groups at 20 months (Hermansky et al., 1999). However, survival at the end of the study was significantly decreased in males at the high-dose

and in females at the mid- and high-dose; increased incidence of chronic nephropathy in the animals that died (see below) was suggested as a possible cause of the late deaths. The mean survival time for males was 642 days in the high-dose group, and 681-684 days in the two control groups. The mean survival time for females was 693 days in both the high- and intermediate-dose groups, compared to 715-718 days in the two control groups. A transient increase in food consumption was seen in all treated females between 5 and 15 months (reported as 8%, data not otherwise shown). Statistically significant increases (19-50%) in mean water consumption were seen in high-dose males at 12 and 18 months and in high-dose females at 12, 18 and 24 months (data not shown). Statistically significant decreases in body weight were seen in high-dose males from 10 months on and females from 19 months on (reported as 6-23% and 6-17%, respectively, data not otherwise shown). Erythrocyte indices (red blood cell counts, hemoglobin, hematocrit) were decreased 4-12% throughout the study in high-dose males compared with controls (statistical analysis not reported). The authors speculated that ethylene diamine may have chelated iron, causing mild anemia in the high-dose males. Hematology results were not reported for low- and mid- dose males or for females. No treatment-related clinical chemistry effects were reported (data not shown). In males exposed to 158 mg/kg-day, mean urine volume was increased concomitant with a decrease in urine specific gravity at 12 and 18 (but not 24) months. Organ weight data were shown only for the liver and kidneys. Absolute and relative liver and kidney weights were generally increased at the mid- and high-dose in both males and females from 12 months onward, although not all of the changes achieved statistical significance. The authors considered these effects to be treatment-related.

Histology data were pooled for animals from the 12, 18 and 24 month sacrifices and those that died during the study (Hermansky et al., 1999). The incidence of hepatocellular pleomorphism was significantly increased ($p < 0.001$, Fisher exact test conducted at SRC) in high-dose males (10/87 versus 1/180 in controls) and females (82/91 versus 0/179); data for the lower doses were not provided. In animals with this lesion, "only a minority of hepatocytes were affected." High-dose animals also exhibited an increased incidence of inflammatory lesions of the upper respiratory tract (rhinitis and tracheitis). The authors speculated that the treatment-related rhinitis and tracheitis may be related to the inhalation of food particles containing ethylene diamine. When the analysis was restricted to animals that died during the study, statistically significant increases in the incidences of nephropathy were seen in high-dose males (18/45, 14/35, 26/42, 22/39 and 39/61 in the control A, control B, low-, mid- and high-dose groups) and females (2/14, 2/16, 3/18, 4/32 and 16/32, respectively). The nephropathy was considered by the researchers to be a possible cause of death in these animals. No evidence of carcinogenicity was observed. No tumor increases were reported (data not shown). The only significant changes in tumor incidence were decreases in pituitary adenomas and interstitial cell adenomas of the testes in high-dose males from the 18 month sacrifice and for a pooled group comprising animals that died during the study and those from the 24 month sacrifice. Among the high-dose males that died during the study, the incidence of neoplasia was significantly reduced compared with controls. In considering the evidence for kidney damage, the authors ruled out

the induction of diuresis because changes in urine volume and specific gravity were not seen until 12 months; these effects were attributed instead to altered renal function preceding nephropathy. This study found dose-related effects on the liver and kidney indicated by changes in organ weight and histopathology in male and female rats at 45 and 158-162 mg/kg-day. There is some evidence to suggest that a late decrease in survival (after 20 months) at these doses may have been related to the renal lesions. The low dose of 9 mg/kg-day is the NOAEL in this study.

