

HEXAMETHYLENEDIAMINE

CAS # 124-09-4

ORAL RISK ASSESSMENT DOCUMENT



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EXECUTIVE SUMMARY

Hexamethylenediamine – Oral Risk Assessment CAS # 124-09-4			
PARAMETER	LEVEL	UNITS	DERIVED
NOAEL (no-observed-adverse-effect level)	150	mg/kg-day	From a 2-generation reproduction study in rats.
Oral RfD (oral reference dose)	2	mg/kg-day	From a 2-generation reproduction study in rats with a 100x uncertainty factor.
TAC (total allowable concentration)	10	mg/L	From the oral RfD, for a 70 kg adult drinking 2L/day with a default 20% Relative Source Contribution for drinking water.
SPAC (single product allowable concentration)	1	mg/L	From the TAC, assuming the default 10 sources of hexamethylenediamine in drinking water.
STEL (short term exposure level)	20	mg/L	From a developmental study in rats, for a 10 kg child drinking 1L/day
KEY STUDY	Short, R.D, F.R. Johannsen, and J.L. Scharden. 1991. A two-generation reproduction study in rats receiving diets containing hexamethylenediamine. Fundamental and Applied Toxicology. 16 : 490-494.		
CRITICAL EFFECT	Decreased parental body weight, decreased pup weight, decreased absolute testicular weight and decreased litter size.		
UNCERTAINTY FACTORS	Uncertainty factors applied in calculating the oral RfD are as follows: • 10x for interspecies extrapolation • 10x for intraspecies variability • 1x for extrapolation from a less-than-lifetime study to lifetime duration • 1x for extrapolation from a LOAEL to a NOAEL • 1x for database deficiencies. Therefore, the total uncertainty factor is 100x.		
TOXICITY SUMMARY	The only human toxicity data were from a metabolism study in which the excretion of hexamethylenediamine was determined to be a rapid process. A possible pathway for the metabolism of hexamethylenediamine to mono-acetylated hexamethylenediamine and 6-aminohexanoic acid was proposed. <i>In vitro</i> metabolism data using mouse fibroblasts suggested that hexamethylenediamine inhibited the activity of ornithine decarboxylase, an enzyme involved in the biosynthesis of polyamines. Toxicological data available for hexamethylenediamine in laboratory animals included short-term, subchronic, two-generation reproduction, developmental and genetic toxicity studies. The critical study chosen to derive the oral RfD was a two-generation reproduction study in which rats were administered hexamethylenediamine in the diet at 0, 50, 150, or 500 mg/kg-day. The NOAEL identified was 150 mg/kg-day, based on decreased parental body weight, decreased pup weight, decreased absolute testicular weight and decreased litter size. Hexamethylenediamine administration did not affect reproductive success. <i>Salmonella typhimurium</i> reverse mutation and <i>in vivo</i> and <i>in vitro</i> chromosomal aberration assays showed hexamethylenediamine was not genotoxic. An <i>in vivo</i> micronucleus assay showed an increase in polychromatic erythrocytes in the total erythrocyte population with no increase in micronucleated normochromatic or polychromatic erythrocytes.		
CONCLUSIONS	Due to the lack of epidemiological data in humans or chronic data in laboratory animals, there is <i>inadequate information to assess the carcinogenic potential</i> of hexamethylenediamine. Decreased parental body weight, decreased pup weight, decreased absolute testicular weight and decreased litter size in the two-generation reproduction study were the most sensitive endpoints of toxicity observed in the available studies. Based on the identified critical effects and the uncertainty factors applied, the drinking water action levels derived in this risk assessment are protective of human health.		

1.0 INTRODUCTION

This document has been prepared to allow toxicological evaluation of the unregulated contaminant **hexamethylenediamine** in drinking water, as an extractant from one or more drinking water system components evaluated 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 that there is a threshold for these endpoints that will not be exceeded if appropriate uncertainty factors (Dourson et al., 1996; U.S. EPA, 2002; WHO/IPCS, 2005) 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, 2003b).

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 to 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 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, 2003c; U.S. EPA, 2005a). 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, 2003c), Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), 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, 2005b).

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 December 16, 2004. This document includes all relevant information retrieved as a result of that search.

2.0 PHYSICAL AND CHEMICAL PROPERTIES

Hexamethylenediamine is an aliphatic amine with synonyms of 1,6-diaminohexane, 1,6-hexandiamine, 1,6-hexylenediamine, HDA and HMDA. It has the following structure and physical and chemical properties listed in Table 1:



Hexamethylenediamine dihydrochloride has synonyms of 1,6-diaminohexane dihydrochloride, 1,6-hexandiamine dihydrochloride, and hexamethylenediammonium dichloride. It has the following structure and physical and chemical properties listed in Table 2:

