**National Toxicology Program**
Toxicity Report Series
Number 24

**NTP Technical Report**
**on Toxicity Studies of**

# 1,6-Hexanediamine Dihydrochloride

**(CAS No. 6055-52-3)**

**Administered by**
**Drinking Water and Inhalation**
**to F344/N Rats and B6C3F$_1$ Mice**

**Charles D. Hébert, PhD, Study Scientist**
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**Post Office Box 12233**
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**NIH Publication 93-3347**
**March 1993**

**United States Department of Health and Human Services**
**Public Health Service**
**National Institutes of Health**

## Foreword

The National Toxicology Program (NTP) is made up of four charter agencies of the United States Department of Health and Human Services (DHHS):

- the National Cancer Institute (NCI) of the National Institutes of Health;
- the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health;
- the National Center for Toxicological Research (NCTR) of the Food and Drug Administration; and
- the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control.

In July 1981, the Carcinogenesis Bioassay Testing Program was transferred from NCI to NIEHS. NTP coordinates the relevant Public Health Service programs, staff, and resources that are concerned with basic and applied research and with biological assay development and validation.

NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

To carry out its mission, NTP designs and conducts studies to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's toxic potential.

The studies described in this toxicity study report were performed under the direction of NIEHS and were conducted in compliance with NTP laboratory health and safety requirements. These studies met or exceeded all applicable federal, state, and local health and safety regulations. Animal care and use were in accord and compliance with the Public Health Service Policy on Humane Care and Use of Animals.

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This NTP Report on the toxicity studies of 1,6-hexanediamine dihydrochloride is based primarily on 2-week drinking water studies conducted in April, 1985, on 2-week inhalation studies conducted in January and February, 1986, and on 13-week inhalation studies that began in June 1987 and ended in September 1987 at Battelle Memorial Laboratories, Columbus, OH.

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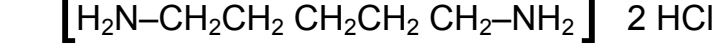
# ABSTRACT

## 1,6-Hexanediamine Dihydrochloride



| | |
|---|---|
| **Molecular Formula** | $C_6H_{16}N_2 \cdot 2HCl$ |
| **CAS Number** | 6055-52-3 |
| **Molecular Weight** | 185.2 |
| **Synonyms** | Hexamethylenediamine dihydrochloride;<br>1,6-diaminohexane dihydrochloride;<br>1,6-hexamethylenediamine dihydrochloride;<br>1,6-hexylenediamine dihydrochloride;<br>1,6-diamino-*n*-hexane dihydrochloride;<br>HMDA; HDA; HDDC. |

1,6-Hexanediamine (HDA) is an aliphatic amine that is produced in large volumes in the United States. HDA is widely used as a corrosion inhibitor in lubricants and as an intermediate in the industrial synthesis of paints, resins, inks, and textiles. Toxicity studies of the dihydrochloride salt of HDA (HDDC) were conducted in male and female Fischer 344/N rats and B6C3F$_1$ mice by the drinking water (2-week studies only) and whole-body inhalation routes (2-week and 13-week studies). Animals were evaluated for histopathology, clinical chemistry, hematology, and reproductive toxicity. In addition, the genetic toxicity of HDA was assessed in *Salmonella typhimurium* and in Chinese hamster ovary cells *in vitro*; HDDC was evaluated in the mouse micronucleus assay *in vivo*.

In the 2-week drinking water studies, groups of 5 rats of each sex received HDDC at doses of 0.75 to 6.7 mg/mL, and groups of 5 mice of each sex received doses of 0.2 to 3.0 mg/mL for 14 or 15 days. All animals survived to the end of the studies. No gross or microscopic pathologic changes and no clinical abnormalities related to HDDC consumption were seen in any dose group. The only statistically significant change was a slight decrease in absolute and/or relative liver weights of female rats in the 1.7, 5.0, and 6.7mg/mL treatment groups, in male rats in the 3.0 mg/mL treatment group, and in female mice in

the 0.8 mg/mL treatment group. Because there was no significant toxicity in these studies, 13-week drinking water studies were not conducted.

In the 2-week inhalation studies, 5 rats and 5 mice of each sex were exposed to 0, 10, 30, 89, 267, or 800 mg HDDC/$m^3$ for 6hours per day for 12 days. In the highest exposure group (800 mg/$m^3$), all male and female rats, all female mice, and 2 male mice died before the end of the studies. In the remaining groups, there was a dose-dependent depression in body weight gain in male and female mice, but not in rats. Clinical signs were primarily related to upper respiratory tract irritation and included dyspnea and nasal discharge in rats and mice. Absolute and relative liver weights were reduced in some male mice, but this did not occur in a dose-dependent manner. In rats, histopathologic lesions that were considered related to chemical exposure included inflammation and necrosis of laryngeal epithelium as well as focal inflammation and ulceration of the respiratory and olfactory nasal mucosa. In mice, focal areas of inflammation and necrosis were present in the respiratory mucosa of the larynx and trachea in the 2 highest exposure groups. Nasal lesions, including focal inflammation and ulceration, and degeneration and necrosis of the olfactory and respiratory epithelium were also seen in mice. In addition, mild testicular degeneration was present in 2 mice from the highest exposure group (800mg/$m^3$).

In the 13-week inhalation studies, 10 rats and 10 mice of each sex were exposed to 0, 1.6, 5, 16, 50, or 160 mg HDDC/$m^3$ for 6 hours per day, 5 days per week for 13 weeks. In addition special groups of 20 male and 40 female rats and mice (mating trial animals) at each exposure level were included to assess the effect of HDDC on reproduction. All rats and mice in the base-study groups survived to the end of the studies, and there were no exposure-related changes in body weight. In the mating trials, 3 female mice exposed to 16 mg/$m^3$ and 1 female and 1 male mouse exposed to 50 mg/$m^3$ died before scheduled termination. These deaths, however, were not considered to be chemical related. In male mice in the base study, liver weights were increased relative to controls in the 2highest exposure groups. No exposure-related changes in absolute or relative organ weights and no exposure-related clinical signs or gross lesions were seen in either species. In female rats, a dose-related decrease in white blood cell count was observed. Chemical-related microscopic lesions in male and female rats and mice were limited to the upper respiratory tract (larynx and nasal passages) in the 2 highest exposure groups and were similar in both species. These lesions included minimal to mild focal erosion/ulceration, inflammation, and hyperplasia of the laryngeal epithelium as well as degeneration of the

olfactory and respiratory nasal epithelium. HDDC caused no significant changes in sperm morphology or in the length of the estrous cycle of rats or mice.

In mating trials, HDDC demonstrated no adverse effects on reproduction of rats. The only statistically significant changes in reproductive parameters of mice were a slight increase in gestation length in the 50 mg/m$^3$ and 160 mg/m$^3$ exposure groups and a decrease in mean pup weight on Day 21 in the highest exposure group. These changes were not considered to be biologically significant.

1,6-Hexanediamine was not mutagenic in 4 strains of *Salmonella typhimurium*, and it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells. These *in vitro* tests were conducted with and without exogenous metabolic activation (S9). Negative results were also obtained in an *in vivo* test that measured the frequency of micronucleated erythrocytes in peripheral blood of male and female mice.

In summary, the toxicity of HDDC to rats and mice resulted from irritant properties of the chemical and was consistent with the effects of other irritant chemicals administered by inhalation. This toxicity was limited to the nose and airways. In the 2-week inhalation studies, deaths occurred in both rats and mice at the highest exposure level (800 mg/m$^3$). In the 13-week studies, the no-observed-adverse-effect-level (NOAEL) for respiratory damage was 5 mg/m$^3$ for rats and mice. HDDC had no adverse effect on reproduction of either species and was not genotoxic.

## PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies on 1,6-hexanediamine dihydrochloride on June 24, 1992 are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members determine if the design and conditions of these NTP studies are appropriate and ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

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# Summary of Peer Review Comments

On June 24, 1992, the Technical Reports Review Subcommittee of the Board of Scientific Counselors for the National Toxicology Program met in Research Triangle Park, NC, to review the draft technical report on toxicity studies of 1,6-hexanediamine dihydrochloride.

