

ETHYLENEDIAMINE

CAS # 107-15-3

ORAL RISK ASSESSMENT DOCUMENT



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TABLE OF CONTENTS

1.0	INTRODUCTION	1
2.0	PHYSICAL AND CHEMICAL PROPERTIES	3
2.1	Organoleptic Properties	3
3.0	PRODUCTION AND USE	4
3.1	Production	4
3.2	Use	4
3.2.1	Industrial Use	4
3.2.2	Food Additive Use	5
3.2.3	Pharmaceutical Use	6
4.0	ANALYTICAL METHODS	6
4.1	Analysis in Water	6
4.2	Analysis in Biological Matrices	6
5.0	SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE	7
5.1	Sources of Human Exposure	7
5.2	Sources of Environmental Exposure	7
6.0	COMPARATIVE KINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS	7
6.1	Absorption	9
6.1.1	Humans	9
6.1.2	Laboratory Animals	9
6.2	Distribution	11
6.2.1	Humans	11
6.2.2	Laboratory Animals	12
6.3	Metabolism	14
6.3.1	Humans	14
6.3.2	Laboratory Animals	14
6.4	Elimination/Excretion	16
6.4.1	Humans	16
6.4.2	Laboratory Animals	16
6.4.3	Summary of Comparative Kinetics between Humans and Laboratory Animals	18
7.0	EFFECTS ON HUMANS	19
7.1	Case Reports	19
7.1.1	Asthma	19

7.1.2	Contact Dermatitis and Skin Sensitization	20
7.2	Controlled Exposure Studies	21
8.0	EFFECTS ON LABORATORY ANIMALS AND <i>IN VITRO</i> TEST SYSTEMS.....	21
8.1	Limited-Exposure Effects	22
8.1.1	Irritation and Sensitization Studies.....	22
8.1.2	Ocular Exposure Studies	22
8.2	Single-Exposure Studies.....	23
8.3	Short-Term Exposure Studies	23
8.3.1	Dietary Exposure Study in F344 Rats.....	23
8.3.2	Gavage Study in F344 Rats	24
8.3.3	Gavage Study in B6C3F1 Mice.....	25
8.3.4	Dietary Exposure Study in B6C3F ₁ Mice	25
8.3.5	Vapor Inhalation Study in Rats.....	26
8.4	Long-Term and Chronic Exposure Studies	27
8.4.1	Subchronic Studies	27
8.4.2	Chronic Oral (Dietary Administration) Study	31
8.4.3	Chronic Dermal Study in Mice.....	33
8.4.4	In vitro assays	34
8.5	Studies of Genotoxicity and Related End-Points	34
8.5.1	Mutagenicity Assays	34
8.5.2	Assays of Chromosomal Damage.....	36
8.5.3	Other Assays of Genetic Damage.....	36
8.6	Reproduction and Developmental Toxicity Studies	38
8.6.1	Two-Generation Reproduction Assay	38
8.6.2	Developmental Toxicity	39
8.7	Studies of Immunological and Neurological Effects.....	42
9.0	RISK CHARACTERIZATION	44
9.1	Hazard Assessment.....	44
9.1.1	Evaluation of Major Non-Cancer Effects and Mode of Action	44
9.1.2	Weight-of-Evidence Evaluation and Cancer Characterization.....	49
9.1.3	Selection of Key Study and Critical Effect.....	50
9.1.4	Identification of Susceptible Populations	51
9.2	Dose-Response Assessment	51
9.2.1	Uncertainty Factor Selection.....	51
9.2.2	Calculation of the Oral RfD	54
9.3	Exposure Characterization.....	54

9.4	TAC Derivation	55
9.5	STEL Derivation.....	55
9.5.1	<i>Selection of Key Study and Critical Effect for the STEL Derivation.....</i>	<i>55</i>
9.5.2	<i>Selection of Uncertainty Factors for the STEL Derivation.....</i>	<i>57</i>
9.5.3	<i>STEL Derivation.....</i>	<i>59</i>
10.0	RISK MANAGEMENT	60
10.1	SPAC Derivation	60
11.0	RISK COMPARISONS AND CONCLUSIONS	60
12.0	REFERENCES	62
12.1	REFERENCES CITED	62
12.2	REFERENCES NOT CITED	
	(Cited/Reviewed by WHO/IPCS, 1999 and/or BUA, 1997).....	70
13.0	APPENDICES	78
13.1	Appendix A: Selected Data Tables	78
13.2	Appendix B: Dose Conversions.....	80
13.2.1	<i>Single-Dose Vapor Inhalation Study with Human Volunteers</i>	<i>80</i>
13.2.2	<i>30-Day Vapor Inhalation Study in Rats.....</i>	<i>80</i>
13.3	Appendix C: Benchmark Dose Results	81
14.0	PEER REVIEW HISTORY	94

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EXECUTIVE SUMMARY

ETHYLENEDIAMINE – Oral Risk Assessment CAS # 107-15-3			
PARAMETER	LEVEL	UNITS	DERIVED:
NOAEL (no observed adverse effect level)	45	mg/kg-day	From a chronic feeding study in rats.
Oral RfD (oral reference dose)	2	mg/kg-day	From a chronic feeding study in rats with a 30x uncertainty factor.
TAC (total allowable concentration)	10	mg/L	From the oral RfD, for a 70 kg adult drinking 2 L/day with a default 20% Relative Source Contribution for drinking water.
SPAC (single product allowable concentration)	2	mg/L	From the TAC, assuming the default 10 sources of ethylenediamine in the drinking water distribution system.
STEL (short term exposure level)	40	mg/L	From a subchronic feeding study in rats, for a 10 kg child drinking 1 L/day.
KEY STUDY	Hermansky, S.J., R.S. Yang, R.H. Garman, and H.W. Leung. 1999. Chronic toxicity and carcinogenicity studies of ethylenediamine dihydrochloride by dietary incorporation in Fischer 344 rats. Food Chem Toxicol 37(7):765-76.		
CRITICAL EFFECT	General toxicity, such as decreased mean terminal body weight gain, organ weights and altered clinical parameters, observed in male and female rats fed ethylenediamine dihydrochloride for two years.		
UNCERTAINTY FACTORS	• 3x for interspecies extrapolation • 10x for intraspecies extrapolation • 1x for extrapolation from a less-than-lifetime study to a lifetime exposure duration • 1x for extrapolation from a LOAEL to a NOAEL • 1x for database deficiencies. Therefore, the total uncertainty factor was 30x.		
TOXICITY SUMMARY	No oral toxicity data for ethylenediamine in humans were located. A number of case reports and controlled studies in humans described the potential for occupational exposure to ethylenediamine to cause contact dermatitis and skin sensitization. Inhalation of ethylenediamine vapors in humans has provoked asthmatic attacks in asthmatics. Oral short-term, subchronic, chronic, reproduction, and developmental toxicity studies in laboratory rodents have been published for ethylenediamine. Short-term to subchronic oral exposure resulted in statistically reduced mean body and organ weights and altered clinical parameters in rats and mice. In the liver, hepatocellular pleomorphism was observed in rats after subchronic and chronic dietary administration, as well as in the F₁ generation rats of a two-generation reproduction feeding study. Since the effect did not progress to liver tumors and was not accompanied by consistent changes in liver weight or enzymes, it was not considered adverse. The critical effect was considered reduced terminal mean body weight gain in male (23%) and female (17%) rats in the chronic feeding study, which occurred at 162 mg/kg-day, with a NOAEL of 45 mg/kg-day. The concurrent reduced organ weights and altered clinical parameters were considered to be secondary to the reduced body weight. The NOAEL approach was selected over the benchmark dose approach to determine the RfD since the reduced body weight gain exceeded 10% and was statistically significant only at the highest dose. Mixed results were obtained in <i>Salmonella typhimurium</i> reverse mutation assays. Ethylenediamine failed to induce forward mutations or sister chromatid exchange in Chinese hamster ovary cells <i>in vitro</i>, and did not affect unscheduled DNA synthesis in cultured rat hepatocytes. Ethylenediamine produced negative results in a rat dominant lethal mutation assay <i>in vivo</i>. The weight of evidence suggests that ethylenediamine has some mutagenic potential in bacterial cells <i>in vitro</i>, but there are insufficient data to evaluate the mutagenic potential of ethylenediamine <i>in vivo</i>. Ethylenediamine was not carcinogenic via the oral or dermal exposure route in rodents. Due to the lack of human epidemiology data or a carcinogenicity bioassay in a second animal species, <i>data are inadequate for an assessment of human carcinogenic potential</i>.		
CONCLUSIONS	Chronic oral exposure to ethylenediamine caused general toxicity, such as reduced mean body weight gain and organ weights, but did not cause cancer in male or female rats. No human epidemiological data were located. Uncertainty factors were used to account for interspecies and intraspecies differences, and drinking water action levels developed in this risk assessment are protective of public health.		

1.0 INTRODUCTION

This document has been prepared to allow toxicological evaluation of the unregulated contaminant **ethylenediamine** in drinking water, as an extractant from one or more drinking water system components tested under NSF/ANSI 61 (2005) or as a contaminant in a drinking water treatment chemical evaluated under NSF/ANSI 60 (2005). Both non-cancer and cancer endpoints have been considered, and risk assessment methodology developed by the U.S. Environmental Protection Agency (U.S. EPA) has been used.