No signs of reproductive toxicity were seen in a two-generation assay in Fischer 344 rats (Yang et al., 1984). Groups of 25 male and 26 female F₀ rats received ethylene diamine dihydrochloride at doses of 50, 150 or 500 mg/kg-day (equivalent to 23, 68 or 226 mg/kg-day of ethylene diamine) in the diet for 100 days prior to and during 15 days of mating. Mortality, body weight and food consumption were monitored. After mating, F₀ males were used in a dominant lethal study (Slesinski et al., 1983). F₀ females continued on their diets through gestation and lactation. Litter size was reduced to 10 on lactational day 4, and F₁ pups were continued on their parents' diets. F₁ rats were mated at 100 days of age to produce F₂ litters. Reproductive parameters measured were fertility index, days from first mating to parturition, gestation index, gestation survival index, number of pups born live and pup body weight on days 4, 14 and 21. Necropsies were performed on F₁ weanlings (5/sex/dose and 10 controls/sex), F₁ adults (10 rats/sex/dose and 20 controls/sex) and F₂ weanlings (5/sex/dose and 10 controls/sex); necropsies included weight determination of 7 organs and histopathology of 40 tissues. Additionally, gross necropsies (including examination for cleft palate) were performed on all dead pups. No mortalities were seen in F₀ or F₁ adult animals. Statistically significant decreased weight gain was seen in all adults exposed to 226 mg/kg-day and in F₀ (but not F₁) females exposed to 68 mg/kg-day. Food consumption was significantly decreased in F₁ males exposed to 68 and 226 mg/kg-day. Statistically significant increases were seen in absolute liver weight of F₁ adult males exposed to 226 mg/kg-day and in relative and absolute kidney weight of F₁ adult females exposed to 68 and 226 mg/kg-day. The incidence of hepatocellular pleomorphism was significantly increased in F₁ males (6/10) and females (10/10) at 226 mg/kg-day; similar lesions were not observed in other treated animals or in controls. Additionally, the incidence of tubular mineralization of the kidneys decreased significantly in high-dose F₁ females compared to controls. No changes in reproductive endpoints were observed in either generation. Statistically significant increases in pup body weight were seen in F₁ pups exposed to 50 mg/kg-day at day 14 and in F₂ pups exposed to 68 and 226 mg/kg-day at day 14 (but not at higher doses or other time points). The authors concluded that ethylene diamine dihydrochloride did not cause reproductive toxicity in rats. This study identifies NOAEL of 23 mg/kg-day of ethylene diamine and a LOAEL of 68 mg/kg-day of ethylene diamine, based on decreased weight gain in F₀ females, decreased food consumption in F₁ males, and increased (relative and absolute) kidney weight in adult F₁ females.

The male F₀ Fischer 344 rats described above (Yang et al., 1984) were used in a dominant lethal study (Slesinski et al., 1983). Males were mated with 1 virgin female per week

for 3 consecutive weeks. Females were sacrificed 13 days after appearance of the vaginal plug. No changes in the number of total and viable implants, litters with all fetuses viable, preimplantation loss or fetal deaths were observed.

A series of oral developmental toxicity studies in Fischer 344 rats detected fetal effects only at doses producing overt maternal toxicity (DePass et al., 1987). Groups of 20 pregnant female rats were fed a diet containing 60, 310 or 1040 mg/kg-day of ethylene diamine hydrochloride (equivalent to 27, 140 or 470 mg/kg-day of ethylene diamine) on gestation days 6-15; 40 control rats were used. Fetuses were delivered by Cesarean section on day 21 and examined for viability (number of live and dead fetuses, corpora lutea and resorptions), gender and body weight and length. Half of each litter was examined for skeletal and half for visceral abnormalities. Statistically significant decreased maternal weight gain was seen for the 310 mg/kg group on gestation days 6-15. Statistically significant weight loss was seen for the 1040 mg/kg group on days 6-11, and significantly decreased body weight gain was seen thereafter (until sacrifice on day 21). These effects correlated with a statistically significant decrease in food consumption for the 310 and 1040 mg/kg groups (6 and 28%, respectively) on days 6-15. At 1040 mg/kg-day, statistically significant decreases in fetal weight (9% in males, 10% in females) and crown-rump length (2.5% in males, 2.6% in females) and an increase in the frequency of litters with at least one resorption were observed. Additionally, at 1040 mg/kg-day, the incidence (of individual fetuses and of litters) for unossified sternbrae, slightly edematous eye bulge, slightly shortened mandible, missing innominate artery and shortened innominate artery were increased; the number of fetuses (but not the number of litters) with increased incidence of "red foci on liver" were significantly increased at 1040 mg/kg-day. Moreover, nonsignificant elevations in delayed ossification of the cervical centra and phalanges were seen at 1040 mg/kg-day, which the authors considered biologically relevant. The authors concluded that the fetal effects were not clearly related to treatment, but might have been secondary to reduced maternal food consumption. Therefore, a "probe" study was conducted in which groups of 10 pregnant female rats received 0 or 1000 mg/kg-day of ethylene diamine hydrochloride (equivalent to 450 mg/kg-day of ethylene diamine) in aqueous solution by gavage on gestation days 6-15, to avoid potential problems with palatability (DePass et al., 1987). Endpoints were maternal weight and food consumption, number of implantations, resorptions and live fetuses (but not fetal examinations). Statistically significant decreases in maternal weight loss and food intake and increases in fetal mortality and resorption incidence were observed and considered dramatic by the authors.