Dr. C.D. Hébert, NIEHS, introduced the short-term toxicity studies of 1,6-hexanediamine dihydrochloride by reviewing the uses and rationale for study of the chemical, the experimental design, and results.

Dr. Silbergeld, a principal reviewer, said the test chemical is a derivative of the widely used high-production volume chemical hexanediamine. She was concerned with the decision to test hexanediamine dihydrochloride, rather than hexanediamine. She said that the rationale appeared to be based on the ability to produce a rather stable compound that could be handled under test conditions; however, public health concerns relate to hexanediamine. Hexanediamine is reportedly toxic to humans, and inhalation/ingestion studies have been conducted in rodents, although actual use of the chloride in these studies could not be ruled out given the preparation method of the chemical. Dr. Hébert responded that he did not have information concerning the actual form of the compound as it occurs in the environment, or more specifically, in the airway epithelium; however, based on its chemical properties, he would expect it to be found as the mono- or dihydrochloride salt. In addition, because hexanediamine forms a precipitate on the walls of the inhalation chambers, it was more practical to test the dihydrochloride salt.

The comments of Mr. Beliczky, a second primary reviewer, who could not attend the meeting, were read by Dr. L. G. Hart, NIEHS. Mr. Beliczky thought that considerable toxicity data were available for hexanediamine, but that the current study still did not provide adequate information on which to base a decision to conduct a 2-year study. Dr. Hébert said that hexanediamine dihydrochloride had a low priority for further studies.

Dr. J. Haartz, NIOSH, requested that the report include more information about the generation and monitoring of inhalation aerosol. Dr. Carlson seconded this request and stated on behalf of the committee that the report would be accepted with the suggested changes.

# INTRODUCTION

## Uses, Production, Exposure, and Physical Properties[1]

1,6-Hexanediamine (HDA) is an aliphatic diamine that is widely used as an intermediate in chemical processes, including the synthesis of nylon-type polyamide resins (especially Nylon 66), the synthesis of oil-modified and moisture area types of urethane coatings, and the manufacture of polyamides for printing inks, paints, dimer acids, and textiles. HDA is also used as a corrosion inhibitor in oil and lubricants and as a curing agent in epoxide resins. Commercially, HDA is prepared by the reduction of adiponitrile with sodium and alcohol. According to the most recent available figures, U.S. manufacturers of HDA produced 837 million pounds in 1972 and 749 million pounds in 1975 (SRI, 1972, 1975); imports amounted to 370,000 pounds in 1972 and 16,000 pounds in 1975 (SRI, 1972, 1975). It has been estimated that as many as 12.8 million pounds of HDA were released into the environment each year in the mid-1970s. According to NIOSH estimates, approximately 1100 workers per year were exposed occupationally between 1972 and 1974 (NIOSH, 1972-1974). Occupational exposure may occur by either dermal or inhalation routes. Estimates for nonoccupational exposure to HDA are not available.

HDA is 1 of several odoriferous compounds produced by *Arum* lilies during flowering (Smith and Meeuse, 1966). No other information on the natural occurrence of HDA was found.

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1 Numerous studies on the toxicity of 1,6-hexanediamine (HDA) have been reported in the literature. In many of these studies, the compound was studied in the form of aqueous solutions of HDA, which are highly basic. Some of these reports describe the HDA solutions as having been neutralized with hydrochloric acid (HCl) before use. Neutralization ofHDA with HCl leads to the formation of, first, the monohydrochloride salt and then, with further addition of HCl, the dihydrochloride salt. The majority of the literature reports do not give the pH of thefinal solutions. As a result, it is not possible to determine the exact form of the compound tested (i.e., free acid, monohydrochloride salt or dihydrochloride salt). Therefore, in the summary of literature, all studies are cited as having been conducted with HDA unless the papers specifically state that the dihydrochloride salt (HDDC) was used. In addition, many studies of HDA toxicity were published in Russian, and translations of the entire articles are unavailable. In those cases in which only the abstracts are available in English, it is difficult to assess the adequacy of study design, details of experimental method, interpretation of results, and accuracy of the abstracts. These citations are indicated as "Abstract" in the reference list.

HDA is a colorless compound with a molecular weight of 116.2, a melting point of 42°C, and a boiling point of 205°C (Verschueren, 1979; NTP, 1991a). The compound in solid form exists as colorless leaflets, which absorb water and carbon dioxide from the atmosphere. HDA is soluble in water (≥ 100 mg/mL at 23°C), ethanol, dimethyl sulfoxide, and benzene. Therefore, the technical product is supplied as a 70% aqueous solution. HDA is strongly alkaline, with a $pK_a$ of 10.7, and is irritating to skin and mucous membranes. 1,6-Hexanediamine dihydrochloride (HDDC) is formed by the neutralization of HDA with hydrochloric acid. HDDC has a molecular weight of 185.2, a melting point of 248°C, and is freely soluble in water. In its solid form, HDDC crystallizes as needles from water or ethanol. No other information on the physical properties of HDDC was found in the literature.

HDA has not been found in U.S. or European drinking water supplies (Commission of the European Communities, 1976; NAS, 1977) or in industrial effluent (US EPA, 1979). No TLV (threshold limit value) for HDA has been established (ACGIH, 1990-91).

## Absorption, Metabolism, and Distribution

The pharmacokinetic profiles of HDA in humans and rodents have been investigated experimentally. HDA (8.2 mg) was given orally to healthy human male volunteers on 2-occasions 3 months apart, and urinary excretion of the compound was monitored (Brorson *et al.*, 1990). Excretion of HDA was found to be virtually complete within 15-hours, with an estimated half-life of elimination of 1.5 hours. In addition to the parent compound, the primary compounds found in the urine were the metabolites 6-aminohexanoic acid and *N*-acetyl-1,6-HDA.

Urine was found to be the principal route of excretion in adult male Fischer 344/N rats dosed by gavage with 1,6-[$^{14}$C]-hexanediamine (0.4 mg/kg body weight) (David and Heck, 1983). Within 72 hours after dosing, urinary excretion accounted for approximately 47% of the total dose; over a 72-hour period, 27% of the dose was excreted in feces; another 20% was recovered as exhaled $^{14}CO_2$. Less than 1.5% of the total dose was retained by the animals. Of the tissues examined, prostate gland contained the highest specific activity of radiolabel, followed by kidney, liver, intestine, and spleen. Similar results were obtained after intravenous administration of [$^{14}$C]-HDA to male and female rats. High levels of radioactivity were found in the prostate gland, intestine, and liver of males 24 hours after dosing. Uteri of female rats contained high concentrations of radiolabel 1 hour after

injection but not 24 hours after injection; ovaries contained little radioactivity at any time. Gas and thin-layer chromatographic analyses of urine from orally dosed rats indicated that 30% of the radioactivity was in the form of parent HDA. No further analyses of the other metabolites were performed.

Decomposition of 1,6-hexanediamine is reported to occur in the presence of intact cells or cell-free extracts of *Bacillus subtilis* (Roi, 1975; Garbara and Rotmistrov, 1982; Gvozdyak *et al.*, 1982). This is the principal method for removal of HDA found in waste water effluents from polyamide fiber production plants in the former Soviet Union. Bacterial diamine oxidases were shown to be incapable of metabolizing HDA (Tanzil and Boenicke, 1969); however, porcine diamine oxidase was able to metabolize HDDC *in vitro* (Bardsley *et al.*, 1970). In addition, Subramanyam *et al.* (1989) found that the metabolic fate of HDA is similar to that of putrescine and cadaverine in that HDA is converted to 6-aminohexanoic acid and caprolactam by rat and rabbit liver aldehyde oxidases via a cyclic intermediate.