Non-cancer endpoints are evaluated using the reference dose (RfD) approach (Barnes and Dourson, 1988; Dourson, 1994; U.S. EPA, 1993; U.S. EPA, 2002), which assumes the threshold for these endpoints will not be exceeded if appropriate uncertainty factors (Dourson et. al., 1996; U.S. EPA, 2002) are applied to the highest dose showing no significant effects. This highest dose is derived from human exposure data when available, but more often is derived from studies in laboratory animals. Either the no-observed-adverse-effect level (NOAEL) taken directly from the dose-response data, or the calculated lower 95% confidence limit on the dose resulting in an estimated 10% increase in response (the LED₁₀ or BMDL₁₀ from benchmark dose programs) can be used (U.S. EPA, 2003a). The lowest-observed-adverse-effect level (LOAEL) can also be used, with an additional uncertainty factor, although the benchmark dose approach is preferred in this case. The RfD is expressed in mg/kg-day. It is defined by the U.S. EPA as “an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime” (Barnes and Dourson, 1988; U.S. EPA, 1993; U.S. EPA, 2005a).

NSF uses the RfD to derive three product evaluation criteria for non-cancer endpoints. The total allowable concentration (TAC), generally used to evaluate the results of extraction testing normalized to static at-the-tap conditions, is defined as the RfD multiplied by the 70 kg weight of an average adult assumed to drink two liters of water per day. A relative source contribution (RSC), to ensure that the RfD is not exceeded when food and other non-water sources of exposure to the chemical are considered, is also applied in calculating the TAC. The relative source contribution should be data derived, if possible. Alternately, a 20% default contribution for water can be used (U.S. EPA, 1991a). The TAC calculation is then as follows:

$$\text{TAC (mg/L)} = \frac{[\text{RfD (mg/kg-day)} \times 70 \text{ kg}] - [\text{total contribution of other sources (mg/day)}]}{2\text{L/day}}$$

or

$$\text{TAC (mg/L)} = \frac{\text{RfD (mg/kg-day)} \times 70 \text{ kg}}{2\text{L/day}} \times 0.2 \text{ (RSC)}$$

The single product allowable concentration (SPAC), used for water treatment chemicals and for water contact materials normalized to flowing at-the-tap conditions, is the TAC divided by the estimated total number of sources of the substance in the drinking water treatment and

distribution system. In the absence of source data, a default multiple source factor of 10 is used. This accounts for the possibility that more than one product in the water and/or its distribution system could contribute the contaminant in question in drinking water.

Finally, a short-term-exposure level (STEL)--at a higher level than the TAC--may be calculated for contaminants such as solvents expected to extract at higher levels from new product, but also expected to decay rapidly over time. The STEL is calculated from the NOAEL or the LED₁₀ of an animal study of 14- to 90-days duration, with uncertainty factors appropriate to the duration of the study. The contaminant level must decay to a level at or below the TAC under static conditions or to a level at or below the SPAC under flowing conditions within 90 days, based on the contaminant decay curve generated from over-time laboratory extraction data.

Endpoints related to cancer are evaluated using modeling to fit a curve to the appropriate dose-response data (U.S. EPA, 1996a; U.S. EPA, 1999; U.S. EPA, 2003b; U.S. EPA, 2005). If there is sufficient evidence to use a non-linear model, the LED₁₀ or BMDL₁₀, divided by the anticipated exposure, is calculated to give a margin of exposure. If there is insufficient evidence to document non-linearity, a linear model drawing a straight line from the LED₁₀ or BMDL₁₀ to zero is used as a default. If a linear model (generally reflecting a genotoxic carcinogen) is used, a target risk range of 10^{-6} to 10^{-4} is considered by the U.S. EPA to be safe and protective of public health (U.S. EPA, 1991a). For the purposes of NSF/ANSI 60 (2005) and 61 (2005), the TAC is set at the 10^{-5} risk level, and the SPAC is set at the 10^{-6} risk level. Use of a higher risk level is not ruled out, but would generally require documentation of a benefit to counteract the additional risk.

The RfD, TAC, SPAC, and STEL values derived in this document are based on available health effects data and are intended for use in determining compliance of products with the requirements of NSF/ANSI 60 (2005) and 61 (2005). Application of these values to other exposure scenarios should be done with care and with a full understanding of the derivation of the values and of the comparative magnitude and duration of the exposures. These values do not have the rigor of regulatory values, as data gaps are generally filled by industry or government studies prior to regulation. Data gaps introduce uncertainty into an evaluation, and require the use of additional uncertainty factors to protect public health.

The general guidelines for this risk assessment include those from the National Research Council (NRC, 1983) and from The Presidential/Congressional Commission on Risk Assessment and Risk Management (1997a, 1997b). Other guidelines used in the development of this assessment may include the following: Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986), Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), draft revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), draft final Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2003b), Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991b), Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996b), Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1998), A Review of the Reference Dose and Reference Concentration Process (U.S. EPA, 2002), Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. EPA, 1988), and Health Effects Testing Guidelines (U.S. EPA, 2006a).

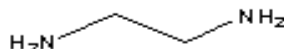
The literature search strategy employed for this compound was based on the Chemical Abstract Service Registry Number (CASRN) and at least one common name. As a minimum, the following data banks were searched:

- ChemID Plus
- Registry of Toxic Effects of Chemical Substances (RTECS)
- Hazardous Substances Data Bank (HSDB)
- GENE-TOX
- Environmental Mutagen Information Center (EMIC)
- Developmental and Reproductive Toxicology (DART)
- TOXLINE – Core and Special
- TRI (Toxics Release Inventory)
- Chemical Carcinogenesis Research Information System (CCRIS)
- Medline (via PubMed)
- Integrated Risk Information System (IRIS)
- Syracuse Research Corporation Online Toxic Substance Control Act Database (TSCATS)
- Current Contents (as requested)

The literature search for this chemical was conducted on August 3, 2004. This document includes all relevant information retrieved as a result of that search.

2.0 PHYSICAL AND CHEMICAL PROPERTIES

Ethylenediamine is an aliphatic diamine with the following structure:



This chemical also has synonyms of 1,2-diaminoethane, 1,2-ethanediamine, 1,2-ethylenediamine, dimethylenediamine, beta-aminoethylamine, dimethylenediamine; EN; ethane-1,2-diamine, ethanediamine, and ethylendiamine (Chemfinder, 2007, ChemIDplus, 2007; HSDB, 2007). It has trade names of Algicode 106L® and Amerstat 274® (ChemIDplus, 2007; HSDB, 2007). The physical and chemical properties of ethylenediamine are listed in Table 1.

2.1 Organoleptic Properties

Ethylenediamine has an ammonia-like odor (Budavari, 1996), and an odor threshold in water of 16,000 ppm (w/v; Amoore and Hautala, 1983). The odor threshold for ethylenediamine in air is 2.5 mg/m³ (BUA, 1997).

Table 1. Physical and chemical properties of ethylenediamine

Property	Data	Reference
Empirical Formula	C ₂ H ₈ N ₂	ChemIDplus, 2007
CAS #	107-15-3	ChemIDplus, 2007
Molecular Weight	60.10	Lide, 2000
Physical State and Color	Water-white liquid Colorless, clear, thick liquid	Flick, 1998 Budavari, 1996
Melting Point	8.5 °C 11 °C	Budavari, 1996 Flick, 1998
Boiling Point	116-117 °C	Budavari, 1996
Density	0.898 g/mL @ 25 °C	Budavari, 1996
Vapor Pressure	10.7 mm Hg @ 20 °C	Flick, 1998
Water Solubility	1 x 10 ⁶ mg/L	Riddick et al., 1985
pK _a	10.71 (step 1) 7.56 (step 2)	BUA, 1997
n-Octanol/Water Partition Coefficient (log K _{ow})	-1.62 (estimated) -1.2 (calculated)	SRC, 2007 BUA, 1997
Henry's Law Constant (air/water partition)	1.73 x 10 ⁻⁹ atm-m ³ /mole	Hine and Mookerjee, 1975

3.0 PRODUCTION AND USE

3.1 Production

Ethylenediamine is listed by the U.S. EPA as a high production volume chemical, indicating that it is produced in or imported to the U.S. in amounts over one million pounds per year (U.S. EPA, 2006b). The Organisation for Economic Co-operation and Development (OECD, 2001) is compiling a Screening Information Data Set (SIDS) on ethylenediamine under the international high production volume chemical program. In 1993, approximately 159,000 tons of ethylenediamine were produced in the United States (BUA, 1997). A more recent value could not be located.

3.2 Use

3.2.1 Industrial Use

Most of the ethylenediamine produced is used to manufacture carbamate fungicides (45%); chelating agents, such as ethylenediamine tetraacetic acid tetrasodium salt (25%); lubricating oil and gasoline additives (5%); polyamides (5%); aminoethylethanol-amine-based surfactants (5%); ethylene (bis)stearamide (5%); and other chemicals, including urethane additives (10%) (Kavaler, 1988). Ethylenediamine is used as a cross-linking agent in derivatization of amines and other compounds (Stone and Perkins, 1984). It has found application as a solvent, emulsifying agent, textile lubricant, and antifreeze inhibitor (HSDB, 2007). Ethylenediamine is used as a solvent for casein, albumin, shellac, and sulfur, and as a stabilizer for rubber latex and aminophylline pharmaceutical injections (Budavari, 1996). It is also used in the manufacture of wetting agents, dispersants, corrosion inhibitors, and detergents (Mackison, et al., 1981). Ethylenediamine is listed with the U.S. EPA under Pesticide Chemical Code 004205 (U.S. EPA, 2001). In January 1999, Komeen™, a complex of elemental copper and ethylenediamine, was

registered with the U.S. EPA for use as a fungicide for potatoes under U.S. EPA Pesticide Chemical Code 024407 (U.S. EPA, 2001).

Of particular significance to NSF, ethylenediamine is an ingredient in elastomer formulations used as drinking water system components certified under NSF/ANSI 61 (2005). Ethylenediamine is also used as a cross-linking agent in epichlorohydrin-dimethylamine polymers certified as coagulants under NSF/ANSI 60 (2005).