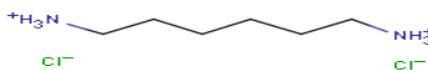


Table 1. The physical and chemical properties of hexamethylenediamine

Property	Data	Reference
Empirical Formula	C ₆ H ₁₆ N ₂	Merck Index, 2001
CAS#	124-09-4	Merck Index, 2001
Molecular Weight	116.2	Merck Index, 2001
Physical State and Color	White crystalline solid	ACGIH, 2001
Melting Point	41.5 °C (experimental)	ChemIDplus, 2005
Boiling Point	205 °C (experimental)	ChemIDplus, 2005
Density	0.89 g/mL at 25 °C	Sigma-Aldrich, 2006
Vapor Pressure	0.12 mm Hg @ 25 °C (extrapolated) 3 mm Hg @ 60 °C	HSDB, 2005 Kennedy, 2005
Water Solubility	Freely soluble 2.46 x 10 ³ g/L @ 5 °C 2.5 g/L	HSDB, 2005; Merck Index, 2001 ChemIDplus, 2005 Kennedy, 2005
pK _a	11.02 (experimental)	ChemIDplus, 2005
n-Octanol/Water Partition Coefficient (log K _{ow})	0.35 (estimated)	HSDB, 2005
Henry's Law Constant (air/water partition)	3.2 x 10 ⁻⁹ atm-m ³ /mol @ 25 °C (estimated)	ChemIDplus, 2005

Table 2. The physical and chemical properties of hexamethylenediamine dihydrochloride

Property	Data	Reference
Empirical Formula	C ₆ H ₁₆ N ₂ •2HCl	Merck Index, 2001
CAS#	6055-52-3	NTP, 1993
Molecular Weight	189.1	ChemFinder, 2005
Physical State and Color	Crystallizes as needles	NTP, 1993
Melting Point	248 °C	Merck Index, 2001
Boiling Point	Not located	
Density	Not located	
Vapor Pressure	Not located	
Water Solubility	Not located	
Dissociation Constant (pK _a)	Not located	
n-Octanol/Water Partition Coefficient (log K _{ow})	Not located	
Henry's Law Constant (air/water partition)	Not located	

2.1 Organoleptic Properties

Hexamethylenediamine has the odor of piperidine, which has a weak fishy odor (Merck Index, 2001). Odor thresholds of 0.0041 and 0.0032 mg/m³ of air have been reported (HSDB, 2005). The olfactory threshold of hexamethylenediamine was reported to be 0.68 parts per billion, without further detail (Kennedy, 2005). No other information on the organoleptic properties of hexamethylenediamine were located.

3.0 PRODUCTION AND USE

3.1 Production

Hexamethylenediamine can be prepared by the reduction of adiponitrile with sodium and alcohol, or by hydrogenation of 1,4-dicyanobutylene produced by chlorination of butadiene followed by reaction with sodium cyanide (HSDB, 2005). Hexamethylenediamine is a high production volume chemical in both the United States and Europe, which indicates it is manufactured or imported in amounts equal to or greater than one million pounds per year (U.S. EPA, 2006; OECD, 2004). The total production capacity in the United States as of January 1, 1996 was 1,330 million pounds (HSDB, 2005). No other data on the production of hexamethylenediamine could be located.

3.2 Use

Hexamethylenediamine is primarily used as a chemical intermediate in the manufacture of polyamide resins for fibers and plastics. The most important resin is Nylon 66, which is produced through polycondensation of hexamethylenediamine and adipic acid (Monsanto, 1984). Hexamethylenediamine is also used in the manufacture of urethane coatings, as a curing agent for epoxide resins, and as a corrosion inhibitor (Brorson et al., 1990; Columbus Laboratories, 1985).

The U.S. Food and Drug Administration lists numerous acceptable uses for hexamethylenediamine as an indirect food additive. Under the U.S. Code of Federal Regulations, Title 21 (U.S. FDA, 2005), hexamethylenediamine is acceptable for use in the manufacture of polyamides used as components of adhesives intended for use in packaging, transporting or holding food (175.105), provided there is a functional barrier between the adhesive and the food. The quantity of adhesive should not exceed the limits of good manufacturing practice for dry packaged food, or trace amounts at the seams and at the edge exposure between packaging laminates for aqueous and fatty foods. Hexamethylenediamine is also acceptable for use as a component of coatings intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting or holding food (175.300). It may be used as a wet strength agent or retention aid in the manufacture of polyamine-epichlorohydrin thermosetting resins used as components of paper and paperboard in contact with aqueous and fatty foods (176.170), and in the manufacture of Nylon 66, 610, 66T, 6I/6T, 612, and 6/69 resins used in processing, handling, and packaging food (177.1500).

4.0 ANALYTICAL METHODS

4.1 Analysis in Water

Hexamethylenediamine has been detected as a hydrolysis product of hexamethylene diisocyanate in water using capillary column gas chromatography with electron capture detection (Berode et al., 1991). The authors did not report the detection limit. NSF analyzes hexamethylenediamine using high performance liquid chromatography with post column derivatization and fluorescence detection. The detection limit is 30 µg/L.