## Toxicity

### HUMAN EFFECTS

At least 2 incidents involving HDA poisoning in humans have been described in the literature. Twenty workers at an Italian nylon manufacturing plant were exposed to HDA and adiponitrile in air. HDA concentrations ranged from 2 to 5.5 mg/$m^3$ during normal plant operations and from 32.7 to 131.5 mg/$m^3$ during autoclaving. Irritation of the conjunctiva and respiratory tract was reported in 8 workers; 1 worker developed contact dermatitis and acute hepatitis, which were believed to be due to HDA exposure. No anemia was seen in any of the workers (Ceresa, 1948; Gallo and Ghiringhelli, 1958). In the second incident, 488 workers in an epoxide resin plant were exposed to HDA. A-variety of symptoms, including itching, allergic rhinitis, bronchial asthma, impairment of bronchial permeability, toxicoallergic hepatitis, gastritis, colitis, hypergammaglobulinemia, increased serum transaminase activity, and eosinophilia of peripheral blood, were reported after prolonged contact (Gul'ko, 1971).

### ANIMAL TOXICITY

The toxicity of HDA in several mammalian and nonmammalian species has been examined using various routes of administration. HDA is reported to be moderately toxic in most

rodent species tested. The single-dose oral $LD_{50}$ for rats has variously been reported as 750 mg/kg (unspecified strain and sex; Vernot *et al.*, 1977), 750 and 800 mg/kg (female and male Sprague-Dawley, respectively; Vernot *et al.*, 1977), 792 and 1127 mg/kg (fasted and unfasted males, respectively; Dashiell and Kennedy, 1984) and 980 mg/kg (male and female Sprague-Dawley; Johannsen and Levinskas, 1987). Clinical signs prior to death in these studies included weakness, malaise, salivation, diarrhea, tremors, and weight loss. In addition, renal hyperemia and gastrointestinal inflammation were reported.

No reports were found on the oral $LD_{50}$ of HDA in mice. However, the $LD_{50}$ in mice by other routes is reported to be 180 mg/kg (intravenous; NDRC, 1942), 320 mg/kg (intraperitoneal; Roi and Garbara, 1978) and 1300 mg/kg (subcutaneous; Izmerov *et al.*, 1982). In addition, the inhalation $LC_{LO}$ is estimated at 750 mg/$m^3$ for a 10 minute exposure (Sax, 1984). Roi and Garbara (1978) also reported that the bacterial degradation products of HDA were 1.7-fold less toxic in mice than the parent compound, and that concentrations of HDA greater than 50 to 70 mg/L in water were lethal to *Daphnia* and *Cyclops* within 5 to 7 minutes.

The single-dose oral $LD_{50}$ for HDA in rabbits is 1110 mg/kg (Vernot *et al.*, 1977). Ceresa and DeBlasiis (1950) found HDA toxic when given orally (in pill form) and subcutaneously (in solution) to guinea pigs. Five of 6 guinea pigs given 0.02 g HDA/day orally died within 20 to 70 days, while the same dose given subcutaneously killed 3 of 3 guinea pigs in 5 to 7 days. Clinical and pathologic findings included weight loss, hemolytic anemia, and kidney and liver degeneration.

Subchronic and chronic studies of HDA toxicity have been conducted in rats and mice. Male and female Sprague-Dawley rats given HDA in feed sufficient to provide daily doses of up to 500 mg/kg body weight for 13 weeks experienced no toxic effects. There were no changes in body weight or in several clinical chemistry parameters examined (Johannsen and Levinskas, 1987). HDA given to rats and mice in drinking water for 1- to2years reportedly caused an increase in the mitotic index of lymphoid tissues at 0.1- and 1.0 mg/kg, but not at 10 mg/kg (Ponomareva and Merkushev, 1978). Marked changes in hemodynamic and bioelectrical properties of the heart were seen in rats exposed to 0.36mgHDA/$m^3$ by inhalation for 3 months (Verich, 1979).

The toxic effects of HDA in rats after inhalation exposure have been characterized. Rats exposed to 1.25 mg HDA/$m^3$ for 4 hours each day for 8 days reportedly had reduced nerve/muscle excitability, increased leukocyte counts, and impairment of hepatic glycogen synthesis and renal excretory capability (Tkachenko, 1976). Izrailet and Laivina (1980) saw no effects on hemoglobin or leukocyte parameters after chronic exposure of rats to 1mgHDA/$m^3$ for unspecified durations. The rat NOEL for inhalation of HDA has been cited as 1 mg/L (equal to 1000 mg/$m^3$) (15 days, 6 hours per day) (Verschueren, 1979). However, albino rats exposed continuously to concentrations of HDA up to 1 mg/$m^3$ for 3 months were said to have experienced growth retardation as well as a number of hematologic alterations, including increases in reticulocytes and leukocytes and decreases in leukocyte phagocytic activity and eosinophils (Kulakov, 1965). In a study published by Johannsen *et al.* (1987), male and female Sprague-Dawley rats were exposed to HDA at concentrations of 0, 12.8, 51, or 215 mg/$m^3$, 6 hours per day, 5 days per week for 13weeks. All rats in the 215 mg/$m^3$ exposure group died or were killed moribund by Week 7 of the study. Inflammation of the airways and lungs and conjunctival irritation were seen at exposure levels of 51 mg/$m^3$ and greater. Body weights were significantly reduced only in the 215 mg/$m^3$ group. After 5 weeks of exposure, erythrocyte counts and hemoglobin and hematocrit values were elevated in the high-exposure group, suggesting possible hemopoietic stimulation by HDA. However, there was no suggestion of hemoconcentration in this or any other exposure group. Rats exposed to 12.8 or 51 mg/$m^3$ showed no treatment-related hematologic changes. Microscopic lesions related to chemical exposure were confined to the trachea, nasal passages, and lungs. The cause of death in rats that died before the end of the study could not be determined.

HDA was shown to suppress immune function, both *in vivo* in rats and *in vitro*. Jobin and Tremblay (1970) found that HDA was able to inhibit collagen- and latex-induced aggregation of human platelets *in vitro*. Luebke *et al.* (1989) recently reported that HDDC suppressed mitogen-stimulated proliferation of mouse lymphocytes *in vitro*; this effect involved inhibition of ornithine decarboxylase (ODC) and polyamine activity, as well as other unidentified processes. In a drinking water study in which HDA was administered to rats for 12 months at concentrations of 0.1, 1.0, or 10.0 mg/L, antibody production was inhibited and the volume of lymphoid tissue in the spleen was reduced by approximately 40% (Shubik *et al.*, 1978).

## BIOCHEMICAL EFFECTS

HDA has been shown to inhibit the growth of human, monkey, and rodent cells *in vitro* (Trakhtenberg *et al.*, 1976; Chapman and Glant, 1980; Yano *et al.*, 1981) and to induce differentiation in sea urchin eggs (Lallier, 1966). Similarly, hexamethylene bisacetamide, a derivative of HDA, has been shown to induce differentiation of murine erythroid cells in culture (Reuben *et al.*, 1976, 1978; Hozumi *et al.*, 1979). Like other diamines, HDA inhibits the activity of ornithine decarboxylase (ODC), an enzyme necessary for synthesis of cellular polyamines. ODC inhibition has been demonstrated *in vivo* (Guha and Janne, 1977; Pegg *et al.*, 1978) and *in vitro* (Guha and Janne, 1977; Kallio *et al.*, 1977; Bethell and Pegg, 1979; Chapman and Glant, 1980), and is believed to be at least partially responsible for HDA-induced inhibition of cell proliferation.

HDA and other polyamines stabilize the structure of polyribonucleotides and increase their melting temperatures, presumably through association with double helical DNA (Szer, 1966; Padmanabhan *et al.*, 1991). This effect, too, may be related to the ability of HDA to inhibit cellular proliferation.