3.2.2 Food Additive Use

There are numerous listings in Title 21 (Food and Drugs) of the U.S. Code of Federal Regulations (21 CFR) for ethylenediamine alone or as a component of a complex with other chemicals (U.S. FDA, 2007). Alone, ethylenediamine can be used at one ppm to control microorganisms in cane-sugar and beet-sugar mills under 21 CFR Part 173.320 .

Derivatives of ethylenediamine or ethylenediamine in combination with other chemicals are permitted under the following sections of 21CFR (U.S. FDA, 2007).

- 21 CFR 169.115, calcium disodium ethylenediamine tetraacetate and/or disodium ethylenediamine-tetraacetate can be used to preserve color and/or flavor in French dressing.
- 21 CFR 175.105, N,N'-distearoyl ethylenediamine can be used as an indirect food additive as a component of adhesives.
- 21 CFR 175.300, ethylenediamine can be used as a catalyst or cross-linking agent for epoxy resins; N,N'-distearoyl ethylenediamine and N,N'-dioleoyl ethylenediamine can be used as release agents.
- 21 CFR 175.320, ethylenediamine can be used as a component of polyamide resins used as coatings for polyolefin films.
- 21 CFR 176.170, ethylenediamine sulfate or acetate may be used with acrylonitrile as a component of paper and paperboard in contact with aqueous and fatty foods at a concentration not to exceed 0.5% by weight.
- 21 CFR 176.180, urea formaldehyde chemically modified with ethylenediamine; N,N'-distearoyl ethylenediamine; or N,N'-dioleoyl ethylenediamine can be used as a component of paper and paperboard in contact with dry food.
- 21 CFR 176.300, N- α -(Nitroethyl)benzyl] ethylenediamine may be used as a slimicide in the manufacture of paper and paperboard that contact food.
- 21 CFR 177.1200, N,N'-dioleoyl ethylenediamine, N,N'-dilinoylethylene-diamine and N-oleoyl-N'linoleoyl ethylene-diamine may be used as a component of cellophane at $\leq 0.5\%$, or with melamine-formaldehyde as an anchor for coatings to substrate.

- 21 CFR 178.1010, tetrasodium ethylenediamine tetraacetate may be used in sanitizing solutions.
- 21 CFR 178.3120, ethylenediamine can be used as a component of animal glue.
- 21 CFR 181.30, ethylenediamine may be used with disodium cyanodithioimidocarbamate and potassium N-methyl dithiocarbamate and/or sodium 2-mercaptobenzothiazole to manufacture paper used in food packaging.

3.2.3 Pharmaceutical Use

Ethylenediamine dihydrochloride is used as a stabilizer in pharmaceuticals, such as aminophylline, a 2:1 mixture of theophylline and ethylenediamine (Yang et al., 1987). It is also used in veterinary medicine as a urine acidifier. Ethylenediamine dihydrochloride was used as a stabilizer in the original formulation of Mycolog™ cream, prescribed for the treatment of candidiasis. Due to allergic contact dermatitis cases linked to ethylenediamine, however, Mycolog™ has been reformulated without ethylenediamine (Hogan, 2001).

4.0 ANALYTICAL METHODS

4.1 Analysis in Water

Direct additives to drinking water under NSF/ANSI 60 (2005) are tested using a gas chromatography method coupled with flame ionization detection, having a detection limit of 50 mg/kg. NSF International uses a liquid chromatography/post-column fluorescence method to detect ethylenediamine as an extractant from drinking water system components tested to NSF/ANSI Standard 61 (2005). The limit of detection is 20 µg/L.

Analytical methods for determination of ethylenediamine in water sources other than potable sources have been reviewed by the BUA (1997). Ethylenediamine can be measured in river and seawater using reverse phase high performance liquid chromatography with ultraviolet detection at 315 nm, following derivatization with acetylacetone. The limit of detection was reported to be 0.00026 mg/L (Nishikawa, 1987). Ethylenediamine can also be analyzed in river water using chemoluminescence with a detection limit of 0.05 mg/L (Matveeva et al., 1987).

4.2 Analysis in Biological Matrices

Ethylenediamine concentrations in human plasma and urine following oral or intravenous administration of aminophylline have been measured by reverse phase high performance liquid chromatography with ultraviolet detection (Cotgreave and Caldwell, 1983; Caldwell et al., 1986). Aminophylline is a 2:1 mixture of theophylline and ethylenediamine. Ethylenediamine was derivatized with N,N'-di(m-toluoyl chloride) or m-toluoyl chloride prior to analysis. The limit of detection was 0.05 mg/L.

Radioactivity in the plasma of rats and mice administered ^{14}C -ethylenediamine dihydrochloride via gavage and/or injection into the trachea, tail vein, and/or heart have been determined through liquid scintillation analysis and chromatographic means (Yang and Tallant, 1982; Leung, 2000).

5.0 SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

5.1 Sources of Human Exposure

Ethylenediamine does not occur naturally (WHO/IPCS, 1999). Human exposure to ethylenediamine is primarily occupational, via dermal contact and inhalation of vapor and/or aerosol at workplaces where ethylenediamine is produced or used (HSDB, 2007). However, a recent exposure estimate was not located. NIOSH (1985) statistically estimated that 64,513 workers, with 21,469 of those being female, were potentially exposed to ethylenediamine in the United States.

Ethylenediamine exposure can occur as a result of food contact with materials covered by one or more of the numerous uses of ethylenediamine, alone or as a complex with other 21 CFR chemicals (U.S. FDA, 2007). Human exposure can also occur through ingestion and eye contact (Sittig, 1985). Ingestion of ethylenediamine as a drinking water contaminant can occur. Ethylenediamine has been detected at normalized concentrations of 1-110 mg/L as a result of its use as a cross-linking agent in epichlorohydrin-dimethylamine polymers certified as coagulants under NSF/ANSI 60 (2005). It has also been detected by NSF International at normalized concentrations of 10-3,300 mg/L as an extractant from certain drinking water system components tested to NSF/ANSI Standard 61 (2005).

5.2 Sources of Environmental Exposure

Since ethylenediamine is produced in large quantities, this chemical may be released into air or in water during its production or through its use as a chemical intermediate (HSDB, 2007). No quantitative data concerning concentrations in effluents could be located. Ethylenediamine may be released to soil, water, and the atmosphere. If released into water, ethylenediamine is expected to adhere to suspended solids and sediment. Based on aerobic screening studies, biodegradation is expected to be the most important degradation process in the environment, but no degradation rates in water or soil were located. Volatilization from water surfaces is not expected to be an important process. As ethylenediamine has two primary amine groups at the pH range found in most environmental waters, it is expected to be partially protonated, and the dissociated form would not volatilize to any appreciable extent (HSDB, 2007).

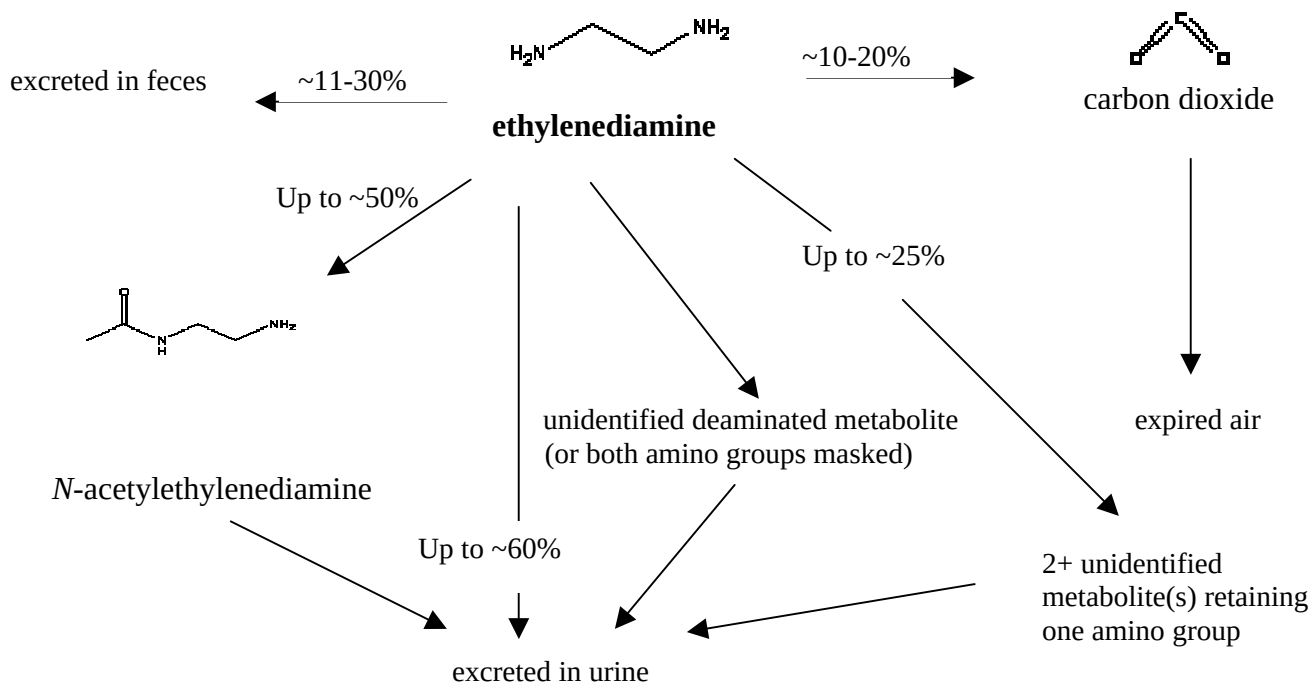
6.0 COMPARATIVE KINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS

In humans, the kinetics of ethylenediamine following oral and intravenous administration have been investigated as a result of the use of the therapeutic agent, aminophylline--a 2:1 theophylline:ethylenediamine mixture. The data in humans indicate that rapid absorption of ethylenediamine via the gastrointestinal tract occurs after oral administration of aminophylline. Ethylenediamine was rapidly removed from plasma, and at least half of the absorbed amount was

excreted in urine, primarily as *N*-acetythylenediamine. The parent compound was also excreted in urine in small amounts.