4.2 Analysis in Biological Matrices

Hexamethylenediamine has been determined in human plasma and urine using gas chromatography with nitrogen-phosphorous detection following intravenous exposure to hexamethylene bisacetamide (Egorin et al., 1987). Hexamethylenediamine has also been detected in human plasma and urine using gas chromatography with an automatic on-column injector and thermionic specific detector (Skarping et al., 1989). It has been analyzed in urine following inhalation exposure of humans to hexamethylene diisocyanate. Analysis was performed using capillary column gas chromatography with a quadrupole mass selective detector in electron impact mode (Maitre et al., 1996). [^{14}C]-Labeled hexamethylenediamine has been analyzed in urine and selected tissues of rats after administration via gavage. The samples were spotted on thin layer chromatography plates and radioactivity was measured using scintillation counting (David and Heck, 1983).

5.0 SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

5.1 Sources of Human Exposure

Exposure to hexamethylenediamine may occur occupationally via dermal or inhalation routes from its use in the manufacture of urethane coatings, polyamides and epoxy resins (NTP, 1993; Brorson et al., 1990). A time-weighted average TLV® of 0.5 ppm has been established for occupational exposure (ACGIH, 2001). According to NIOSH, 1,100 workers were exposed occupationally each year between 1972 and 1974 (NTP, 1993). The general population may be exposed to hexamethylenediamine through its incorporation into polymers having food or drinking water contact.

5.2 Sources of Environmental Exposure

Production of hexamethylenediamine and its use in nylon and other materials may result in its release to the environment through air or wastewater streams. An estimated 12.8 million pounds of hexamethylenediamine per year were released to the environment in the mid 1970's (NTP, 1993). Whether release to air or water predominated was not reported.

6.0 COMPARATIVE KINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS

Data on the kinetics and metabolism of hexamethylenediamine following oral administration are available in humans and laboratory animals. Data in humans suggest a possible metabolic pathway for hexamethylenediamine following ingestion involving the formation of mono-acetylated hexamethylenediamine and 6-aminohexanoic acid. According to the animal data, the distribution of hexamethylenediamine one hour following administration was highest in the intestine followed by the liver and kidney. Twenty-four hours following administration, the concentration of hexamethylenediamine was highest in the prostate followed by the intestine and liver, and at 72 hours, the concentration was highest in the prostate, followed by the kidney and liver. *In vitro* data obtained from rat liver preparations suggest hexamethylenediamine is converted to 3,4,5,6-tetrahydro-2H-azepine, which in turn is converted to 6-aminohexanoic acid

and caprolactam. *In vitro* data using mouse fibroblasts suggest hexamethylenediamine suppressed ornithine decarboxylase activity. Data in rats suggest the elimination of hexamethylenediamine is greatest via the kidneys with significant amounts also eliminated via the gastrointestinal tract.

6.1 Absorption

No studies regarding absorption of hexamethylenediamine could be located. However, at least partial absorption is inferred based on the detection of hexamethylenediamine or its metabolites in tissues and urine, the detection of hexamethylenediamine-derived carbon dioxide in expired air following oral administration to rats (David and Heck, 1983; Monsanto, 1984), and rapid urinary excretion of hexamethylenediamine or its 6-aminohexanoic acid metabolite in humans.

6.2 Distribution

Four male F-344 rats were administered 25 μCi 1,6- ^{14}C hexamethylenediamine-dihydrochloride (purity and vehicle unspecified) via gavage at a reported dose of 0.4 mg/kg hexamethylenediamine (David and Heck, 1983). Food and water were available *ad libitum*. After 72 hours, the animals were sacrificed. The following tissues were collected: prostate, intestine, kidney, liver, spleen, testes, lungs, heart, brain and blood. The prostate contained the highest amount of radioactivity (250 ± 30 nmol/g) followed by the kidney (194 ± 27 nmol/g) and liver (171 ± 26 nmol/g).

Two F-344 rats per sex were intravenously administered 100 μCi ^{14}C hexamethylenediamine (purity and vehicle unspecified) in order to investigate the distribution of hexamethylenediamine using whole-body autoradiography (David and Heck, 1983). One animal per sex was sacrificed one hour and 24 hours following injection. The following tissues were collected: prostate, intestine, kidney, liver, spleen, testes, lungs, heart, brain and blood. One hour after injection, the male intestine contained the highest amount of radioactivity followed by the liver and kidney. The prostate gland contained far less than the liver, and the blood contained less than the prostate. At one but not 24 hours post-injection, the uterus of the female had a high concentration of ^{14}C hexamethylenediamine. Results in the uterus could be dependent on the estrous cycle. Little radioactivity was seen in the brain of either sex or in the testes of males. Twenty-four hours following injection, the prostate gland contained the highest concentration of radioactivity followed by the intestine and the liver. The authors indicated this high concentration of ^{14}C from hexamethylenediamine could be due to a slow accumulation in the prostate, or could be attributed to persistence of the compound. The authors stated since the ^{14}C concentration in the prostate after one hour was relatively low compared to the concentration 24 hours after injection, a metabolite of ^{14}C hexamethylenediamine could be involved. The prostate is rich in polyamines and ^{14}C hexamethylenediamine may have been incorporated into higher polyamines.