Subsequently, a "paired-feeding" study was conducted (DePass et al., 1987). A group of 20 pregnant female rats were fed 1000 mg/kg-day of ethylene diamine dihydrochloride (equivalent to 450 mg/kg-day of ethylene diamine) on gestation days 6-15; a group of 20 controls (with a staggered starting date) received the same amount of food consumed by the treatment group on the corresponding gestation days. An additional control group was fed *ad libitum*. The same endpoints used in the feeding study were used, except that all fetuses were used to detect

visceral abnormalities (skeletal abnormalities not assayed) and the length of the innominate artery was measured. Treated and pair-fed control dams exhibited statistically significant reductions in food consumption, weight loss on days 6-8, and decreased weight gain on days 8-15 compared to *ad libitum* controls. In ethylene diamine-exposed fetuses, statistically significant decreases in body weight, crown-rump length and innominate arterial length were observed compared to either control group. Fetal body weight was lower for pair-fed fetuses than for *ad libitum* control fetuses. Additionally, two fetuses from both treated and pair-fed control groups (but none from the *ad libitum* controls) were missing innominate arteries. Other endpoints were not affected. The authors considered the missing and shortened innominate arteries to be biologically significant and possibly related to malnutrition, but did not consider the shortening to be a teratological effect because no functional deficit would have resulted. The authors concluded that ethylene diamine hydrochloride was not teratogenic in Fischer 344 rats in these experiments, and that the fetal effects observed were a result of growth retardation. The maternal LOAEL was the lowest dose tested, 140 mg/kg-day of ethylene diamine, based on decreased weight gain and food consumption. The fetal NOAEL was 140 mg/kg-day of ethylene diamine and the fetal LOAEL was 450 mg/kg-day, based on decreased body weight, body length and innominate arterial length.

An NTP study (Heindel et al., 1993; Price et al., 1993) did not report signs of developmental toxicity. Female pregnant New Zealand White rabbits were given 0, 22, 89 or 178 mg/kg-day of ethylene diamine hydrochloride in aqueous solution (equivalent to 0, 10, 40 or 80 mg/kg-day of ethylene diamine) on gestation days 6-19. Maternal body weight, clinical condition and food intake were measured. On day 30, Caesarean section was performed and fetal endpoints of implant survival, weight, gender and morphological external, visceral and skeletal abnormalities were determined; maternal liver, kidney and uterine weights were recorded. No maternal or developmental toxicity was reported. Therefore, the fetal and maternal NOAEL for this study is 80 mg/kg-day of ethylene diamine.

Additionally, a preliminary developmental toxicity assay of 60 chemicals was conducted in CD-1 mice (Hardin et al., 1987). Groups of 50 pregnant female mice received 0 or 400 mg/kg-day of ethylene diamine on gestation days 6-13. Pups were monitored until lactational day 3. No significant changes were seen in maternal survival, the number of viable litters, number of live births per litter or pup survival. A statistically nonsignificant decrease in maternal body weight (18%) was detected. Statistically significant decreases were observed in pup birth weight (6%) and pup weight gain (3%). The authors did not discuss the significance of these results; it appears that the developmental effects are secondary to maternal toxicity. Because these effects are slight, the test dose of 400 mg/kg-day of ethylene diamine is considered a developmental and maternal NOAEL for this study.

None of the studies identified regarding sensitization were useful for the derivation of provisional toxicological values. Most animal studies regarding hypersensitization have used

dermal exposure. One study investigated whether oral pre-treatment of female Hartley albino guinea pigs might decrease dermal sensitization, but failed to observe this effect (Eriksen, 1979). The study was not designed to measure other endpoints of toxicity. Another study observed that intravenous injection of ethylene diamine (concentration and frequency of dosing not reported) reduced serum complement levels in BALB/c mice and increased the delayed hypersensitivity response of mice to sheep red blood cells injected cutaneously (Klerx et al., 1985). The authors concluded that ethylene diamine exhibited immunological adjuvant activity in mice.