In rodents, oral, endotracheal, intracardial, and intravenous studies with ethylenediamine have been conducted. The data indicate that ethylenediamine was rapidly absorbed after oral administration, and that ethylenediamine and/or its metabolites were widely distributed throughout the body. Distribution of ethylenediamine to the major organs was greater after dermal application (up to 17% of the administered dose) compared to gavage administration (up to 3% of the dose), possibly due to hepatic first-pass metabolism. Elimination occurred through the urine, as carbon dioxide in expired air, and--to a limited extent--in the feces. As in humans, *N*-acetythylenediamine was a urinary metabolite in the rat and mouse. Data suggested that saturation of this pathway occurred at higher doses, such that alternative metabolic pathways may become involved, resulting in greater urinary elimination of unchanged ethylenediamine. Based on the results of the kinetic and metabolism studies in humans and laboratory animals, a metabolic pathway for ethylenediamine following oral administration--proposed by the authors of this risk assessment--is presented in Figure 1.

**Figure 1. Metabolic pathway for ethylenediamine following oral administration
(proposed by the authors of this risk assessment)**



6.1 Absorption

6.1.1 Humans

No studies specifically examining gastrointestinal bioavailability of ethylenediamine in humans were located. Absorption of ethylenediamine after oral administration can be inferred, however, based on the results of studies which examined the plasma kinetics of aminophylline after oral administration (Caldwell and Cotgreave, 1983; Caldwell and Cotgreave, 1982). Aminophylline is a 2:1 mixture of theophylline and ethylenediamine.

6.1.2 Laboratory Animals

6.1.2.1 Gavage, Endotracheal, and Intravenous Studies in Wistar Rats

[¹⁴C]-Ethylenediamine dihydrochloride (> 99% radiochemical purity) in distilled water with a specific activity of 8 mCi/mM was administered to groups of four male Hilltop Wistar rats at 5, 50, or 500 mg/kg via gavage in distilled water, by injection into a trachea tube in 0.9% sodium chloride, or by injection into the tail vein in 0.9% sodium chloride (Yang and Tallant, 1982). The amounts studied were equivalent to ethylenediamine doses of 2, 23, or 225 mg/kg. Food and water were available *ad libitum*. Blood was collected periodically over 24 hours. Ethylenediamine concentrations were plotted against time, and the area under the curve was used to estimate bioavailability and total plasma clearance (Table 2).

Table 2. Route-specific pharmacokinetic estimates for ethylenediamine in Wistar rats (Yang and Tallant, 1982)

Dose (mg/kg)	Bioavailability (%) ¹			Total Plasma Clearance (mL/hr) ²			Area under the Curve (µg/mL-hr) ³		
	Oral	Endo	iv	Oral	Endo	iv	Oral	Endo	iv
2	60	58	100	179	189	173	4	4	6
23	95	108	100	216	204	227	47	54	50
225	80	130	100	120	101	154	703	1,120	860

¹ Oral or endotracheal (Endo) bioavailability: area under concentration versus time curve for oral or endotracheal routes plus area under curve for intravenous route; bioavailability via the intravenous route was assumed to be 100%.
² Total plasma clearance: area under curve plus the fraction of dose absorbed x dose; mean of 4-8 rats
³ Plasma concentration versus time.

Ethylenediamine concentration versus time curves appeared to follow triphasic first-order kinetics. The authors suggested that the “first-pass effect” by the liver via oral and the lung via endotracheal administration could explain the differences in bioavailability of ethylenediamine between the low dose and the two higher doses. The bioavailability data at the high-dose suggested a reduced capacity for absorption by the gastrointestinal tract as compared to the lung. According to the study authors, since a single bolus of 500 mg/kg is unlikely in a chronic study, it can be assumed that all routes examined are likely equivalent at relatively low dose levels, such as those equivalent to ≤ 23 mg/kg ethylenediamine.

6.1.2.2 Gavage Study in F344 Rats

A single dose of [^{14}C]-ethylenediamine dihydrochloride (radiochemical purity > 99% in distilled water) was administered to three to five F344 rats per sex and dose via gavage at 0 or 50 mg/kg, equivalent to ethylenediamine doses of 0 or 23 mg/kg (Yang et al., 1984a). Blood was collected periodically over 24 hours, and the absorption rate constant was determined. The study was conducted parallel to a chronic feeding assay, so this procedure could be repeated after 6 and 18 months to determine whether there were kinetic differences in relation to age, sex, and/or dosing duration. The rats used at each time interval (0, 6, or 18 months) for the kinetic study were randomly chosen from either the control (age- and sex-related differences) or 50 mg/kg (age-, sex-, and exposure duration-related differences) groups used in the chronic study. Thus, treated rats in the 6- and 18-month groups were administered unlabelled ethylenediamine dihydrochloride daily for the respective durations as compared to the corresponding controls at 6 and 18 months, which received only one dose of [^{14}C]-ethylenediamine. A three-way analysis of variance was used to compare each of the kinetic parameters to age, sex, and duration of exposure.

There was no significant difference in the absorption rate constant for ethylenediamine with respect to age, sex, or duration of exposure.

6.1.2.3 Gavage and Intravenous Studies in Swiss Webster Mice

In a study by Leung (2000), two male Swiss Webster mice per dose and sacrifice interval were administered a single gavage dose of 50 mg/kg ^{14}C -ethylenediamine dihydrochloride (> 99% radiochemical purity in physiological saline) or an intravenous injection of 50 mg/kg ^{14}C -ethylenediamine dihydrochloride directly into the cardiac muscle. Food and water were available *ad libitum*. Blood was collected periodically over 24 hours. Various pharmacokinetic parameters were estimated and included maximum plasma concentration, time to reach maximum concentration, area under the plasma concentration versus time curve, bioavailability, total plasma clearance, and terminal half-life.

Ethylenediamine was rapidly absorbed following oral administration since the peak plasma concentration of 0.0048 mg/mL was reached within one hour compared to 0.00275 mg/mL after intravenous injection. Bioavailability was 87% (assuming 100% for intravenous), and the area under the plasma concentration versus time curve was 0.0205 mg-h/mL compared to 0.0235 mg-h/mL for intravenous administration. Total plasma clearance was 32 and 33.5 mL/hour for oral and intravenous administration, respectively. Terminal half-lives were 6.8 and 5.0 hours for oral and intravenous administration, respectively. The volume of distribution was estimated to be 0.55 L/kg after intravenous administration.

6.1.2.4 Dermal Study in Wistar Rats

A 0.2 mL solution of 10, 25, or 50% ^{14}C -ethylenediamine dihydrochloride (> 99% radiochemical purity in distilled water) was applied to the clipped intact dorsal skin of four to six male Wistar rats per dose (Yang et al., 1987). Assuming 100% purity and based on a density of 0.898 and a median rat weight of 0.350 kg, each rat was treated with approximately 51, 128, or 256 mg/kg

^{14}C -ethylenediamine dihydrochloride, or approximately 23, 58, or 115 mg/kg ethylenediamine. Occluded patches were placed over the treatment area. Blood was taken periodically over 24 hours. The absorption rate constant and terminal plasma half-life were determined, and the area under the plasma concentration versus time curve was calculated.

The absorption of ethylenediamine was estimated to be 12, 55, and 61% in animals given 10, 25, and 50% ethylenediamine, respectively. The pharmacokinetic parameters could not be adequately measured in the 10% group due to analytical limitations. At 25 and 50%, the absorption rate constants for ethylenediamine were approximately 0.99 and 0.93 hours⁻¹, respectively. At 25 and 50%, the terminal plasma half-lives of ethylenediamine were approximately 4.4 and 4.9 hours, respectively. At 25 and 50%, the areas under the plasma concentration versus time curves were approximately 161.5 and 307.7 µg/mL-hour, respectively. The authors concluded that a two-compartment model with first-order absorption could clearly describe the plasma kinetics of ethylenediamine. Further, the authors concluded that the skin absorption of ethylenediamine was relatively low and concentration-dependent, possibly due to epidermal damage caused by higher concentrations.

6.2 Distribution

6.2.1 Humans

Plasma concentrations of ethylenediamine were examined after oral administration of aminophylline (purity unspecified in saline) in three healthy male volunteers, ages 22-26 years and weighing 65-69 kg (Caldwell and Cotgreave, 1982; Caldwell and Cotgreave, 1983). As each volunteer received 43 mg ethylenediamine from the aminophylline dose, the final oral dose of ethylenediamine was approximately 0.6-0.7 mg/kg, based on reported body weights. Blood was collected at regular intervals up to six hours after ingestion. A two-compartment open model was used to analyze the ethylenediamine plasma concentration: time relationships.

After oral administration of aminophylline, the plasma concentration of ethylenediamine reached a maximum after 45 minutes (0.4 µg/mL) and decreased exponentially with time. Ethylenediamine was only detected in the plasma for the first two hours after dosing. The mean plasma half-life was one hour, and bioavailability was reported to be 34%.

Plasma concentrations of ethylenediamine were examined after a single intravenous injection of aminophylline (purity unspecified) in water in six healthy human volunteers. The volunteers consisted of four males and two females ages 21-47 years old (Caldwell et al., 1986). Assuming 100% purity and based on an administered volume of 10 mL and weights between 51-84 kg, each volunteer received 56 to 92 mg/kg. Blood was taken periodically over 24 hours. Plasma elimination half-life, volume of distribution, and clearance were estimated.

The plasma ethylenediamine concentration versus time curve was biphasic, and the data fit a two-compartment open model. The plasma elimination half-life was 114 ± 58 minutes. The volume of distribution was 374 ± 45 mL/kg, and plasma clearance was 8.7 mL/min-kg.