6.3 Metabolism

6.3.1 Study in Humans

The metabolism of hexamethylenediamine and formation of its metabolites, 6-aminohexanoic acid and mono-acetylated hexamethylenediamine, were investigated in six healthy non-smoking male volunteers between the ages of 25 and 50 years (Brorson et al., 1990). Prior to administration, urine was collected for background analysis. Each subject was orally administered 8.2 mg (approximately 0.1 mg/kg) hexamethylenediamine on two separate occasions three months apart, always following an overnight fast. The purity was not specified and the vehicle was described as a slightly acidic liquid. On a separate occasion, the volunteers were given a single oral dose of 4,4'-diamino diphenylsulfone (Dapsone or DDS) to determine whether they were slow or fast acetylators. Dapsone is acetylated by N-acetyltransferase in the liver to its major metabolite, monoacetyl dapsone (MADDS). Slow acetylators were defined as having plasma acetylation ratios (MADDS/DDS) less than 0.3 and rapid acetylators had ratios greater than 0.3. Following administration of hexamethylenediamine, urine was collected every second hour for the first 15 hours, and then at unspecified intervals up to 24 hours.

The pre-exposure urine samples did not contain hexamethylenediamine or 6-aminohexanoic acid. Hexamethylenediamine and 6-aminohexanoic acid were detected in hydrolyzed urine, and hexamethylenediamine and mono-acetylated hexamethylenediamine (N-acetyl-1,6-hexamethylenediamine) were detected in non-hydrolyzed urine. There were higher amounts of 6-aminohexanoic acid in the hydrolyzed urine compared to hexamethylenediamine, that the authors attributed to the enzymatic oxidation of orally administered hexamethylenediamine in the gastrointestinal tract, which has high levels of diamine oxidase.

The possible metabolic pathway proposed by the study authors is shown in Figure 1.

6.3.2 *In Vitro* Conversion to Metabolites

The *in vitro* metabolic conversion of hexamethylenediamine (purity unspecified) was investigated by Subramanyam et al. (1989). Livers from male Sprague-Dawley rats were homogenized and incubated with diamine oxidase and hexamethylenediamine (2.5×10^{-3} M). This yielded 3,4,5,6-tetrahydro-2H-azepine as the only metabolite detected. 3,4,5,6-Tetrahydro-2H-azepine was then combined with a supernatant fraction of rat liver and partially purified rabbit liver aldehyde oxidase. The resulting compounds were 6-aminohexanoic acid and caprolactam. Twice as much 6-aminohexanoic acid was formed compared to caprolactam (475 nmol/g liver versus 279 nmol/g liver per 30 minutes). The authors suggested that hexamethylenediamine metabolites might interact with the polyamine system, especially with respect to regulation of cell growth, based on the fact that hexamethylenediamine served as a substrate for diamine oxidase. The proposed metabolic pathway of hexamethylenediamine based on the *in vitro* studies of Subramanyam et al. (1989) is shown in Figure 2.

Figure 1: Proposed metabolic pathway of hexamethylenediamine in humans (Brorson et al., 1990)

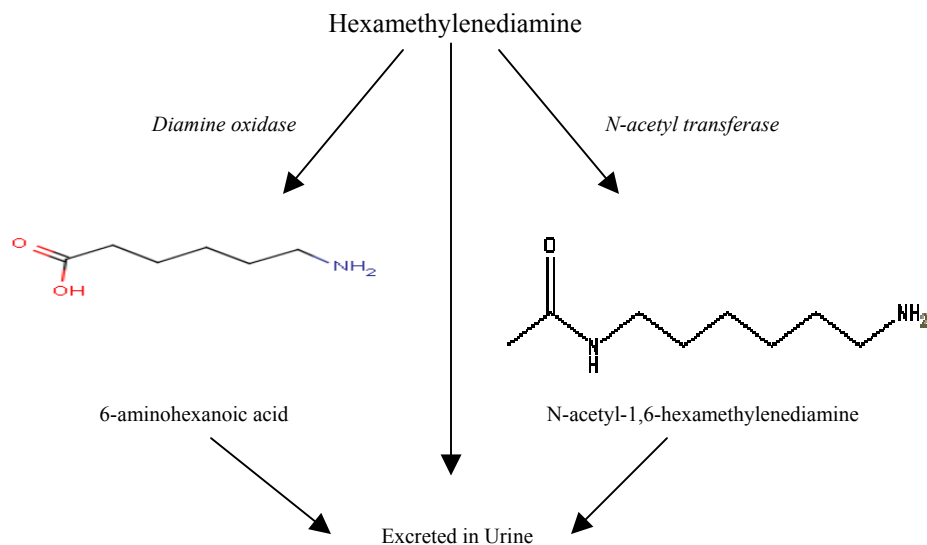
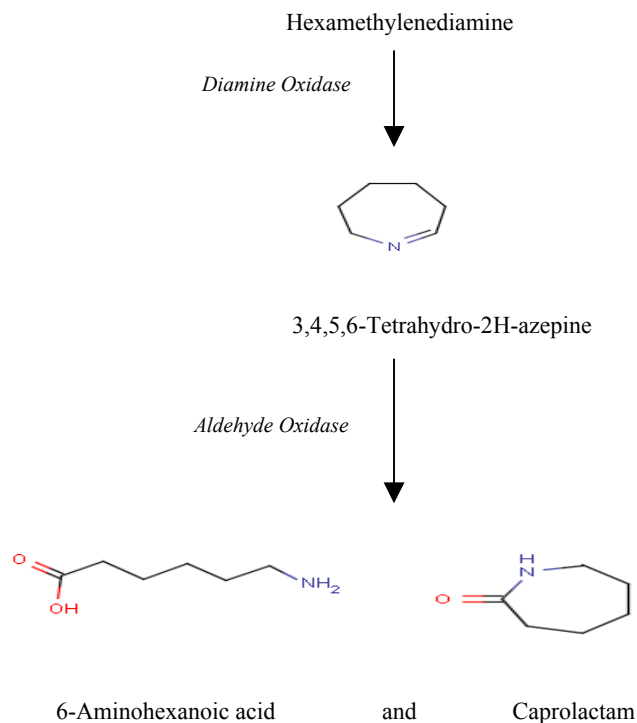