Only one animal inhalation study was identified. Groups of 15 male and 15 female Sherman rats were exposed to 0, 59, 132, 225 or 484 ppm (0, 145, 325, 553 or 1190 mg/m³) of ethylene diamine 7 hours per day, 5 days per week, for 30 days (Pozzani and Carpenter, 1954). The nominal concentrations were 0, 125, 250, 500 and 1000 ppm (0, 307, 615, 1229, and 2458 mg/m³); the authors attributed the decrease to hygroscopicity and the chemical reactivity of ethylene diamine with atmospheric carbon dioxide. The authors described the methodology for generating and measuring the test atmosphere, and noted that “solid white reactant product of ethylene diamine and carbon dioxide condensed in the inlet and outlet pipes as well as on the chamber walls. A water scrubber had to be installed between the chamber and the exhaust pump to prevent damage.” This suggests that the vapor was caustic, and that animals may have been exposed to degradation products of ethylene diamine. Liver and kidney weights were measured; histological examinations were performed for the liver, kidneys and lung of the 145 mg/m³ group and the lungs, heart, liver, kidney, adrenal gland and spleens of all other treatment groups. Survival was 30/30, 30/30, 26/30, 4/30 and 0/30 (genders not specified). The mean days of death for the 553 and 1190 mg/m³ groups were 17.2 and 11.4, respectively (data not provided for other dose groups). In the 553 mg/m³ group, lung infection was detected in 10 of the deceased animals, so the authors considered only 16 of the deaths to be clearly treatment-related. In the 325 mg/m³ group, lung infection was found in all 4 animals that died, and therefore the authors did not consider these deaths to be treatment-related. No mortalities were seen at 145 mg/m³. No lung infection was observed in the control, high-dose or low-dose animals.

Depilation was observed continuously beginning on the 6th day of exposure in the 1190 mg/m³ group, was apparent “to a lesser degree” at 553 mg/m³, and was “slight” at 325 mg/m³, but not at 145 mg/m³ or in control animals (Pozzani and Carpenter, 1954). Lung congestion was observed in a third of control rats and rats exposed to 553 mg/m³ and in 17/28 animals exposed to 1190 mg/m³ (not statistically significant). Aside from the 4 animals that died from lung infection, no lung lesions were seen at 145 or 325 mg/m³. At 1190 mg/m³, histopathological examination observed statistically significant increases in the incidences of cloudy swelling in the liver (23/28), cloudy swelling and degeneration of the convoluted tubules and cloudy swelling of the loop (7/28), and congestion of the adrenal cortex (5/28). At 553 mg/m³, cloudy swelling of the liver and of the loop and convoluted tubules of the kidneys was observed “in most cases.” The authors considered 325 mg/m³ to be a NOAEL, but noted that slight depilation had persisted at this dose. The most sensitive effect seen in this study, depilation, is not clearly an

inhalation effect and instead may be caused by dermal exposure to vapors. Since the authors noted that a solid reactant product condensed on the equipment used in this study, presumably some amount also condensed on the skin and in the cages of the animals. Therefore, the data are not sufficiently clear to conclude whether 325 mg/m³ is a NOAEL or LOAEL. Additionally, the lowest concentration at which frankly toxic effects occur is unclear. A dose-response for reduced survival is seen at 325, 553 and 1190 mg/m³. However, it is not clear whether or not the mortalities seen at 325 mg/m³ are exposure-related; although the authors attributed the deaths to lung infection, ethylene diamine exposure may have contributed directly or indirectly (by increasing susceptibility to infection).