6.2.2 Laboratory Animals

6.2.2.1 Gavage, Endotracheal, and Intravenous Studies in Wistar Rats

[¹⁴C]-Ethylenediamine dihydrochloride (> 99% radiochemical purity in distilled water) with a specific activity of 8 mCi/mM was administered to groups of four male Hilltop Wistar rats at 5, 50, or 500 mg/kg via gavage in distilled water, injection into a trachea tube in 0.9% sodium chloride, or intravenous injection in 0.9% sodium chloride (Yang and Tallant, 1982). These doses were equivalent to ethylenediamine doses of 2, 23, or 225 mg/kg. Food and water were available *ad libitum*. Twenty-six tissues were analyzed for radioactive concentrations of ethylenediamine and its metabolites, but only data for the liver, kidneys, brain, lung, muscle, fat (unspecified), thyroid, bone marrow, and adrenal gland were presented. The remaining tissues analyzed were not indicated.

After intravenous administration of 2, 23, and 225 mg/kg ethylenediamine, the mean percent of the administered radioactivity distributed to the nine noted organs varied between 0.09 mg/kg (brain) to 1.09 mg/kg (thyroid) in rats administered 2 mg/kg ethylenediamine. The tissue concentrations of radioactivity increased in direct proportion with dose (1:10:100). Only data for the intravenous route were provided, since the results following gavage or endotracheal administration were reportedly similar. According to the authors, although radioactivity was found throughout the body, the thyroid and bone marrow contained relatively higher levels than the other tissues via all three routes of administration. The study authors concluded that the data suggested that the dose levels employed were within the limit of linear kinetics with respect to tissue distribution.

6.2.2.2 Gavage Study in F344 Rats

A single dose of [¹⁴C]- ethylenediamine dihydrochloride (radiochemical purity > 99%) in distilled water was administered to three to five F344 rats per sex and dose via gavage at 0 or 50 mg/kg, equivalent to ethylenediamine doses of 0 or 23 mg/kg (Yang et. al., 1984a). Blood was collected over 24 hours for determination of plasma terminal half-life, area under the plasma concentration versus time curve, and volume of distribution. Radioactivity in the plasma, brain, lung, heart, thymus, liver, kidney, adrenal, spleen, pancreas, esophagus, stomach and contents, small intestine, colon, thyroid, salivary gland, trachea, ovary or testes, lymph node, bone marrow, fat, muscle, and carcass was determined after 24 hours. The study was conducted parallel to a chronic feeding assay, so this procedure could be repeated after 6 and 18 months in order to determine whether there were kinetic differences in relation to age, sex, and/or duration of exposure. The rats used at each time interval (0, 6, or 18 months) for the kinetic study were randomly chosen from either the control (age- and sex-related differences) or 50 mg/kg (age-, sex-, and exposure duration-related differences) groups used in the chronic study. Thus, treated rats in the 6- and 18-month groups have been administered unlabelled ethylenediamine dihydrochloride daily for the respective durations as compared to the corresponding controls at 6 and 18 months, which have received only one dose of [¹⁴C]-ethylenediamine. A three-way analysis of variance was used to compare each of the kinetic parameters to age, sex, and duration of exposure.

Based on the area under the plasma concentration versus time curve, a two-compartment model adequately described the plasma kinetics of ingested ethylenediamine. There was no significant difference between the terminal half-life of ethylenediamine with respect to age, sex, or duration of exposure. However, the area under the curve was statistically higher for older rats than for very young ones, which means that there was more ethylenediamine in the systemic circulation of older versus younger rats. The authors suggested that this finding may be related to the fact that body fat content increases with age. Thus, since the doses were administered per kg body weight and the test compound is non-lipophilic, higher doses may have been available to older rats. This theory is supported by the fact that the age-related increase in area under the curve corresponded to a statistical decrease in volume of distribution, such that older rats had smaller values for volume of distribution than younger ones. The study authors suggested that this result indicated that ethylenediamine is distributed through a smaller circulatory and tissue volume in older rats.

Chronically dosed rats, especially females, had lower ethylenediamine concentrations in peripheral tissues compared to the concurrent control rats. The study authors suggested this finding may be due to competition from the non-labeled compound taken up via the diet. That is, the intake of ^{14}C -ethylenediamine dihydrochloride in treated rats at 6 and 18 months may have been limited since these rats had been receiving ethylenediamine dihydrochloride daily in their diet through the parallel feeding study.

6.2.2.3 Gavage and Intravenous Studies in Swiss Webster Mice

In a study by Leung (2000), four male Swiss Webster mice per dose were administered 5, 50, or 500 mg/kg ^{14}C -ethylenediamine dihydrochloride of > 99% radiochemical purity in physiological saline (equivalent to ethylenediamine doses of 2, 23, or 225 mg/kg) via gavage. The concentration of radioactivity in the brain, kidney, liver, lung, plasma, muscle, and fat was measured after 48 hours. At the highest dose, the concentration of radioactivity was also determined in the adrenal gland, bladder, stomach, heart, small intestine, colon, lymph nodes, skin, spleen, stomach, testes, and thymus. The experiment was repeated once for unspecified reasons and the results of both experiments were combined by the study authors.

In mice administered 2 mg/kg ethylenediamine, the mean amount of ethylenediamine distributed to the seven organs examined was approximately 0.003 mg ethylenediamine/gram tissue, with the highest concentrations found in the liver (0.0009 mg/g) and kidney (0.0007 mg/g).

In mice administered 23 mg/kg ethylenediamine, the mean amount of ethylenediamine distributed to the seven organs examined was approximately 0.019 mg ethylenediamine/gram tissue, again with the highest concentrations found in the liver (0.007 mg/g) and kidney (0.005 mg/g).

In mice administered 225 mg/kg ethylenediamine, the mean amount of ethylenediamine distributed to the 19 organs examined was approximately 0.68 mg ethylenediamine/gram tissue, with the highest concentrations found in the small intestine (0.08 mg/g), liver (0.07 mg/g), and kidney (0.06 mg/g).

6.2.2.4 Dermal Study in Rats

A 0.2 mL solution of 10, 25, or 50% ^{14}C -ethylenediamine dihydrochloride (> 99% radiochemical purity in distilled water) was applied to the clipped intact dorsal skin of four to six male Wistar rats per dose (Yang et al., 1987). Assuming 100% purity and based on a density of 0.898, each rat was treated with approximately 18 mg ^{14}C -ethylenediamine dihydrochloride. The “major organs” (considered the liver, kidney, lung, and brain) and carcass were analyzed for radioactivity.

Total recovery of the administered dose averaged 70, 75, and 83% in the 10, 25, and 50% groups, respectively. The authors suggested the low recovery was possibly due to volatilization of ethylenediamine from the skin. Of the total radioactivity recovered, means of 0.7, 2.4, and 2.9% were recovered from the “major organs” and means of 3.4, 10.6, and 17.9% were recovered from the carcasses of animals administered 10, 25, and 50%, respectively. No further details regarding tissue distribution were available.

6.3 Metabolism

6.3.1 Humans

No metabolism studies in humans were identified.

6.3.2 Laboratory Animals

6.3.2.1 Gavage Study in F344 Rats

A single dose of [^{14}C]-ethylenediamine dihydrochloride (radiochemical purity > 99% in distilled water) was administered to three to five F344 rats per sex via gavage at 0 or 50 mg/kg, equivalent to ethylenediamine doses of 0 or 23 mg/kg (Yang et al., 1984a). $^{14}\text{CO}_2$ production was measured for 48 hours. Urine was collected at 24-hour intervals for 48 hours for the identification of urinary metabolites. The study was conducted parallel to a chronic feeding assay, so this procedure could be repeated after 6 and 18 months, in order to determine whether there were kinetic differences in relation to age, sex, and/or dosing duration. The rats used at each time interval (0, 6, or 18 months) for the kinetic study were randomly chosen from either the control (age- and sex-related differences) or 50 mg/kg (age-, sex-, and exposure duration-related differences) groups used in the chronic study. Thus, treated rats in the 6- and 18-month groups have been administered unlabelled ethylenediamine dihydrochloride daily for the respective durations as compared to the corresponding controls at 6 and 18 months, which have received only one dose of [^{14}C]-ethylenediamine. A three-way analysis of variance was used to compare each of the kinetic parameters to age, sex, and duration of exposure.

Ten to 22% of the administered radioactivity was eliminated via expired air in the form of $^{14}\text{CO}_2$. Up to 50% of the administered radioactivity was detected in the urine as unchanged ethylenediamine, N-acetyethylenediamine, and at least two other unidentified metabolites. The amount of unchanged compound excreted in the urine increased with age, but the amount of N-

acetylenediamine excreted in the urine did not vary according to age, sex, or dosing duration.

6.3.2.2 Gavage, Endotracheal, and Intravenous Study in Wistar Rats

[¹⁴C]-Ethylenediamine dihydrochloride (> 99% radiochemical purity in distilled water) with a specific activity of 8 mCi/mM was administered to groups of four male Hilltop Wistar rats at doses of 5, 50, or 500 mg/kg via gavage in distilled water, injection into the trachea in 0.9% sodium chloride, or injection into the tail vein in 0.9% sodium chloride (Yang and Tallant, 1982). The dichloride salt doses were equivalent to ethylenediamine doses of 2, 23, or 225 mg/kg. Food and water were available *ad libitum*. Urine was collected at 24-hour intervals for 48 hours to identify the metabolites.