Figure 2: Proposed metabolic pathway of hexamethylenediamine *in vitro* (Subramanyam et al., 1989)



6.4 Elimination/Excretion

6.4.1 Humans

Six healthy non-smoking male volunteers between the ages of 25 and 50 years were administered 8.2 mg (approximately 0.1 mg/kg) hexamethylenediamine on two separate occasions three months apart, always following an overnight fast (Brorson et al., 1990). Prior to administration, urine was collected for background analysis. The purity was not specified and the vehicle was described as a slightly acidic liquid. On a separate occasion, the volunteers were given a single oral dose of 4,4'-diamino diphenylsulfone (Dapsone or DDS) to determine whether they were slow or fast acetylators. Dapsone is acetylated by N-acetyltransferase in the liver to its major metabolite, monoacetyl dapsone (MADDS). Slow acetylators were defined as having plasma acetylation ratios (MADDS/DDS) less than 0.3 and rapid acetylators as having ratios greater than 0.3. Following administration of hexamethylenediamine, urine was collected every second hour for the first 15 hours, and then at unspecified intervals up to 24 hours.

The pre-exposure samples did not contain hexamethylenediamine or 6-aminohexanoic acid. The amount of hexamethylenediamine in the urine peaked 2-5 hours following administration with levels ranging from 0.08 mg to 0.19 mg (1 to 6% of the administered dose, respectively). 6-Aminohexanoic acid peaked 4-6 hours following administration with levels ranging from 0.2 to 0.9 mg (< 1 to 27% of the administered dose, respectively). In four of the six volunteers, no hexamethylenediamine could be detected after 10 hours, and in one of the six volunteers, 6-aminohexanoic acid could be detected after 12 hours. The elimination half-life of hexamethylenediamine was determined to be 1.5 hours. There was considerable intra- and inter-individual variation in the results, especially in the elimination of 6-aminohexanoic acid. The slow acetylators excreted lower amounts of the administered dose in the urine than the rapid acetylators (2% and 5% of a given dose, respectively). However, no correlation between the excretion of 6-aminohexanoic acid and acetylating phenotype was identified. Over 90% of the urinary hexamethylenediamine and 6-aminohexanoic acid detected was present in samples taken within 10 hours of dose administration.

6.4.2 Laboratory Animals

Four male F-344 rats were administered 25 μCi [^{14}C] hexamethylenediamine dihydrochloride (purity and vehicle unspecified) via gavage (David and Heck, 1983). Food and water were available *ad libitum*. Urine, feces, and expired air were collected for 72 hours. According to the authors, 47% of the administered dose was excreted in the urine, 27% was excreted in the feces, and 20% was excreted as carbon dioxide. After 72 hours, less than 1.5% of the administered dose was retained in the animals.

Three Sprague-Dawley rats per sex were administered single gavage doses of 11.96 μCi [^{14}C] hexamethylenediamine (unspecified purity and vehicle), averaging 7.3 mg/kg for males and 8.5 mg/kg for females (Monsanto Co., 1984). Urine, feces, and expired air were collected 0-8, 8-16, 16-24, 24-48, and 48-72 hours following administration. An average of 49.75% of the administered dose was found in male urine and 58.65% in female urine. The majority of the compound was detected in the 0-8-hour sample. The feces contained an average of 26.61% of

the administered dose for males and 15.4% for females. Percentages of the administered dose recovered in expired air averaged 8.7 for males and 13.35 for females. Total elimination of the administered dose averaged 85.28% for males and 88.89% for females. The authors concluded [^{14}C] hexamethylenediamine and its metabolites were quantifiably excreted within 72 hours following oral administration, and that the major route of elimination was through the urine followed by the feces.