Experiments in isolated animal tissues and *in vitro* have demonstrated that ethylene diamine causes depression of the central nervous system, mediated through γ -aminobutyric acid (GABA) (U.S. EPA, 1988; Krantis et al., 1990; McKay and Krantis, 1991). Notably, similar anticholinergic action is seen with the structurally analogous drug piperazine (diethylenediamine), and this activity is responsible for its activity in humans and animals as an antihelminic agent: the parasite loses motility and passes live from the gastrointestinal tract (NIH, 2002; Trochimowicz et al., 2001). No studies were found regarding the neurological effects of ethylene diamine in animals following oral or inhalation exposure; therefore it is unknown whether ethylene diamine would exhibit similar activity. Acute intraperitoneal injection induced lethargy in frogs, mice, guinea pigs and rabbits (Barbour and Hjort, 1920; Contreras and Tamayo, 1985). These studies provide suggestive evidence that the neurological effects seen in humans following exposure to complex mixtures (Marino et al., 1994; Su et al., 2000; Valeeva et al., 1979) may have been caused in part by ethylene diamine.

A chronic dermal assay in male C3H mice did not detect evidence of carcinogenicity (DePass et al., 1984). Two samples of ethylene diamine (purity \geq 99.1%, from different industrial sources) were diluted in deionized water to 1%, and applied dorsally in 25 μ l aliquots to groups of 50 mice (housed individually) 3 times per week until death. A group of 40 mice was treated with a solution of 0.1% 3-methylcholanthrene in acetone as a positive control. Two control groups treated with deionized water were used; a group of 50 mice were housed individually and a group of 40 mice were housed 5/cage. Animals were examined daily and assessed for tumors monthly. Dorsal fur was clipped weekly. An interim sacrifice of 10 mice from each group was conducted after 18 months of treatment. Necropsies were performed on all animals. Histological analyses of the dorsal skin for all animals and the liver, kidneys and lungs of animals sacrificed at 18 months were performed. Survival incidence after 600 days was significantly decreased in one of the ethylene diamine-treatment groups compared to controls. Tumor incidence was increased significantly in the positive control group (39/40) but not in the ethylene diamine-treated groups, and the overall incidence of tumors was low (\leq 1/50/group). The ethylene diamine sample that did not affect survival caused a statistically significant increase in dermal fibrosis; no other treatment-related pathology was observed. The authors concluded that their findings provided no evidence for cutaneous oncogenicity of ethylene diamine in mice.

Notably, the authors speculate that the systemic absorption of ethylene diamine in this study was “very limited.”

Studies of the genotoxicity of ethylene diamine have generally been negative. One study reported “slight activity” in *Salmonella typhimurium* TA100 (Hulla et al., 1981). Another study detected activity in *Salmonella* strain TA1535 but not in TA98 or 1537, tested only without activation (Gee et al. 1998). Other studies saw no activity with or without metabolic activation in strains TA98, 100, 1535, 1537 or 1538 (Hedenstedt, 1978; Leung, 1994). The ability of ethylene diamine to induce mutations without metabolic activation in a series of *Salmonella* strains especially sensitive to base substitutions was assayed; the results were negative in TA 7001, 7002 and 7003 and positive in TA 7004, 7005, 7006 and a mixture of these strains (Gee et al. 1998). Assays in mammalian cells were negative, including the sister chromatid exchange assay in CHO hamster ovary cells, gene mutation in CHO cells and unscheduled DNA synthesis in rat hepatocytes (Leung, 1994; Slesinski et al., 1983). Negative results were obtained in a micronucleus test of polychromatic erythrocytes from Swiss-Webster mice injected intraperitoneally (Leung, 1994), recessive lethal testing in *Drosophila* (Zimmering et al., 1985), and dominant lethal testing in rats (Slesinski et al., 1983).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR ETHYLENE DIAMINE

Human studies have provided suggestive evidence of oral corrosivity (Marino et al., 1994; Su et al., 2000) for ethylene diamine. Although oral exposure has not been strongly associated with allergic hypersensitization (Hatfield, 1987), the concern exists that oral exposure may cause allergic hypersensitivity in susceptible individuals (FDA, 2002d), even if previous exposures occurred via other routes (e.g. inhalation, dermal, injection). The p-RfD values calculated here may not be protective of individuals hypersensitive to ethylene diamine, and it is not known whether any safe level of oral exposure exists for this subpopulation.