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY AND CARCINOGENICITY

A number of studies have been conducted to investigate the reproductive and developmental toxicity of HDA in rats (David and Heck, 1983; Johannsen and Levinskas, 1987; Short *et al.*, 1991) and mice (Manen *et al.*, 1983). These included a double generation study of rats given HDA in the diet (Short *et al.*, 1991). In rats, HDA doses as high as 900 mg/kg/day (during gestation) or 150 mg/kg/day (over 2 generations) had no effect on copulatory behavior, gestation length, fertility, number of corpora lutea, litter size, incidence of resorptions, pup survival, pup weight, sex ratios, or nesting or nursing behavior of dams. Similarly, HDA did not affect testis weight or copulatory behavior of male rats. HDA administered intraperitoneally to adult male CD-1 mice caused no adverse reproductive effects. HDA given to pregnant female CD-1 mice at 0.89 mM/kg was not feticidal; however, it caused a decrease in fetal body weight after administration on gestation Days 10, 11, or 12 (Manen *et al.*, 1983) as well as a retardation of supraoccipital bone development. No teratogenic effects of HDA have been described, and no information was found in the literature on the carcinogenic potential of HDA.

GENETIC TOXICITY

1,6-Hexanediamine was not mutagenic in any of several strains of *Salmonella typhimurium* tested with a preincubation protocol in the presence or the absence of S9 activation (Mortelmans *et al.*, 1986). 1,6-Hexanediamine was also tested (in the dihydrochloride form, HDDC, pH 4.2) for direct mutagenic activity in 7 *Salmonella* tester strains; HDDC was tested after reaction with sodium nitrite (Murphey-Corb *et al.*, 1983). In this study, HDDC failed to form a nitrosamine after incubation with nitrite, and caused neither direct mutations in *Salmonella* nor frameshift activity in tester strain TA1952.

## Study Rationale and Design

The U.S. Environmental Protection Agency nominated HDA for toxicity testing by the National Toxicology Program because of the large production volume of the chemical, the potential for occupational and nonoccupational human exposure, and the lack of information on the toxicity, mutagenicity, teratogenicity, and carcinogenicity of HDA. Inhalation and drinking water were chosen as administration routes because these are the major routes of potential occupational and nonoccupational exposure, respectively, in humans. Mating trials were included because inadequate data were available in the literature to assess the potential reproductive effects of HDA.

In the NTP toxicity studies of HDA, all solutions were converted from the free diamine to the dihydrochloride salt (HDDC) for the following reasons:

- HDA solutions are highly basic and, as such, are extremely caustic. HDA has a very high $pK_a$ (10.7) and would become protonated very rapidly upon contact with tissues or fluids at physiologic pH, causing local necrosis.
- Nonneutralized HDA solutions have a pungent odor and would be unpalatable in drinking water.
- HDA strongly absorbs carbon dioxide from the atmosphere. Stability studies conducted by the NTP indicated that under inhalation exposure conditions, HDA tended to deposit on the walls of the inhalation chambers and become converted to the carbamate form. HDDC was found to be much more stable in the aerosol form than HDA.
- HDDC has the same organic backbone as HDA and its use would allow detection of any specific toxicity associated with that backbone while avoiding the causticity, palatability, and stability problems that would be encountered with the use of HDA.

Two-week drinking water studies were performed on male and female rats and mice using HDDC. Because gross and histopathologic examinations of animals from the 2-week drinking water studies revealed no specific target tissues, 13-week drinking water studies were not conducted. Two-week and 13-week whole body inhalation studies of HDDC toxicity were conducted on male and female rats and mice. Gross and histopathologic examinations and sperm morphology and vaginal cytology evaluations were performed on rats and mice, and clinical pathology analyses were performed on rats in the 13-week inhalation studies. In addition, supplemental groups of rats and mice were exposed to HDDC by inhalation for 13 weeks and used in mating trials to assess the reproductive toxicity of HDDC. The genetic toxicity of HDDC was evaluated in the *in vivo* mouse micronucleus assay using mice in the 13-week inhalation study, and the genetic toxicity of HDA was evaluated in *in vitro* assays in *S. typhimurium* and in Chinese hamster ovary cells.

# MATERIALS AND METHODS

## Procurement and Characterization of 1,6-Hexanediamine

1,6-Hexanediamine was purchased from E. I. DuPont de Nemours and Company, Inc. (Wilmington, DE), in 2 lots (Lot No. PT-011882 and Lot No. PT-031985), and was shipped to the study laboratory, Battelle Columbus (Columbus, OH), from Midwest Research Institute (Kansas City, MO). Lot PT-011882 was used for the 2-week drinking water and inhalation studies; Lot PT-031985 was used for the 13-week inhalation studies. The chemical was identified as HDA by infrared spectroscopy. Purity analyses performed by gas chromatography indicated a purity of 101% for Lot PT-011882 (purchased in solid form) and 70.9% for Lot PT-031985 (purchased as a 70% aqueous solution). Bulk chemical was stored at room temperature in amber or foil-wrapped bottles; periodic chemical reanalyses at 4-month intervals indicated no breakdown of the chemical during storage.

## Dose Formulations for Drinking Water Studies

Drinking water solutions of hexanediamine were prepared in deionized water. All solutions, including the dosed water for the control group, were adjusted to pH 4.5 to 5.5 with 5.0N hydrochloric acid. At this pH, virtually all the hexanediamine exists in the form of the dihydrochloride salt (HDDC). Solutions were prepared weekly and stored in Nalgene® containers in the dark at room temperature. Analyses of the dose formulations were performed by gas chromatography; all dose formulations were found to be within 10% of target concentrations.

## Aerosol Generation for Inhalation Studies

For the inhalation studies, 1,6-hexanediamine was converted to 1,6-hexanediamine dihydrochloride (HDDC) by acidification with concentrated hydrochloric acid under a stream of nitrogen. The final pH was adjusted within the range of 4.5 to 5.5 before storage and again before use in the inhalation chambers.

The 70% aqueous HDDC solution was placed in a 9-liter glass reservoir and pressurized with $N_2$ gas. HDDC was delivered to 5 Sonimist Ultrasonic Spray Nozzles (Model HS600-2, Heat Systems-Ultrasonics, Inc., Farmingdale, NY) by a positive displacement metering

pump. Up to this point, stainless steel lines carried the test substance. The nebulizer reservoir was kept in a separate exposure chamber (H-1000, Hazelton Systems, Inc., Aberdeen, MD). This chamber served as a mixing plenum where large droplets and nonnebulized liquid were impacted or sedimented out of the test atmosphere before the aerosol was delivered to the inhalation chambers. The HDDC aerosol was mixed with compressed breathing air that had been filtered through an ENMET (ENMET Air Filtration Panel, Model AFP-82, Enmet Co., Ann Arbor, MI) and supplied at 50 psi to generate an aerosol at a concentration equal to the highest exposure concentration. The resulting aerosol was transported to the inhalation chambers through a manifold constructed of 3-inch diameter PVC tubing. At each chamber, a metered amount of aerosol was removed from the manifold and mixed with the appropriate amount of HEPA/charcoal-filtered room air to obtain the desired test concentration, then delivered to the inhalation chamber. After exiting the chambers, the test atmospheres were delivered to a common duct and cleansed of the test substance by a Mystaire HS-7CM scrubber (Heat Systems Ultrasonics).

## Concentration Monitoring

Concentrations of HDDC in the exposure chamber, exposure room, and exhaust were monitored by measuring the forward light scatter with RAM-S real-time aerosol monitors (GCA Corporation, Technology Division, Bedford, MA) and by gravimetric analyses of filter samples collected from each exposure chamber. Six RAM-S readings and 3 gravimetric samples were taken from each exposure chamber on each day of exposure. Gravimetric sampling was conducted with 25 mm glass fiber filter paper (Gelman Sciences, Inc., Ann Arbor, MI). Gravimetric analysis was performed on a Perkin Elmer AS-2Zmicrobalance (Perkin Elmer, Norwalk, CT) by weighing filters to the nearest 0.01 mg before and after sampling and again after storing the filters in a desiccator overnight. Twice monthly during the 13-week studies, glass fiber filter samples from each chamber were analyzed by gas chromatography with flame ionization detection for total hexanediamine, using the technique supplied by Midwest Research Institute. Measured concentrations of HDDC in the exposure chambers were within 6% of the target concentrations in all samples.