6.5 Conclusions Regarding Comparative Kinetics and Metabolism

The only data available in humans regarding kinetics and metabolism indicate that fast acetylators excrete more of the administered dose of hexamethylenediamine than slow acetylators. There were no data obtained regarding acetylation in laboratory animals and thus comparisons between humans and animals could not be made.

7.0 EFFECTS ON HUMANS

7.1 Case Reports

Several instances of occupational exposure to hexamethylenediamine have been reported. At an Italian nylon plant, 20 workers were exposed to hexamethylenediamine and adiponitrile in the air. The concentrations of hexamethylenediamine ranged from 2-5.5 mg/m³ during normal plant operations and 32.7-131.5 mg/m³ during autoclaving. Symptoms included irritation of the conjunctiva and respiratory tract in eight workers, and contact dermatitis and acute hepatitis in one worker (Ceresa, 1948; Gallo and Ghiringhelli, 1958; NTP, 1993). Dermatitis of the hands and face was noted in four male workers at a nylon manufacturing plant following exposure to hexamethylenediamine. Following treatment, the condition reappeared within hours of returning to their jobs (Duverneuil and Buisson, 1952; Kennedy, 2005).

7.2 Epidemiological Studies

No epidemiological studies on hexamethylenediamine were located.

7.3 Inhibition of Antitrypsin

A study on the inhibition of antitrypsin by hexamethylenediamine was conducted by Berode et al. (1988). Alpha-1-antitrypsin is a serum protease that inactivates elastase secreted by neutrophils. Serum samples from 26 volunteers were collected and analyzed for α_1 -antitrypsin phenotypes and total trypsin inhibiting activity using isoelectric focusing on polyacrylamide gel. The serum (0.5 mL) was incubated overnight with 0, 1, 2, 2.5, 3, 4, or 5 mM hexamethylenediamine (purity unspecified), and the analyses for inhibiting activity and phenotypes was repeated. At 5 mM, hexamethylenediamine caused a significant decrease in heterozygous antitrypsin activity with lower decreases in activity in individuals with homozygous antitrypsin phenotypes. The authors concluded heterozygous antitrypsin carriers might be more prone to the effects of inhaled amines, including hexamethylenediamine.

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

Available data in laboratory animals include limited and single exposure studies (dermal, LD₅₀, and approximate lethal dose), short-term exposure studies (via oral and inhalation routes), subchronic exposure studies (via oral and inhalation routes), a two-generation reproduction study (via oral route), and developmental toxicity studies (via oral, intraperitoneal and inhalation routes). The data also include several genotoxicity studies including *Salmonella typhimurium* reverse mutation assays, *in vivo* and *in vitro* chromosomal aberration assays, a micronucleus assay, and a sister chromatid exchange assay.

The limited and single exposure studies indicated hexamethylenediamine had LD₅₀ values ranging from 410 to 1,127 mg/kg and that it was corrosive to the skin upon dermal contact. Orally administered hexamethylenediamine was associated with changes in organ weights after short-term exposure and decreased weight gain after subchronic exposure. Irritation, ulceration, and histopathology of the respiratory tract, along with various organ weight changes, were associated with inhalation exposure to hexamethylenediamine.

The weight of evidence suggests that hexamethylenediamine has low genotoxic potential. No statistical increases in mutation frequency were observed in the *Salmonella typhimurium* reverse mutation assays, and no increases in the frequency of chromosomal aberrations were observed *in vivo* or *in vitro*. An *in vivo* micronucleus assay indicated no increases in micronucleated polychromatic or normochromatic erythrocytes and lastly, hexamethylenediamine did not statistically increase the number of sister chromatid exchanges observed *in vitro*.

The two-generation reproduction feeding study showed statistically decreased parental body weights, decreased pup weights, and decreased litter sizes. The developmental studies indicated that orally administered hexamethylenediamine was associated with decreased dam weight and weight gain, decreased fetal body weight, and an increased incidence of pups with spotty livers. Inhaled hexamethylenediamine did not adversely affect development.

8.1 Limited-Exposure Effects

8.1.1 Irritation and Sensitization Studies

A mixture of 30.5% 1,2-diaminocyclohexane and 69.5% 1,6-hexamethylenediamine (unspecified purity and vehicle) was applied to the shaved backs and trunks of adult male albino rabbits at concentrations from 3,400 mg/kg to 10,000 mg/kg (E.I. Dupont, 1974). The mixture was applied under occluded patch and left for 24 hours, after which the application site was washed with water and dried. Animals were observed daily and survivors were sacrificed after 14 days. One rabbit dosed at 3,400 mg/kg and another dosed at 5,000 mg/kg were lethargic. The rabbit dosed at 7,500 mg/kg died on Day 2 and the two rabbits dosed at 10,000 mg/kg died on Day 1. The skin on all the rabbits became necrotic after 24 hours. The authors concluded the mixture of 1,2-diaminocyclohexane and 1,6-hexamethylenediamine was a corrosive skin irritant.