Up to 9% of the administered radioactivity was eliminated via expired air in the form of ¹⁴CO₂ for all three routes. Three characteristically similar peaks were observed following all three routes of administration (Table 3). The first peak appeared to represent a completely deaminated metabolite (little or no cation-exchange properties), or one in which both amino groups were masked, but the metabolite was not identified. The second peak, which represented more than half of the urinary radioactivity, was N-acetylenediamine. The third peak represented unmetabolized ethylenediamine. N-acetylenediamine was the major urinary metabolite at doses up to 23 mg/kg, but saturation of this pathway occurred at higher doses resulting in greater urinary elimination of unchanged ethylenediamine. At a dose of 500 mg/kg, the presence of an additional unidentified peak close to the peak of N-acetylenediamine implied an alternate or secondary pathway at higher doses. There were no substantial route-specific differences.

Table 3. Urinary metabolites of ethylenediamine in Wistar rats (Yang and Tallant, 1982)¹

Dose (mg/kg)	Gavage (%)			Endotracheal (%)			Intravenous		
	2	23	225	2	23	225	2	23	225
Unchanged ethylenediamine	8.4	22	44	2.1	21	46	10	29	48
N-acetylenediamine	65	62	37	67	59	40	63	52	39
Peak #1 (unidentified)	20	13	10	25	15	11	22	15	10

¹ Results represent mean percent of recovered radioactivity over 48 hours (combined 0-24 and 24-48 hour samples).

6.3.2.3 Gavage Study in Swiss Webster Mice

In a study by Leung (2000), four male Swiss Webster mice per dose were administered 5, 50, or 500 mg/kg ¹⁴C-ethylenediamine dihydrochloride in physiological saline, equivalent to ethylenediamine doses of 2, 23, or 225 mg/kg, via gavage. Urine and feces were collected at 24-hour intervals for 48 hours, and expired ¹⁴CO₂ was measured in 24-hour intervals for 48 hours.

Following gavage administration, up to 10% of the administered radioactivity was eliminated via expired air in the form of ¹⁴CO₂. Five metabolites were detected in the urine (Table 4). Peak #1 appeared to be a deaminated metabolite of ethylenediamine, based on little or no cation-exchange properties. Peaks #2 and #3 appeared to retain one of the amino groups. Peak #4,

identified as acetylenediamine (*N*-acetylenediamine), was the main metabolite. Peak #5 was the parent compound, ethylenediamine.

N-acetylenediamine was the major urinary metabolite at oral doses up to 23 mg/kg, but saturation of this pathway occurred at higher doses, resulting in greater urinary elimination of unchanged ethylenediamine.

Table 4. Urinary metabolites for ethylenediamine after gavage administration in mice (Leung 2000) ¹

Dose (mg/kg)	Mean percent of recovered radioactivity		
	2	23	225
Unchanged ethylenediamine	15.1	19.7	64.3
<i>N</i> -acetylenediamine	57.6	35.3	12.8
Peak #1 (unidentified)	11.7	24.9	7.3
Peak #2 (unidentified)	Not detected	2.1	0.5
Peak #3 (unidentified)	13.1	20.3	17.9

¹ Results represent mean percent of recovered radioactivity over 48 hours (combined 0 to 24- and 24 to 48-hour samples).

6.4 Elimination/Excretion

6.4.1 Humans

No elimination or excretion studies in humans were identified.

6.4.2 Laboratory Animals

6.4.2.1 Gavage Study in F344 Rats

A single dose of [¹⁴C]-ethylenediamine dihydrochloride (radiochemical purity > 99% in distilled water) was administered to three to five F344 rats per sex via gavage at 0 or 50 mg/kg, equivalent to ethylenediamine doses of 0 or 23 mg/kg (Yang et al., 1984a). The ¹⁴CO₂ production rate constant was determined after 48 hours. Urine and feces were collected at 24-hour intervals for 48 hours. The study was conducted parallel to a chronic feeding assay, so this procedure could be repeated after 6 and 18 months in order to determine whether there were kinetic differences in relation to age, sex, and/or dosing duration. The rats used at each time interval (0, 6, or 18 months) for the kinetic study were randomly chosen from either the control (age- and sex-related differences) or 50 mg/kg (age-, sex-, and exposure duration-related differences) groups used in the chronic study. Thus, treated rats in the 6- and 18-month groups have been administered unlabelled ethylenediamine dihydrochloride daily for the respective durations as compared to the corresponding controls at 6 and 18 months, which received only one dose of [¹⁴C]-ethylenediamine. A three-way analysis of variance was used to compare each of the kinetic parameters to age, sex, and duration of exposure.

Ten to 22% of the administered radioactivity was eliminated via expired air in the form of ¹⁴CO₂. The ¹⁴CO₂ production rate constant was higher in females than in males. The authors suggested

that this result could be due to differing contributions of this pathway in rats of different sex. Thirty-nine to 51% of the administered radioactivity was excreted in the urine. *N*-acetylenediamine accounted for approximately half of the urinary radioactivity, with unchanged ethylenediamine accounting for much of the other half. The amount of unchanged compound excreted in the urine increased with age, but the amount of *N*-acetylenediamine excreted in the urine did not vary according to age, sex, or dosing duration. At least two other minor metabolites were detected in the urine, but were not identified. Eleven to 30% of the administered radioactivity was excreted in the feces.

6.4.2.2 Gavage, Endotracheal, and Intravenous Study in Wistar Rats

[¹⁴C]-Ethylenediamine dihydrochloride (> 99% radiochemical purity in distilled water) with a specific activity of 8 mCi/mM was administered to groups of four male Hilltop Wistar rats at doses of 5, 50, or 500 mg/kg via gavage in distilled water, injection into the trachea in 0.9% sodium chloride, or injection into the tail vein in 0.9% sodium chloride (Yang and Tallant, 1982). These dichloride salt doses were equivalent to ethylenediamine doses of 2, 23, or 225 mg/kg. Food and water were available *ad libitum*. Urine and feces were collected at 24-hour intervals for 48 hours.

Most of the radioactivity in the urine, feces, and expired air was recovered during the first 24-hour interval. The combined 48-hour results suggest that the elimination of ethylenediamine through the urine, feces, and expired air after oral, endotracheal, and intravenous administration is similar (Table 5).

Table 5. Route-specific elimination of ethylenediamine in Wistar rats (Yang and Tallant, 1982)¹

Dose (mg/kg)	Gavage (%)			Endotracheal (%)			Intravenous (%)		
	Urine	Feces	Air ²	Urine	Feces	Air ²	Urine	Feces	Air ²
2	57	5	9	65	13	7	57	5	9
23	58	13	6	62	13	8	58	13	6
225	48	17	7	62	10	9	48	17	7

¹ Results represent mean percent of recovered radioactivity over 48 hours (combined 0 to 24- and 24- to 48-hour samples).
² As ¹⁴CO₂.

6.4.2.3 Gavage Study in Swiss Webster Mice

In a study by Leung (2000), four male Swiss Webster mice per dose were administered 0, 5, 50, or 500 mg/kg ¹⁴C-ethylenediamine dihydrochloride in physiological saline, equivalent to ethylenediamine doses of 0, 2, 23, or 225 mg/kg, via gavage. Urine and feces were collected at 24-hour intervals for 48 hours, and expired ¹⁴CO₂ was measured periodically for 24 hours. The experiment was repeated once for unspecified reasons, and the results were reported as the mean of both experiments.

As the gavage dose increased, the portion of unmetabolized parent compound eliminated in the urine increased, with a corresponding decrease in the percentage of acetylene diamine and the other urinary metabolites, suggesting saturation kinetics (Table 6). Since one of the unidentified

urinary metabolites (peak #2 retaining one amino group) occurred only in mice receiving 23 or 225 mg/kg, an alternate or secondary pathway may have been involved at doses above 2 mg/kg. The authors concluded that the metabolic fate of ethylenediamine in mice was similar to that in rats (Yang and Tallant, 1982), although mice may be more metabolically active with respect to ethylenediamine.

Table 6. Elimination of ethylenediamine after gavage administration in mice (Leung, 2000)¹

Dose (mg/kg)	% of administered dose		
	Urine	Feces	Air ²
2	63	14	10
23	72	5	10
225	56	12	9

¹ Mean percent of recovered radioactivity over 48 hours (combined 0 to 24- and 24- to 48-hour samples).
² As ¹⁴CO₂

6.4.2.4 Dermal Study in Wistar Rats

A 0.2 mL solution of 10, 25, or 50% ¹⁴C-ethylenediamine dihydrochloride (> 99% radiochemical purity in distilled water) was applied to the clipped intact dorsal skin of four to six male Wistar rats per dose (Yang et al., 1987). Assuming 100% purity and based on a density of 0.898, each rat was treated with approximately 18 mg ¹⁴C-ethylenediamine dihydrochloride. At 10, 25, and 50%, the mean recovery of administered radioactivity in the urine was 6.7, 36.3, and 32.2%, and the mean recovery of administered radioactivity in the feces was 0.4, 2.2, and 1.9%, respectively.

6.4.3 Summary of Comparative Kinetics between Humans and Laboratory Animals

The kinetics studies in humans are the result of administration of the therapeutic agent, aminophylline, which is a 2:1 mixture of theophylline:ethylenediamine. Nonetheless, since ethylenediamine plasma concentrations were measured in these studies, a summary of some kinetic parameters after oral administration in humans and laboratory animals can be made (Table 7). Based on the available data, it appears that total plasma clearance (mL/min-kg) is similar between humans and rats, but the plasma elimination half-life of ethylenediamine in humans was four times faster than rats administered approximately the same oral dose of ethylenediamine (≤ 2 mg/kg). Due to limited data in humans, no other kinetics comparisons between humans and laboratory animals could be made.