Spatial homogeneity of the aerosol within the exposure chambers was determined using the calibrated RAM-S monitors. Chamber concentrations were measured at 12 points within each chamber and then were compared to a fixed reference point. Time spans required to reach stable concentrations after start up and to reach background concentrations at the end of exposure were determined by taking measurements of aerosol

concentrations every 60 seconds. The time span required after start up to reach 90% of the target concentration was identified as the $T_{90}$; the time span required after the end of the exposure period to reach 10% of the target concentration was identified as the $T_{10}$.

Triplicate particle size measurements were obtained for each exposure chamber once in the first week and monthly thereafter, using an APS 3300 aerodynamic particle sizer (TSI, Inc., Minneapolis, MN). In addition, a CFM Ambient Impactor (Flow Sensor, McLean, VA) cascade impactor was used to determine the particle size distribution in the highest exposure level chamber once during the 13-week studies. The mass median aerodynamic diameter values for each chamber ranged from 1.62 to 1.72 microns, with a geometric standard deviation of 1.52 to 1.53. All control chamber respirable mass concentration values were less than 0.005 mg/m$^3$.

## Study Design

Fischer 344/N rats and B6C3F$_1$ mice used for the 2-week drinking water studies were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). Animals used for the 2-week inhalation studies were from Frederick Cancer Facility (Frederick, MD); those used in the 13-week inhalation base studies and mating trials were from Taconic Laboratory Animals and Services (Germantown, NY). Rats and mice were shipped to the study laboratory at approximately 4 weeks of age, quarantined at the study laboratory for 11 to 14 days, and placed on study at 6 to 7 weeks of age. Blood samples were collected at the beginning and end of the studies. Serum samples from 3 male and female rats and mice in the 2-week inhalation studies were analyzed for viral titers, as were samples from 5 male and female rats and mice in the 13-week studies. Data from 5 viral screens performed in rats and 12 viral screens performed in mice (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b) showed no positive antibody titers. Additional details concerning study design are provided in Table1.

After the quarantine period, rats and mice were weighed and randomly assigned to exposure groups using a Xybion® computer program (Xybion Medical Systems Corp., Cedar Knolls, NJ).

In the 2-week drinking water studies, animals were housed individually in polycarbonate cages suspended from stainless steel drawer-type racks; in the 2-week and 13-week inhalation studies, animals were housed in individual compartments of multi-compartment

stainless steel wire mesh cages. Pelleted NIH-07 feed (Zeigler Brothers, Inc., Gardners, PA) and water were available *ad libitum* on a continuous basis in the drinking water studies and during nonexposure periods in the inhalation studies. At all times (except during exposure periods in the inhalation studies), animal rooms were maintained at 72° ± 3°F and 50% ± 15% relative humidity with 12 to 15 fresh air changes per hour and 12 hours of subdued fluorescent light per day. In the inhalation studies, animals were housed in Hazelton H-2000 stainless steel and glass exposure chambers (Hazelton Systems, Inc., Aberdeen, MD) of 2 $m^3$ volume, with 15 air changes per hour (500 L/min). During inhalation exposures, chambers were maintained at 72° to 78°F and 70% to 80% relative humidity.

In the drinking water studies, groups of 5 rats and 5 mice of each sex received drinking water solutions containing 1,6-hexanediamine dihydrochloride *ad libitum* on a continuous basis for 14 days (mice) or 15 days (rats). Doses were selected based on reported literature values for oral $LD_{50}$ in rats (Vernot *et al.*, 1977) and intraperitoneal $LD_{50}$ in mice (Roi and Garbara, 1978), and on estimated water consumption. The doses for female rats were 11%higher than for males because female rats have been reported to be slightly less sensitive to HDA toxicity than males (Vernot *et al.*, 1977). The concentrations used were: 0, 0.75, 1.5, 3.0, 4.5, and 6.0 mg/mL for male rats; 0, 0.83, 1.7, 3.3, 5.0, and 6.7 mg/mL for female rats; and 0, 0.2, 0.4, 0.8, 1.5, and 3.0 mg/mL for male and female mice (Table1). Body weights were recorded on the day before dosing began and on Days 8 and 15 of the study.

In the inhalation studies, animals were housed continuously in exposure chambers with chamber doors closed except during animal husbandry procedures. In all inhalation studies, rats and mice were treated in the same chambers and, therefore, received the same exposure concentrations. For the 2-week inhalation studies, groups of 5 rats and 5 mice of each sex were administered HDDC by whole-body inhalation exposure for 12days, 6 hours plus $T_{90}$ (30 minutes) per day, 5 days per week. The total mass concentrations of aerosol for both rats and mice were 0, 31, 94, 282, 847, and 2540mg/$m^3$ (equivalent to 0, 10, 30, 89, 267, and 800 mg HDDC/$m^3$). These concentrations were chosen based on the reported inhalation $LC_{LO}$ of 750 mg/$m^3$ in mice and because of the lack of information on inhalation toxicity of HDDC in rats. For the 13-week inhalation studies, HDDC was administered to 10 animals/sex/species/exposure group (Base Study Groups) and 20 male animals and 40 female animals/species/exposure group (Mating Trial Groups). Because of the

weight gain depression and the inflammation and ulceration of the nasal cavity and larynx seen in both sexes of rats and mice at the higher concentrations in the 2-week studies, the concentrations used in the 13-week studies were 0, 1.6, 5, 16, 50, and 160mg HDDC/$m^3$. Exposures took place for 6 hours plus $T_{90}$ (30minutes) per day, 5 days per week for 13 weeks. Body weights were recorded at study start, weekly, and at the end of the studies. Clinical signs for animals in the base study groups were recorded weekly.

At study termination, a complete necropsy was performed on all treated and control animals in the 2-week drinking water studies and the 2-week and 13-week inhalation base studies. The thymus, heart, right kidney, lungs, brain, liver, and right testis of each animal were weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. No chemical-related gross or microscopic lesions were identified in the 2-week drinking water studies. For the inhalation studies, all tissues from control and high-exposure groups were examined microscopically. On the basis of these examinations, the mesenteric, mediastinal, mandibular, and peribronchiolar lymph nodes, spleen, thymus, nose/nasal cavity, larynx, testis, ovary, pancreas, and trachea were examined to a no-effect level in lower exposure groups in the 2-week inhalation studies. In the 13-week studies, nose/nasal cavity and larynx were examined in lower exposure groups to a no-effect level. Nasal sections were taken at 3 standard sites (Levels I, II, and III) for all animals (Boorman *et al.*, 1990). Tissues and groups examined for rats and mice are listed in Table 1.

Upon completion of the histologic evaluation by the laboratory pathologist, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory for quality assessment; the results were reviewed and evaluated by the NTP Pathology Working Group (PWG). The final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

# Supplemental Evaluations

## HEMATOLOGY AND CLINICAL CHEMISTRY

No clinical pathology studies were performed on rats or mice in the 2-week drinking water studies or the 2-week inhalation studies, nor on mice in the 13-week inhalation studies of HDDC. Clinical pathology analyses were performed on rats in the 13-week inhalation studies, as described below.

Blood samples were collected from all 13-week inhalation base-study rats at the end of the study. In addition, blood samples were taken from 10mating-trial rats/sex/exposure group after 3 and 13 exposures (Days 4 and 18). Animals were anesthetized with a $CO_2$:$O_2$ (70:30) gas mixture, and blood samples were drawn from the retroorbital sinus. Blood for hematology was collected in Microtainers® (Becton-Dickinson and Co., Rutherford, NJ) containing sodium EDTA as an anticoagulant. Samples for clinical chemical chemistry evaluations were collected in serum separator Microtainers® devoid of anticoagulant, allowed to clot at room temperature, centrifuged, and the serum was removed. Blood for methemoglobin determination was collected in Microtainers® containing heparin as anticoagulant.

Hematology determinations were performed with an Ortho ELT-8 Laser Hematology Counter (Ortho Instruments, Westwood, MA). Smears of peripheral blood were stained with Brechers stain and counterstained with a modified Romanowsky stain, then examined microscopically for determination of differential leukocyte counts and reticulocyte counts. Erythrocyte, leukocyte, and platelet morphologies were evaluated during the leukocyte differential count. Methemoglobin concentrations were measured with a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, OH). Clinical chemistry variables were measured with an Hitachi Automatic Chemistry Analyzer (Boehringer-Mannheim, Indianapolis, IN). Clinical pathology that were evaluated are listed in Table 1.