Hexamethylenediamine (unspecified purity) was applied in 0.05 mL aliquots to the shaved shoulder skin of 10 male albino guinea pigs (E.I. Dupont, 1969). Assuming 100% purity, the

approximate equivalent dose was 45 mg. Within one hour, necrosis was seen on the treated areas of all 10 guinea pigs. The necrotic areas were blackish-brown within one hour. A day later the areas had a white superficial covering. The authors concluded hexamethylenediamine caused severe necrosis within a short time period.

Hexamethylenediamine (unspecified purity) was gently rubbed into the application sites, on the shaved trunk and lateral area of groups of six male albino rabbits, as 0.5 mL of an aqueous solution (E.I. Dupont, 1969). A 6% hexamethylenediamine solution (approximately equivalent to 27 mg) was applied to the left area and a 10% solution (approximately equivalent to 45 mg) was applied to the right area. After a contact period of 30 seconds or one minute, the solution was washed from the skin with water and dried. Observations were made 24 and 48 hours following dosing. The authors reported when the test material was washed from the skin within 30 seconds or one minute of application, no skin irritation was observed. The criteria to assess skin irritation were not mentioned.

8.1.2 Ocular Exposure Studies

No ocular exposure studies on hexamethylenediamine were located. Based on the reviewed skin irritation studies, the compound is expected to be irritating and/or corrosive to the eye.

8.2 Single-Exposure Studies

8.2.1 LD₅₀ Studies

Five Long Evans rats per sex and dose were administered a 40% solution of hexamethylenediamine (99.8% purity and unknown vehicle) via gavage at 485, 679, 950, 1,330 or 1,860 mg/kg (Proctor & Gamble Co., 1977). Mortality and clinical signs were recorded 15, 30, 60, 120 and 240 minutes following administration and daily thereafter for 14 days. All surviving animals were weighed at study termination and two rats from each dose group were observed for gross abnormalities and necropsied. Occasionally, surviving animals were necropsied and given a microscopic evaluation. The authors did not specify the tissues that were examined. At all dose levels, there was an immediate decrease in motor activity, an increase in respiratory rate, coarse body tremors, and abdominal griping. The authors stated these symptoms were slight at the two lowest doses, more moderate at the next two doses, and extreme at the highest dose, with all 10 rats dying within the first 24 hours. Necropsies indicated severe mucous membrane irritation of the gastrointestinal tract. The authors determined the oral LD₅₀ to be 830 mg/kg with 95% confidence limits of 730-950 mg/kg.

Five Long Evans rats per sex and dose were administered a 40% solution of hexamethylenediamine dihydrochloride (99.8% purity and unknown vehicle) via gavage at 950, 1,330, 1,860, 2,600 or 3,640 mg/kg (Proctor & Gamble Co., 1977). Mortality and clinical signs were recorded 15, 30, 60, 120 and 240 minutes following administration and daily thereafter for 14 days. All surviving animals were weighed at study termination and two rats from each dose group were observed for gross abnormalities and necropsied. The authors did not specify the tissues that were examined. Occasionally, surviving animals were necropsied and given a microscopic evaluation. The authors stated the symptoms were similar to those observed using

hexamethylenediamine for those rats that died. The compound appeared to be slightly irritating to the stomach but the authors did not identify how this was determined. The LD₅₀ was 1,860 mg/kg for hexamethylenediamine dihydrochloride. When expressed as the amount of hexamethylenediamine present, the oral LD₅₀ was 1,140 mg/kg with 95% confidence limits of 930-1,420 mg/kg.

Ten fasted male Charles River rats per dose were administered 500, 700, 800 or 1,000 mg/kg hexamethylenediamine (3-10% suspension of 90.5% purity in corn oil), and 10 non-fasted rats per dose were administered 800, 1,000, 1,100, 1,200, 1,250, 1,300, 1,400, 1,600, or 2,000 mg/kg hexamethylenediamine (3-10% suspension in corn oil) via gavage (Dashiell and Kennedy, 1984). Fasted rats were not allowed food 24 hours prior to dosing and non-fasted rats were allowed food and water *ad libitum*. Surviving rats were weighed and observed for 14 days prior to sacrifice. No pathological examinations were conducted. Observations included weakness, salivation, staining of perineal area and weight loss. Seven deaths occurred at 800 mg/kg in the fasted rats but no deaths occurred at this dose in the non-fasted rats. There were 8 deaths in the fasted group at 1,000 mg/kg, whereas 5 deaths occurred in the non-fasted group. All deaths occurred within two days of dosing. The authors determined the LD₅₀ to be 792 mg/kg for fasted rats with 95% confidence limits of 704-896 mg/kg and 1,127 mg/kg for non-fasted rats with 95% confidence limits of 594-1,344 mg/kg.