**Table 7. Summary of comparative oral kinetics
between humans and laboratory animals**

Study type and ethylenediamine dose (reference)	Bioavailability (% of administered dose)	Total plasma clearance (mL/min-kg)	Plasma elimination T _{1/2} (minutes)
Human males, oral, 0.6-0.7 mg/kg (Caldwell and Cotgreave, 1982; Caldwell and Cotgreave, 1983)	Not determined	8.7	114 (1.9 hours)
Male mice, gavage, 23 mg/kg (Leung, 2000)	87	17.8 ¹	408 (6.8 hours)
Male rats, gavage, 2 mg/kg (Yang and Tallant, 1982)	60	8.5 ²	456 (7.6 hours)
Male rats, gavage, 23 mg/kg (Yang and Tallant, 1982)	95	10.3 ³	318 (5.3 hours)
¹ reported as 32 mL/hr; assuming 0.03 kg mouse, 32 mL/hr x 1 hr/60 minutes x 1 mouse/0.03 kg = 17.8 mL/min-kg			
² reported as 179 mL/hr; assuming 0.35 kg rat, 179 mL/hr x 1 hr/60 minutes x 1 rat/0.35 kg = 8.5 mL/min-kg			
³ reported as 216 mL/hr; assuming 0.35 kg rat, 216 mL/hr x 1 hr/60 minutes x 1 rat/0.35 kg = 17.8 mL/min-kg			

7.0 EFFECTS ON HUMANS

No oral human exposure data for ethylenediamine were located. Numerous case reports were located detailing the potential for dermal occupational exposure to ethylenediamine to cause contact dermatitis, which was confirmed by positive patch test reactions. Inhalation of ethylenediamine vapors has also been shown to induce asthma in asthmatics.

This section includes only studies not included in the qualitative risk assessments for ethylenediamine conducted by the WHO/IPCS (1999) or BUA (1997); or studies which may have been included, but were considered to be of particular significance in an oral risk assessment.

7.1 Case Reports

7.1.1 Asthma

A large number of cases of occupational asthma potentially caused by exposure to ethylenediamine were reviewed by WHO/IPCS (1999) and BUA (1997). The WHO/IPCS (1999) concluded that the mechanism of respiratory tract hypersensitivity and provocation of asthma were suggestive of an immunological mechanism at sub-irritant concentrations, based on the skin sensitizing potential of ethylenediamine and the limited evidence of immunological involvement in workers with ethylenediamine-provoked asthma.

A study by Nakazawa and Matsui (1990), which was included in the WHO/IPCS (1999) review, provided strong evidence of ethylenediamine-induced respiratory tract hypersensitivity and provocation of asthma, and implicated an immunological mechanism. Two male asthmatic factory workers, 18 and 37 years old, exhibited late asthmatic responses to ethylenediamine vapor. After several months of exposure to ethylenediamine, both patients developed coughing, wheezing, and dyspnea. Provocative inhalation tests using ethylenediamine (purity unspecified in saline) evoked late asthmatic responses in both patients, but did not evoke any asthmatic

symptoms in healthy subjects. In an intracutaneous test, when 1 mM ethylenediamine (purity unspecified in saline) was injected, wheal and flare reactions were observed in both subjects, but not in healthy volunteers. A Prausnitz-Kustner test was also performed, in which serum from each subject was intracutaneously injected into a healthy volunteer. Forty-eight hours later, each volunteer received a challenge intracutaneous injection of ethylenediamine. Positive reactions in the volunteers were observed at the injection sites with serum from both subjects. Immunoglobulin E antibodies to ethylenediamine were detected in both the intracutaneous and Prausnitz-Kustner tests. Patch tests were also performed and revealed no evidence of dermal sensitization after a 48-hour observation period. No further details were provided. Based on the collective results, the authors concluded that late asthmatic responses observed in this study were mainly due to immediate-type allergic reactions. They were uncertain as to whether immunocomplex-mediated hypersensitivity and cell-mediated hypersensitivity played a role, since Immunoglobulin G antibodies to ethylenediamine were not detected in the serum of the subjects and since patch tests were negative.

7.1.2 Contact Dermatitis and Skin Sensitization

A large number of case reports and studies have been published documenting the skin sensitizing properties of ethylenediamine or ethylenediamine dihydrochloride following clinical or occupational use. These studies have been reviewed by WHO/IPCS (1999) and/or BUA (1997). These reports include cases of eczema in pharmacists using aminophylline, eczema in patients using a skin cream containing ethylenediamine, and contact dermatitis from the use of floor polish remover, coolant oils, or in personnel employed in a wire-drawing facility--all of which were traced back to exposure to ethylenediamine (WHO/IPCS, 1999). In many studies, patch tests with ethylenediamine were conducted, and positive reactions were observed in up to 60% of the individuals.

A few case reports regarding the potential for ethylenediamine to cause contact dermatitis were not included in the reviews by WHO/IPCS (1999) and/or BUA (1997). These studies reconfirm that occupational exposure to ethylenediamine can cause contact dermatitis.

A 33-year old female nurse developed acute dermatitis while preparing and administering systemic aminophylline (Corazza et al., 1994). The dermatitis was located on the middle and distal phalanges of the left thumb, forefinger, and middle finger, and the right forefinger and middle finger. The patient was patch tested, and was positive to ethylenediamine dihydrochloride, but not sensitive to emulsifiers, gloves, or theophylline. Patch tests confirmed that the contact dermatitis was due to the ethylenediamine and not the theophylline component of aminophylline.

Arens et al. (1998) reported on a 44-year old male with a history of hand eczema over the past several months. The man was patch tested with an extended standard series (unspecified methodology). A positive reaction to ethylenediamine was observed. Upon review of the material safety data sheets, it was noted that a widely used metal polisher contained more than 1% ethylenediamine as a solvent. Minimal erythema was elicited by patch tests with the polisher at 0.1 and 1.0%. His condition improved after he discontinued use of the polisher. No further details were available

Three cases of hand dermatitis or eczema in metallurgical workers, 20-30 years old, were described by Camarasa and Alomar (1978). No previous exposure to ethylenediamine-containing products was known. Standard and special series patch tests with 1% ethylenediamine (purity unspecified) in petrolatum were positive in all three cases. No additional details were available.

7.2 Controlled Exposure Studies

Four volunteers (unspecified age and sex) inhaled ethylenediamine vapor (unspecified purity and vehicle) at 100, 200, or 400 ppm (245, 490 or 980 mg/m³; Appendix B) for five to ten seconds (Pozzanni and Carpenter, 1954). The volunteers found the low concentration to be “inoffensive”. At the mid concentration, slight tingling sensations of the face and slight irritation of the nasal mucosa were reported. At the high concentration, “definitely intolerable irritation” of the nasal mucosa was reported.

8.0 EFFECTS ON LABORATORY ANIMALS AND *IN VITRO* TEST SYSTEMS

Ethylenediamine is a skin irritant and sensitizer, and an eye irritant. Short-term oral exposures to ethylenediamine in rodents were associated with decreased mean body weight gain and absolute and/or relative liver weights, increased mean absolute and/or relative kidney weights, and renal tubular degeneration and/or necrosis. Although some short-term studies also reported decreases in kidney weights, these effects may be secondary to reduced body weight.

After subchronic dietary exposures, hepatocellular pleomorphism in male and female rats, along with general toxicity, such as reduced body and organ weights and altered clinical parameters, were observed. Hepatocellular pleomorphism was not observed after subchronic gavage exposures in rats, but dose-related eye lesions, including retinal atrophy, were observed. Hepatocellular pleomorphism was not observed after subchronic gavage exposures in mice, but increased mean absolute and relative kidney weights and renal cortical tubular degeneration and/or necrosis were observed. In a chronic feeding study in rats, a dose-related increase in the incidence of hepatocellular pleomorphism was observed in female rats at doses that preceded and included general toxicity, such as reduced mean body weight gain and reduced organ weights. The pleomorphism was not accompanied by an increase in liver tumors.

The weight of evidence suggests that ethylenediamine is not genotoxic. Mixed results were obtained for ethylenediamine in *Salmonella typhimurium* reverse mutation assays. Ethylenediamine did not induce forward mutations or sister chromatid exchanges in Chinese hamster ovary cells, and did not affect unscheduled DNA synthesis in primary rat hepatocytes *in vitro*. Ethylenediamine was negative in a *Drosophila* sex-linked recessive lethal mutation assay and rat dominant lethal mutation assay *in vivo*. Although most of these studies were reviewed by WHO/IPCS (1999) and/or BUA (1997), a comprehensive summary of each study was not included. Due to their significance in the development of an oral RfD for ethylenediamine, the genotoxicity studies for ethylenediamine were also reviewed by NSF.

A two-generation reproduction feeding study in rats, as well as developmental studies in rabbits and mice via gavage and in rats via daily feed, have been conducted. All of the studies were

reviewed by WHO/IPCS (1999) and/or BUA (1997), but due to their significance in the development of an oral RfD for ethylenediamine, NSF also reviewed the studies. The results indicate that ethylenediamine does not cause reproductive or developmental toxicity, but high doses caused maternal and paternal toxicity, such as decreased food consumption and body weight. In the F₁ generation of the two-generation reproduction study, increased mean absolute and relative kidney weights were observed in female rats, and an increase in the incidence of hepatocellular pleomorphism was observed in male and female rats. A developmental feeding study in rats was inconclusive. Arterial defects were observed in the presence of maternal toxicity in a conventional study and in a pair-feeding study. However, developmental defects were not observed in gavage studies in rabbits. Thus, the weight of evidence suggests that ethylenediamine does not cause developmental effects at non-maternally toxic doses.

8.1 Limited-Exposure Effects

8.1.1 Irritation and Sensitization Studies

Neat ethylenediamine (unspecified purity) or 40% ethylenediamine hydrochloride (unspecified purity in distilled water) was applied to the skin of an unspecified number of rabbits or five rabbits (unspecified strain, sex, and site), respectively. Neat ethylenediamine was classified as a Grade 6 (out of 10) irritant, indicating that necrosis was observed. No irritation was observed on the skin of the five rabbits in which 40% ethylenediamine dihydrochloride was applied. No further details were available (Smyth et al., 1962 as reported by Yang et al., 1983a).