## REPRODUCTIVE SYSTEM EVALUATIONS

### Sperm Morphology and Vaginal Cytology in Rats and Mice

In the 13-week inhalation studies, sperm morphology and vaginal cytology evaluations (SMVCE) were performed on base-study rats and mice from the control group and the 3highest exposure groups (0, 16, 50, and 160 mg/$m^3$). To screen for potential reproductive toxicity, epididymal sperm motility was evaluated at necropsy; vaginal

cytology was evaluated during the week preceding necropsy using procedures outlined in the NTP's SMVCE protocol (modified October, 1984). Vaginal saline lavage was performed on females for 7 consecutive days prior to scheduled termination. The relative preponderance of leukocytes, nucleated epithelial cells, and large squamous epithelial cells in the lavage fluid were used to identify the stages of the estrous cycle.

Sperm motility was evaluated at necropsy as follows: The right epididymal tail (cauda epididymis) was removed at the junction of the vas deferens and the epididymal body (corpus epididymis), and a small cut was made in the distal border of the epididymal tail. A small amount of sperm was extruded, and the number of motile and nonmotile sperm in 5 microscopic fields were counted. After sperm sampling for motility evaluation, the epididymal tail was placed in sterile phosphate-buffered saline (PBS), finely minced, and swirled to release the contents. The tissue was incubated in PBS and then heat fixed at 65° C. Sperm density was determined using a hemacytometer. Four sperm morphology slides were prepared for each animal evaluated. An aliquot of killed sperm suspension was stained in a test tube, spread on a microscope slide, coverslipped, and examined.

## Mating Trials in Rats and Mice

Mating trials were performed on rats and mice from the control group and from the 3-highest exposure groups (0, 16, 50, and 160 mg/$m^3$) in the 13-week inhalation studies. These exposure groups were selected based on the lack of significant clinical findings (body weight changes or clinical signs of toxicity) in all exposure groups. Mating trial animals were bred for 10 nights (approximately study days 68 to 80, weekdays only) prior to the end of the 13-week exposure period. Females were removed from the inhalation chambers and housed overnight in polycarbonate cages with males from the same treatment group (2 females per male). Trios selected for breeding were not altered during the mating trial. These animals were returned to the inhalation chambers each day and exposed in the same manner as the base-study animals. Each morning during the mating period, females were examined for evidence of copulation by vaginal lavage. Females not showing evidence of copulation were mated again each night until they were sperm positive or for a maximum of 10 nights. Day 0 of gestation was considered to be the day sperm were observed in the lavage samples. Females not showing signs of copulation by the end of the breeding period were monitored for signs of pregnancy for an additional 23 days. If no clinical signs of pregnancy were seen, the animals were killed, and the uteri were examined for signs of pregnancy. If implantation was not evident, the uterus was stained with

ammonium sulfide and was examined for signs of early implantation. Following the last day of exposure, females were housed individually in polycarbonate cages until parturition. Male rats were killed at the end of the breeding period and were discarded without further examination. The day of parturition was considered to be lactation Day 0. Females and pups were killed on lactation Day 21.

Adult females were weighed on gestation Days 0 and 20. Adult males were weighed at the end of the mating period. Dams and pups were individually weighed on lactation Days 0, 5, 14, and 21. Pups were examined at birth for morphological abnormalities, viability, and gender. The number of live/dead offspring, percent neonatal survival, mean live pup weight, and sex ratio were recorded on lactation Days 0, 5, 14, and 21.

Necropsies were performed only on mating-trial females selected for breeding and examined for pregnancy 23 days after the conclusion of breeding as described above. Tissues from mating-trial animals were not fixed or retained.

## Genetic Toxicity Studies

### *SALMONELLA TYPHIMURIUM* MUTAGENICITY TEST PROTOCOL

Mutagenicity studies of 1,6-hexanediamine (HDA; CAS Number 124-09-4) in *Salmonella typhimurium* were conducted as described in Mortelmans *et al.* (1986). Briefly, HDA was supplied to the laboratory as a coded aliquot and was tested for mutagenicity in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, using a preincubation assay in both the absence and presence of Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. The compound was tested on 2 separate occasions in the same laboratory under different code numbers. HDA was tested at doses up to 10,000 µg/plate in both studies.

### CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1987). HDA was provided to the testing laboratory as a coded aliquot. Chinese hamster ovary cells (CHO) were incubated with HDA for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix.

## MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

At the end of the 13-week inhalation study, smears were prepared from peripheral blood samples obtained by cardiac puncture of all exposed and control mice. The slides were stained with Hoechst 33258/pyronin Y (MacGregor *et al.*, 1983). Ten thousand normochromatic erythrocytes and 2000 polychromatic erythrocytes from each animal were scored for micronuclei.

**TABLE 1 Experimental Design and Materials and Methods in the Drinking Water and Inhalation Studies of 1,6-Hexanediamine Dihydrochloride**

| | |
|---|---|
| **EXPERIMENTAL DESIGN** | |
| **Study Laboratory** | Battelle Memorial Institute, Columbus Division |
| **Size of Study Groups** | 2-Week Drinking Water Studies:<br>5 males and 5 females of each species per dose group<br>2-Week Inhalation Studies:<br>5 males and 5 females of each species per exposure group<br>13-Week Inhalation Studies:<br>Base Study Group:<br>10 males and 10 females of each species per exposure group<br>Mating Trial Group:<br>20 males and 40 females of each species per exposure group |
| **Chemical Source** | E.I. DuPont de Nemours, Inc., Wilmington, DE |
| **Doses/Duration of Dosing** | 2-Week Drinking Water Studies:<br>Male Rats:<br>0, 0.75, 1.5, 3.0, 4.5, or 6.0 mg/mL<br>(0, 750, 1500, 3000, 4500, or 6000 ppm)<br>Female Rats:<br>0, 0.83, 1.7, 3.3, 5.0, or 6.7 mg/mL<br>(0, 830, 1700, 3300, 5000, or 6700 ppm)<br>Male and Female Mice:<br>0, 0.2, 0.4, 0.8, 1.5, or 3.0 mg/mL<br>(0, 200, 400, 800, 1500, or 3000 ppm)<br>2-Week Inhalation Studies:<br>Male and Female Rats and Mice:<br>0, 10, 30, 89, 267, or 800 mg HDDC/$m^3$,<br>6 hours plus $T_{90}$/day, 5 days/week; total 12 days<br>13-Week Inhalation Studies:<br>Male and Female Rats and Mice:<br>0, 1.6, 5, 16, 50, or 160 mg HDDC/$m^3$,<br>6 hours plus $T_{90}$/day, 5 days/week; total 13 weeks |
| **Date of First Dose/Exposure** | 2-Week Drinking Water Studies:<br>Rats: 2 April 1985<br>Mice: 1 April 1985<br>2-Week Inhalation Studies:<br>Rats: 28 January 1986<br>Mice: 29 January 1986<br>13-Week Inhalation Studies:<br>Rats: females, 2 June 1987; males, 3 June 1987<br>Mice: females, 9 June 1987; males, 10 June 1987 |