Animal studies indicate that the liver is a target organ following oral exposure, as demonstrated by hepatic pleomorphisms and altered liver weight (Hermansky et al., 1999; Yang et al., 1983, 1984), increased alanine aminotransferase and aspartate aminotransferase (Yang et al., 1983), and “red foci on liver” (DePass et al., 1987). Additional support is provided by the inhalation study, which reported a dose-response for cloudy swelling of the liver (Pozzani and Carpenter, 1954). The kidney also appears to be a target organ; observed effects include changes in urine pH and urine triple phosphate (Yang et al., 1983), kidney lesions (unpublished NTP data reported by Hermansky et al., 1999), increased kidney weight (Hermansky et al., 1999; Yang et al., 1984), increased nephropathy (Hermansky et al., 1999), and histopathology evidence of cloudy swelling and degeneration of the convoluted tubules and cloudy swelling of the loop (Pozzani and Carpenter, 1954). Notably, the use of ethylene diamine dihydrochloride reportedly

decreases urine pH (Yang et al., 1983); therefore, it is not clear to what degree the use of the salt instead of the parent compound may have contributed to these kidney effects. Two oral studies (Hermansky et al., 1999; Yang et al., 1983) also reported respiratory damage (tracheitis and rhinitis, presumably due to inhalation or aspiration of the treated feed) and hematological effects (decreased red blood cell counts and hematocrit). A two-generation reproductive study (Yang et al., 1984) did not observe evidence of impaired reproductive performance in rats even at high doses that produced systemic effects. Fetal toxicity was observed only at high doses that also produced overt maternal effects in rats (DePass et al., 1987). No developmental toxicity was observed in mice or rabbits (Hardin et al., 1987; Heindel et al., 1993).

For subchronic oral toxicity, two studies (Yang et al., 1983, 1984) were selected as the most reliable and sensitive from which to derive a subchronic p-RfD. The first, a 3-month feeding study in male and female rats, identified a NOAEL of 23 mg/kg-day of ethylene diamine and a LOAEL of 117 mg/kg-day of ethylene diamine, based on elevated serum alanine aminotransferase in male rats (Yang et al., 1983). The second, a 2-generation study, identified a NOAEL of 23 mg/kg-day of ethylene diamine (Yang et al., 1984). The LOAEL of 68 mg/kg-day was based on decreased weight gain in F₀ females treated subchronically (22 weeks total). The first study included clinical chemistry and hematology, and both studies included comprehensive histopathology. Supporting subchronic data include the interim sacrifices (at 6 and 12 months) performed in the chronic study (Hermansky et al., 1999), and an unpublished 13 week study (described in Hermansky et al., 1999).

A **subchronic p-RfD of 0.2 mg/kg-day** is derived by applying an uncertainty factor of 100 (10 to extrapolate from rats to humans and 10 to protect sensitive individuals) to the NOAEL of 23 mg/kg-day, as follows:

$$\begin{aligned}\text{subchronic p-RfD} &= \text{NOAEL} / \text{UF} \\ &= 23 \text{ mg/kg-day} / 100 \\ &= 0.2 \text{ mg/kg-day or } 2\text{E-}1 \text{ mg/kg-day}\end{aligned}$$

Confidence in the key studies is high. Two studies, each with a comprehensive array of endpoints in a sufficient number of animals, identified the same NOAEL and roughly similar LOAELs. Confidence in the database is high; adequate developmental and reproductive toxicity studies are available in addition to supporting subchronic studies. High confidence in the subchronic p-RfD values results.

Relevant to chronic oral toxicity, a 2-year study in male and female rats (Hermansky et al., 1999) observed a NOAEL of 9 mg/kg-day for ethylene diamine. At higher doses (45 and 158-162 mg/kg-day), observed effects were liver and kidney toxicity, as indicated by changes in organ weight and histopathology, and a decrease in late-term survival that may have been related to the renal lesions.