Male and female albino rats were administered hexamethylenediamine as a 10% aqueous solution (unspecified purity) in single gavage doses (Johannsen and Levinskas, 1987). The authors did not indicate the number of rats or the exact doses. The rats were observed for 14 days before sacrifice, followed by gross examination of the viscera of all rats. An unspecified number of deaths occurred during the study from several hours to four days following dosing. Clinical signs included malaise, salivation, diarrhea and tremors. Of those rats sacrificed at the end of the study, renal hyperemia and gastrointestinal inflammation were noted. The authors concluded the LD₅₀ in rats was 980 mg/kg with 95% confidence limits of 850-1,130 mg/kg.

Male Sprague-Dawley rats were given oral doses of hexamethylenediamine (unspecified purity and vehicle) ranging from 470 to 1,360 mg/kg and female rats were given oral doses ranging from 510 to 1,100 mg/kg (Vernot et al., 1977). Information regarding type of oral exposure, number of rats exposed, and observations and symptoms were not mentioned. The LD₅₀ for male rats was determined to be 800 mg/kg with 95% confidence limits of 470-1,360 mg/kg and for female rats was 750 mg/kg with 95% confidence limits of 510-1,100 mg/kg.

Five Long Evans rats per sex and dose were intravenously administered a 10% solution of hexamethylenediamine (99.8% purity and unknown vehicle) at 250, 375, 415, or 560 mg/kg via the femoral vein (Proctor & Gamble Co., 1977). Mortality and clinical signs were recorded 15, 30, 60, 120 and 240 minutes following administration and daily thereafter for 14 days. All surviving animals were weighed at study termination, and two rats from each dose group were observed for gross abnormalities and necropsied. The authors did not specify the tissues that were examined. Occasionally, surviving animals were necropsied and given a microscopic evaluation. The authors stated similar effects were seen in the rats intravenously administered the 10% solution as those orally administered the 40% solution. However no internal organ

abnormalities or mucous membrane irritation were observed. The authors determined the intravenous LD₅₀ to be 410 mg/kg with 95% confidence limits of 380-570 mg/kg.

Three female New Zealand albino rabbits per dose were exposed to hexamethylenediamine via dermal exposure at doses ranging from 600 to 2,120 mg/kg (Vernot et al., 1977). Information regarding observations and symptoms was not noted. The authors determined the LD₅₀ to be 1,110 mg/kg with 95% confidence limits of 600-2,120 mg/kg.

8.2.2 Approximate Lethal Dose Studies

A mixture of 30.5% 1,2-diaminocyclohexane and 69.5% 1,6-hexamethylenediamine (unspecified purity and vehicle) was applied to the shaved back and trunk of five adult male albino rabbits at concentrations from 3,400 mg/kg to 10,000 mg/kg (E.I. Dupont, 1974). The mixture was applied under occluded patches and left for 24 hours. Surviving animals were observed and sacrificed after 14 days. Since the rabbit at the 7,500 mg/kg dose died on Day 2, and the two rabbits given 10,000 mg/kg died on Day 1, the authors determined the approximate lethal dose for the mixture to be 7,500 mg/kg.

A single gavage dose of hexamethylenediamine (unspecified purity in corn oil) was administered to one male Charles River rat per dose at 670, 1,000, 1,500, 2,250 or 3,400 mg/kg (Kennedy et al., 1986). The rats were weighed and observed daily for 14 days prior to sacrifice. The rats dosed at 670 and 1,000 mg/kg survived until Day 14. The rats at the remaining doses died on the day of dosing. The authors concluded hexamethylenediamine was slightly toxic and the approximate lethal dose was 1,500 mg/kg.

A summary of acute toxicity results is presented in Table 3.

Table 3. Summary of LD₅₀ values and approximate lethal doses (ALDs) for hexamethylenediamine

Species	Route	LD ₅₀ (mg/kg)	Reference
Long Evans rats	Oral	830	Proctor & Gamble Co., 1977
Long Evans rats	Oral	1,860*	Proctor & Gamble Co., 1977
Charles River rats (Fasted)	Oral	792	Dashiell and Kennedy, 1984
Charles River rats (Non-Fasted)	Oral	1,127	Dashiell and Kennedy, 1984
Albino rats	Oral	980	Johannsen and Levinskas, 1987
Sprague-Dawley rats (male)	Oral	800	Vernot et al., 1977
Sprague-Dawley rats (female)	Oral	750	Vernot et al., 1977
Long Evans rats	Intravenous	410	Proctor & Gamble Co., 1977
New Zealand Albino rabbits	Dermal	1,110	Vernot et al., 1977
Species	Route	ALD (mg/kg)	Reference
Albino rabbits	Dermal	7,500	E.I. Dupont, 1974
Charles River Rat	Oral	1,500	Kennedy et al., 1986

* Represents hexamethylenediamine dihydrochloride. When expressed as hexamethylenediamine, the LD₅₀ is 1,140 mg/kg