**TABLE 1 Experimental Design and Materials and Methods in the Drinking Water and Inhalation Studies of 1,6-Hexanediamine Dihydrochloride (continued)**

| | |
|---|---|
| **EXPERIMENTAL DESIGN (continued)** | |
| **Date of Last Dose/Exposure** | 2-Week Drinking Water Studies:<br>Rats: 16 April 1985<br>Mice: 15 April 1985<br>2-Week Inhalation Studies:<br>Rats: 12 February 1986<br>Mice: 13 February 1986<br>13-Week Inhalation Studies:<br>Rats: females, 2 September 1987; males, 3 September 1987<br>Mice: females, 9 September 1987; males, 10 September 1987 |
| **Necropsy Dates** | 2-Week Drinking Water Studies:<br>Rats: 17 April 1985<br>Mice: 16 April 1985<br>2-Week Inhalation Studies:<br>Rats: 13 February 1986<br>Mice: 14 February 1986<br>13-Week Inhalation Studies:<br>Rats: females, 3 September 1987; males, 4 September 1987<br>Mice: females, 10 September 1987; males, 11 September 1987 |
| **Type and Frequency of Observation** | 2-Week Drinking Water Studies:<br>Observed 2 times per day. Animals were weighed just prior to being placed on study and on Days 8 and 15 of the study.<br>2-Week Inhalation Studies:<br>Observed 2 times per day. Animals were weighed just prior to being placed on study and on Days 8 and 15 of the study.<br>13-Week Inhalation Studies:<br>Body weights were recorded at study start, weekly, and at study termination. Clinical signs recorded weekly for animals in the base-study groups. |
| **Necropsy and Histologic Examinations** | Necropsy performed; tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with H&E for microscopic examination. The following tissues were examined microscopically from all high-exposure and control animals: adrenal gland, bone and bone marrow, brain, bronchial lymph node, cecum, clitoral/preputial glands, colon, duodenum, epididymis, esophagus, gallbladder (mice), heart, ileum, jejunum, kidney, larynx, lung and mainstem bronchi, liver, mammary gland, mandibular lymph node, mediastinal lymph node, mesenteric lymph node, nasal cavity and nasal turbinates, ovary, pancreas, prostate gland, pituitary gland, parathyroid gland, rectum, salivary gland, skin, spleen, stomach, seminal vesicle, testis, thyroid gland, thymus, trachea, urinary bladder, uterus, and all gross lesions. |

**TABLE 1 Experimental Design and Materials and Methods in the Drinking Water and Inhalation Studies of 1,6-Hexanediamine Dihydrochloride (continued)**

**EXPERIMENTAL DESIGN (continued)**

**Supplemental Evaluations**

Hematology and Clinical Chemistry:

Blood samples were collected from all 13-week inhalation base-study rats at study termination. In addition, blood samples were taken from 10 mating-trial rats/sex/exposure group after 3 and 13 exposures. The following hematology parameters were evaluated: erythrocyte (RBC), leukocyte (WBC), and platelet (PLAT) counts, hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and methemoglobin (METH). Smears of peripheral blood were stained with Brechers stain and counterstained with a modified Romanowsky stain, then examined microscopically. Leukocyte differentials were determined on 100 cells; the absolute counts for each leukocyte type were obtained as the product of the corresponding percentage and the total leukocyte count. Reticulocytes were counted from the slides prepared for the leukocyte counts. Relative numbers of reticulocytes, determined by microscopic examination of approximately 1000 erythrocytes, were converted to absolute counts based on the total erythrocyte count. Erythrocyte, leukocyte, and platelet morphologies were evaluated during the leukocyte differential count.

The following clinical chemistry assays were performed: urea nitrogen (UN), creatinine, alanine aminotransferase (ALT), alkaline phosphatase (AP), sorbitol dehydrogenase (SDH), and glucose.

Sperm Morphology/Vaginal Cytology and Mating Trials

Sperm morphology and vaginal cytology evaluations (SMVCE) and mating trials were performed at the end of the 13-week studies. Sperm morphology and vaginal cytology were evaluated in base-study rats and mice from the control, 16, 50, and 160 mg HDDC/$m^3$ exposure groups. Mating trials were performed on supplemental rats and mice exposed to 0, 16, 50, or 160 mg HDDC/$m^3$.

**ANIMALS AND ANIMAL MAINTENANCE**

**Strain and Species**

F344/N Rats
$B6C3F_1$ Mice

**Animal Source**

2-Week Drinking Water Studies:
Simonsen Labs, Inc., Gilroy, CA
2-Week Inhalation Studies:
Frederick Cancer Facility, Frederick, MD
13-Week Inhalation Studies:
Taconic Farms, Inc., Germantown, NY

**Time Held Before Study**

11-14 days

**Age When Placed on Study**

6-7 weeks

**TABLE 1 Experimental Design and Materials and Methods in the Drinking Water and Inhalation Studies of 1,6-Hexanediamine Dihydrochloride (continued)**

| | |
|---|---|
| **ANIMALS AND ANIMAL MAINTENANCE (continued)** | |
| **Age When Killed** | 2-Week Drinking Water Studies:<br>8-9 weeks<br>2-Week Inhalation Studies:<br>8-9 weeks<br>13-Week Inhalation Studies:<br>19-20 weeks |
| **Method of Animal Distribution** | Animals were weighed and randomized (by partitioning algorithm) into groups by sex and assigned to cages; cages were assigned to dose groups. |
| **Diet** | 2-Week Drinking Water Studies:<br>NIH 07; available *ad libitum*<br>2-Week and 13-Week Inhalation Studies:<br>NIH 07; available *ad libitum* except during exposure periods |
| **Animal Room Environment** | 2-Week Drinking Water Studies:<br>Temperature was maintained at 72° ± 3°F and relative humidity at 50% ± 15% with 12 - 15 room air changes per hour. Fluorescent light was provided for 12hours per day.<br>2-Week and 13-Week Inhalation Studies:<br>Temperature was maintained at 72° ± 3°F and relative humidity at 50% ± 15% with 12 - 15 room air changes per hour. Fluorescent light was provided for 12 hours per day. During inhalation exposure, temperature was maintained at 72° - 78°F and relative humidity at 70% - 80%. |

# Statistical Methods

## ANALYSIS OF CONTINUOUS VARIABLES

In the 13-week studies, two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry and hematology data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or, at most, half of the next smallest value.

### ANALYSIS OF VAGINAL CYTOLOGY DATA

Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous state), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels.

### ANALYSIS OF MATING TRIAL DATA

Data from the mating trials were grouped into 3 categories and analyzed statistically. Continuous, quantitative data, such as body weights, were analyzed by Dunnett's *t*-test for multiple comparisons to a single control group. Discrete, counting data, such as litter counts, were analyzed by the Mann-Whitney U nonparametric test. Percentage data, such as the fertility and survival indices, were analyzed by the Chi Square test.

### ANALYSIS OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

A positive response in the *Salmonella typhimurium* assay was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any 1-strain/activation combination. An equivocal response was defined as an increase in revertants that was not dose related, not reproducible, or not of sufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

### ANALYSIS OF CHINESE HAMSTER OVARY CELL CYTOGENETICS DATA

For the SCE data, statistical analyses were conducted on the slopes of the dose-response curves (Galloway *et al.,* 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at 1 dose point is less than 0.01; the

probability for such a chance occurrence at 2 dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at 2 or more doses resulted in a determination that the trial was positive. A statistically significant trend (P≤0.05) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal aberration data are presented as percentage of cells with aberrations. Statistical analyses were conducted on both the dose-response curve and individual dose points (Galloway *et al.*, 1987). For a single trial, a statistically significant (P≤0.05) difference for 1 dose point and a significant trend (P≤0.015) were considered weak evidence for a positive response; significant differences for 2 or more doses indicated the trial was positive. A positive trend test in the absence of a statistically significant increase at any 1 dose resulted in an equivocal call (Galloway *et al.*, 1987).

### ANALYSIS OF MOUSE PERIPHERAL BLOOD MICRONUCLEUS DATA

Log transformation of the normochromatic erythrocyte (NCE) data, and testing for normality by the Shapiro-Wilk test and for heterogeneity of variance by Cochran's test were performed before statistical analyses. The frequency of micronucleated cells among NCEs was analyzed by analysis of variance using the SAS GLM procedure. The NCE data for each dose group were compared with the concurrent solvent control using Student's *t*-test. The frequency of micronucleated cells among polychromatic erythrocytes (PCEs) was analyzed by the Cochran-Armitage trend test, and individual dose groups were compared to the concurrent solvent control by Kastenbaum-Bowman's (1970) binomial test. The percentage of PCEs among total erythrocytes was analyzed by an analysis of variance on ranks (classed by sex) and individual dose groups were compared with the concurrent solvent control using a *t*-test on ranks.