As reported by the WHO/IPCS (1999), an early study by Smyth et al. (1951) indicated that the application of 0.01 mL doses of undiluted ethylenediamine (purity unspecified) to the shaved backs of albino rabbits produced skin necrosis within 24 hours, and a study by Dubinina et al. (1997) also indicated that ethylenediamine was a skin irritant. No further details were available. Numerous dermal sensitization studies in guinea pigs using standard methodologies such as the Magnusson and Kligman maximization, Buehler, optimization, and Draize tests have demonstrated the skin sensitizing potential of ethylenediamine. These studies have been extensively reviewed by the WHO/IPCS (1999) and BUA (1997). Sensitization to ethylenediamine was observed in guinea pigs after dermal induction, but not after oral or intravenous induction. Both reviews concluded that clear evidence for a sensitization response to topical ethylenediamine existed at non-irritating concentrations, confirming the sensitization reactions observed in humans after dermal exposures to ethylenediamine. No studies were identified on respiratory sensitization in animals.

8.1.2 Ocular Exposure Studies

Ethylenediamine (unspecified purity) was instilled into rabbit eyes (unspecified strain, sex, and number) and subsequently categorized as causing Grade 8 (out of 10) corneal injury, since corneal injury was observed after the application of an “excess volume of a solution between 5 - 15%” (Carpenter and Smyth, 1946 as reported by Yang et al., 1983a).

Trace corneal injury was observed on one of five eyes after the instillation of 0.5 mL of a 40% solution of ethylenediamine dihydrochloride (unspecified purity in distilled water). No further details were reported (Carpenter and Smyth, 1946 as reported by Yang et al., 1983a).

As reported by the WHO/IPCS (1999), an early study by Carpenter and Smyth (1946) indicated that the instillation of 0.005 mL solutions of 5% or greater concentrations of ethylenediamine (purity and vehicle unspecified) produced corneal injury in an unspecified animal. Dubinina et al. (1997) reported that inflammatory responses in the rabbit eye were induced by one drop of ethylenediamine (purity and vehicle unspecified). No further details were available.

8.2 Single-Exposure Studies

The BUA (1997) reviewed numerous acute toxicity studies for ethylenediamine or ethylenediamine dihydrochloride which determined LD₅₀ or LC₅₀ values in rats, mice, rabbits, cats, and guinea pigs via the oral, inhalation, dermal, subcutaneous, intravenous, and/or intraperitoneal routes of exposure. Additional LD₅₀ or LC₅₀ values were located in the published literature. Oral LD₅₀ values were approximately 500 to 1,850 mg/kg in rodents, 470 mg/kg in guinea pigs, and 450 mg/kg in cats (BUA, 1997; Izmerov et al., 1982). Although there were route- and species-specific differences, ethylenediamine had a slight order of acute toxicity overall according to criteria defined by Gosselin et al. (1976).

Ethylenediamine as a monohydrochloride or dichlorohydrate salt in water (purity unspecified) was administered to five Fischer 344 rats per sex and dose at single gavage doses of 0, 200, 400, 800, 1,200, or 1,800 mg/kg. The monochloride salt studies were equivalent to 0, 124, 248, 497, 744, or 1,116 mg/kg ethylenediamine. The dichloride salt studies were equivalent to 0, 90, 180, 360, 541, or 812 mg/kg ethylenediamine. Mortality was attributed to treatment at 1,800 mg/kg (9/10) and 1,200 mg/kg (2/10). An LD₅₀ was not calculated. Clinical signs observed at 1,200 mg/kg included emaciation, bluish extremities, and diarrhea. Upon necropsy, no gross pathology was observed (Dow, 1992; NTP, 1982a).

Ethylenediamine dichlorohydrate (purity unspecified) in distilled water was administered to five B6C3F1 mice per sex and dose at single gavage doses of 200, 400, 800, 1,200, or 1,800 mg/kg, equivalent to 0, 90, 180, 360, 541, or 812 mg/kg ethylenediamine. Mortality was attributed to treatment at 1,800 mg/kg (10/10), 1,200 mg/kg (10/10), and 800 mg/kg (3/5 males and 4/5 females). An LD₅₀ was not calculated. The only clinical sign observed prior to death was moribundity. Necropsies conducted 14-days post dosing indicated no gross pathology. Thus, histopathology was not performed (NTP, 1982b).

8.3 Short-Term Exposure Studies

8.3.1 Dietary Exposure Study in F344 Rats

Ethylenediamine dihydrochloride (purity unspecified) was administered in feed to groups of five albino F344 rats per sex at mean measured doses of 0, 200, 630, or 1,940 mg/kg-day in males and 0, 240, 820, or 2,470 mg/kg-day in females for seven days (Yang et al., 1983a). These doses were equivalent to ethylenediamine doses of 0, 90, 284, or 875 mg/kg-day in males and 0, 108,

370, or 1,114 mg/kg-day in females. Food and water were available *ad libitum*. Mortality and food and water intake were monitored daily. Body, liver, and kidney weights were measured at study termination. Relative kidney and liver weight to body weight ratios were calculated.

One high-dose female died on Day 6. Hyperactivity, followed by collapse and depressed respiratory rate, were evident before death. Distention of the gastrointestinal tract and “respiratory embarrassment” (unspecified) was observed upon necropsy.

No statistically significant differences compared to controls were observed in low-dose male or female or mid-dose male rats. In mid-dose females, a statistically significant increase in mean relative kidney weight (5%) was observed.

In high-dose males, a statistically significant decrease in mean body weight gain (63%) was observed at study termination. In high-dose females, statistically significant decreases in mean body weight gain (89%) and in mean absolute liver (25%) and kidney (13%) weights, and an increase in mean relative kidney weight (7%) were observed. Based on the doses utilized, the authors did not identify a NOAEL, but suggested that it was probably close to 450 mg/kg-day ethylenediamine in both sexes.

8.3.2 Gavage Study in F344 Rats

Ethylenediamine dihydrochloride (purity unspecified in distilled water) was administered via gavage to groups of five F344 rats per sex at doses equivalent to 0, 100, 200, 400, 800, or 1,600 mg/kg-day ethylenediamine five days per week for a total of 12 doses (Dow, 1992; NTP, 1982a). Body weight was measured at unspecified intervals. Organs were weighed at study termination, and relative organ-to-body-weight ratios were calculated. Although the organs weighed were unspecified, they included the thymus and liver. Gross necropsies and histological examinations were performed on all surviving animals at study termination. Although a complete list of assayed organs was not provided, the organs examined included the kidney. No further methodological details were available.

At 100 mg/kg-day, mean body weight gain was reduced in males (26%) and females (8%).

At 200 mg/kg-day, mean body weight gain was reduced in males (23%) and females (7%). Very minor regeneration of the tubular epithelium was observed in the kidneys of 20% of the animals, but the sex was not specified.

At 400 mg/kg-day, mean body weight gain was reduced in males (39%) and females (24%). Minor dilation of the tubular lumen, degeneration and regeneration of the tubular epithelium, and necrosis of the tubular epithelium cells were observed in the kidneys of an unspecified number of animals.

At 800 mg/kg-day, one male and two females died before study termination, and lymphoid depletion and/or necrosis were observed in these animals upon histological examination. All surviving rats were reported to have rough haircoats, thin appearances, and pale eyes. Mean body weight gain was reduced in males (74%) and females (73%). Relative thymus and liver

weights were reduced in all animals, but the magnitude and statistical significance was not specified. The authors considered these effects biologically significant. The only observation upon gross necropsy was a thin appearance in all of the animals. Dilation of the tubular lumen, degeneration and regeneration of the tubular epithelium, and necrosis of the tubular epithelium cells were observed in the kidneys of an unspecified number of animals.

At 1,600 mg/kg-day, all rats died before the third dose was administered. Inactivity, weakness, and diarrhea were reported prior to death. No observations were made upon gross necropsy, and histological examinations were not performed. The authors did not identify a NOAEL or LOAEL.

8.3.3 Gavage Study in B6C3F1 Mice

Ethylenediamine dihydrochloride (unspecified purity in distilled water) was administered via gavage to five B6C3F1 mice per sex and dose at doses equivalent to 0, 50, 100, 400 or 600 mg/kg-day ethylenediamine five days per week for 12 total doses (NTP, 1982b). Gross necropsies and histopathology were conducted at study termination.

At 50 mg/kg-day, male and female mice had a negative increase in mean body weight gain compared to controls. The magnitude could not be determined, since absolute weights were not specified.

At 100 mg/kg-day, male mice had a negative increase in mean body weight gain compared to controls. The magnitude could not be determined, since absolute weights were not specified. Renal tubule regeneration was observed. Minimal evidence of tubular regeneration was also observed (unspecified sex and number affected).

At 200 mg/kg, a male mouse died and, upon necropsy, a severe lesion affecting the tubules of both kidneys was observed. Male mice had a negative increase in mean body weight gain compared to controls. The magnitude could not be determined, since absolute weights were not specified. Renal tubule nephrosis and minimal evidence of tubular regeneration were observed (unspecified sex and number affected).

At 400 mg/kg-day, 3/5 females died. Male mice had a decrease in mean body weight gain of ~90%. Lymphoid depletion in and necrosis of splenic follicles were observed (unspecified sex and number affected). Renal lesions of the tubular epithelium were observed in 5/10 mice.

At 600 mg/kg, all mice died before the fourth day of dosing. Inactivity and weakness from the first day until death were observed. Necropsies or histopathological examinations, however, were not conducted. This summary was based on details provided by NTP (1982b). Further details of the original study and the citation were not provided.

8.3.4 Dietary Exposure Study in B6C3F₁ Mice

Ethylenediamine dihydrochloride (purity unspecified) was administered through feed to groups of five B6C3F₁ mice per sex and dose at mean measured doses of 0, 160, 630, or 2,180 mg/kg-