A **chronic p-RfD of 0.09 mg/kg-day** is derived by applying to the NOAEL of 9 mg/kg-day an uncertainty factor of 100 (10 to extrapolate from rats to humans and 10 to protect sensitive individuals), as follows:

$$\begin{aligned} \text{p-RfD} &= \text{NOAEL} / \text{UF} \\ &= 9 \text{ mg/kg-day} / 100 \\ &= 0.09 \text{ mg/kg-day or } 9\text{E-}2 \text{ mg/kg-day} \end{aligned}$$

Confidence in the key study is medium. The study included a comprehensive array of endpoints in a sufficient number of animals and identified a NOAEL and LOAEL, but results were not reported in adequate detail. Confidence in the database is medium-to-high; adequate developmental and reproductive toxicity studies are available, although supporting chronic studies are lacking. Medium-to-high confidence in the chronic p-RfD value results.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR ETHYLENE DIAMINE

Studies of human inhalation exposure to ethylene diamine have observed respiratory symptoms frequently diagnosed as asthma or allergic sensitization (Aldrich et al., 1987; Boas-Traube et al., 1948; Lewinsohn and Ott, 1991; Dernehl, 1951; Hagmar et al., 1982; Nakazawa and Matsui, 1990). In several cases of exposure to complex mixtures, sensitization to ethylene diamine specifically was verified by dermal testing or controlled inhalation exposure (Hagmar et al., 1982; Lam and Chan-Yeung, 1980; Nakazawa and Matsui, 1990). The only quantitative chronic measurements of exposure were provided by Aldrich et al. (1987); these data were not suitable for derivation of an p-RfC because exposure levels were not associated with observed health effects and because exposure was potentially confounded by smoking and n-butyl amine. One animal inhalation study was identified, a 30-day study in rats (Pozzani and Carpenter, 1954). The HEED considered the concentration of 145 mg/m³ to be a NOAEL, and used this as the basis for deriving a subchronic inhalation RfD of 1 mg/m³. The animal study (Pozzani and Carpenter, 1954) did not observe histological effects comparable to symptoms seen in humans. The dose-response is not sufficiently clear to support a quantitative risk assessment. The lowest dose tested, 145 mg/m³ of ethylene diamine, was non-toxic and the highest two doses tested, 553 and 1190 mg/m³, were clearly toxic. The most sensitive effect seen in this study, depilation, was observed at 325 mg/m³. However, this is not clearly an inhalation effect and instead may be caused by dermal exposure to vapors. Moreover, an apparent dose-response was seen for decreased survival (0/30, 0/30, 4/30, 26/30 and 30/30 deaths in the control, 145, 325, 553 and 1190 mg/m³ groups, respectively). Although the authors attributed all 4 of the deaths seen at 325 mg/m³ and 10/26 deaths at 553 mg/m³ to lung infection, inhalation exposure to ethylene diamine may have contributed directly or indirectly (by increasing susceptibility to infection). Other

adverse effects (lung congestion, liver and kidney histopathology) were seen only at 553 mg/m³ and above. Therefore, the data are not sufficient to evaluate the effects at 325 mg/m³.

In summary, no suitable human or animal studies were identified. Therefore, derivation of subchronic and chronic inhalation p-RfC values for ethylene diamine is precluded.

DERIVATION OF A PROVISIONAL CARCINOGENICITY ASSESSMENT FOR ETHYLENE DIAMINE

The human carcinogenicity data for ethylene diamine are limited to a single study (Wilkins and Sinks, 1990). A 2-year chronic feeding study did not observe increased tumor incidences in male or female rats fed the dihydrochloride salt equivalent of 9, 45 or 158-162 mg/kg-day of ethylene diamine (Hermansky et al., 1999). This study was adequate, in that it was well-designed and well-conducted, included a comprehensive array of endpoints and was of sufficient statistical power. Toxicity was observed at the mid- and high-dose, indicating that the maximum tolerated dose was achieved. Survival was high (over 75%) in all groups through 20 months of the study, but the authors speculated that treatment-related reduced survival during the last 4 months in high-dose males and mid- and high-dose females may have affected observed tumor incidence in these groups. However, no toxicity or effects on survival were found in the low-dose groups. The study was limited in that only one species was used and tumor incidences were described summarily. An 18-month dermal study in mice detected no evidence of skin tumorigenicity (DePass et al., 1984); however, the authors speculated that systemic absorption was low, and histopathology was limited to the skin, liver, kidneys and lungs. Assays for mutagenicity of ethylene diamine in bacteria produced mixed results, while an array of tests conducted in mammalian cells *in vitro* and in rodents and fruit flies *in vivo* were all negative. The available data are inadequate for an assessment of human carcinogenic potential according to the U.S. EPA (2005) cancer guidelines.

Derivation of quantitative estimates of cancer risk for ethylene diamine is precluded by the absence of data demonstrating carcinogenicity.

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