## Quality Assurance

The studies of 1,6-hexanediamine dihydrochloride were performed in compliance with the United States FDA Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of Battelle Columbus Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies. The operations of the Quality Assurance Unit were monitored by the NTP.

# RESULTS

## 2-Week Drinking Water Study in F344/N Rats

All rats survived to the end of the study. No clinical abnormalities related to chemical exposure occurred. Water consumption was reduced for male rats in the 2 highest dose groups (4.5 and 6.0mg/mL) and for females in the 3highest dose groups (3.3, 5.0, and 6.7 mg/mL). This decrease in water consumption was attributed to poor palatability of the drinking water solutions. The total estimated dose of HDDC consumed by each dose group, based on average water consumption and mean body weights, is given in Table 2. Although the total amount of compound consumed did increase with larger concentrations of HDDC in the water, this increase was not linear because of the reduced water intake at the higher concentrations. Mean body weight gains of treated rats were similar to those of controls (Table 2).

**TABLE 2 Survival, Weight Gain, Water Consumption, and Compound Consumption in F344/N Rats in the 2-Week Drinking Water Study of 1,6-Hexanediamine Dihydrochloride**

| Dose (mg/mL) | Survival[1] | Mean Body Weight (grams) Initial | Final | Change[2] | Final Weight Relative to Controls (%)[3] | Average Water Consumption (g/day) | Average Dose of HDDC (mg/kg/day) |
|---|---|---|---|---|---|---|---|
| **MALE** | | | | | | | |
| 0 | 5/5 | 105 | 168 | 63 | | 16.8 | |
| 0.75 | 5/5 | 105 | 169 | 65 | 101 | 17.5 | 96 |
| 1.5 | 5/5 | 106 | 172 | 66 | 103 | 17.3 | 187 |
| 3.0 | 5/5 | 105 | 167 | 61 | 99 | 16.2 | 357 |
| 4.5 | 5/5 | 107 | 161 | 55 | 96 | 13.4 | 449 |
| 6.0 | 5/5 | 108 | 158 | 50 | 94 | 12.1 | 545 |
| **FEMALE** | | | | | | | |
| 0 | 5/5 | 90 | 122 | 33 | | 17.6 | |
| 0.83 | 5/5 | 89 | 122 | 33 | 100 | 16.1 | 126 |
| 1.7 | 5/5 | 89 | 121 | 32 | 99 | 16.2 | 263 |
| 3.3 | 5/5 | 90 | 124 | 35 | 102 | 13.7 | 422 |
| 5.0 | 5/5 | 90 | 119 | 29 | 97 | 10.8 | 517 |
| 6.7 | 5/5 | 90 | 115 | 26 | 94 | 9.7 | 634 |

1 Number surviving at 2 weeks/number of animals per dose group.

2 Mean weight change.

3 (Dosed group mean/control group mean) x 100.

Mean liver weights and liver-weight-to-body-weight ratios of rats receiving HDDC in drinking water for 2 weeks are given in Table 3. Female rats in the 1.7, 5.0, and 6.7-mg/mL treatment groups showed significant decreases in absolute and relative liver weights. The only significant difference in liver weights between control and treated male rats was a decrease in the mean relative liver weight of the 3.0 mg/mL dose group. The weights of the other organs were not affected. No treatment-related gross or microscopic lesions were present.

**TABLE 3 Liver Weights and Liver-Weight-to-Body-Weight Ratios of F344/N Rats in the 2-Week Drinking Water Study of 1,6-Hexanediamine Dihydrochloride[1]**

| | Dose (mg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.75 | 1.5 | 3.0 | 4.5 | 6.0 |
| **MALE** | | | | | | |
| Necropsy body weight (g) | 175 | 175 | 178 | 171 | 170 | 169 |
| Liver | | | | | | |
| Absolute (g) | 9.793 | 9.561 | 9.470 | 8.500 | 8.892 | 8.786 |
| Relative (mg/g) | 55.97 | 54.48 | 53.09 | 49.53** | 52.30 | 51.76 |

| | Dose (mg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.83 | 1.7 | 3.3 | 5.0 | 6.7 |
| **FEMALE** | | | | | | |
| Necropsy body weight (g) | 125 | 124 | 123 | 128 | 124 | 123 |
| Liver | | | | | | |
| Absolute (g) | 6.912 | 6.420 | 5.884* | 6.460 | 5.997* | 5.748** |
| Relative (mg/g) | 55.48 | 52.09 | 48.06* | 50.57 | 48.43* | 46.56 |

[1] n=5. Liver weights and body weights are given in grams; relative liver weights (liver-weight-to-body-weight ratios) are given as mg liver weight/g body weight.

* Significantly different (P≤0.05) from the control group by Dunnett's test.

** Significantly different (P≤0.01) from the control group by Dunnett's test.

## 2-Week Drinking Water Study in B6C3F$_1$ Mice

All mice survived to the end of the 2-week drinking water study. No clinical abnormalities related to HDDC exposure were observed in any dose group. Water consumption by treated mice was similar to that of controls. The estimated daily dose of HDDC consumed by each treatment group is shown in Table 4. There was a linear increase in total estimated chemical intake with increasing concentration of HDDC in the drinking water. Mean body weights and body weight gains of treated mice were similar to those of controls (Table 4). The only statistically significant difference in absolute or relative organ weights was a decrease in the relative liver weight of females in the 0.8 mg/mL dose group (Table5). No chemical-related gross or microscopic lesions were present.

**TABLE 4 Survival, Weight Gain, Water Consumption, and Compound Consumption in B6C3F$_1$ Mice in the 2-Week Drinking Water Study of 1,6-Hexanediamine Dihydrochloride**

| Dose (mg/mL) | Survival[1] | Mean Body Weight (grams) Initial | Final | Change[2] | Final Weight Relative to Controls (%)[3] | Average Water Consumption (g/day) | Average Dose of HDDC (mg/kg/day) |
|---|---|---|---|---|---|---|---|
| **MALE** | | | | | | | |
| 0 | 5/5 | 22.5 | 25.2 | 2.7 | | 4.6 | |
| 0.2 | 5/5 | 22.4 | 25.3 | 2.9 | 100 | 4.3 | 36 |
| 0.4 | 5/5 | 22.0 | 25.4 | 3.4 | 101 | 3.9 | 66 |
| 0.8 | 5/5 | 22.0 | 25.1 | 3.1 | 100 | 4.1 | 139 |
| 1.5 | 5/5 | 21.8 | 25.4 | 3.6 | 101 | 4.2 | 267 |
| 3.0 | 5/5 | 21.6 | 25.2 | 3.6 | 100 | 4.4 | 564 |
| **FEMALE** | | | | | | | |
| 0 | 5/5 | 17.6 | 20.6 | 3.0 | | 4.4 | |
| 0.2 | 5/5 | 17.1 | 20.1 | 3.0 | 98 | 4.5 | 48 |
| 0.4 | 5/5 | 17.5 | 20.2 | 2.7 | 98 | 5.5 | 116 |
| 0.8 | 5/5 | 17.2 | 19.8 | 2.6 | 96 | 4.8 | 208 |
| 1.5 | 5/5 | 18.0 | 20.3 | 2.3 | 99 | 5.0 | 391 |
| 3.0 | 5/5 | 17.3 | 20.4 | 3.1 | 99 | 4.0 | 632 |

[1]Number surviving at 2 weeks/number of animals per dose group.
[2]Mean weight change.
[3](Dosed group mean/control group mean) x 100.

**TABLE 5 Liver Weights and Liver-Weight-to-Body-Weight Ratios of B6C3F$_1$ Mice in the 2-Week Drinking Water Study of 1,6-Hexanediamine Dihydrochloride[1]**

| | Dose (mg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.2 | 0.4 | 0.8 | 1.5 | 3.0 |
| **MALE** | | | | | | |
| Necropsy body weight (g) | 25.3 | 25.2 | 25.1 | 24.4 | 25.7 | 24.8 |
| Liver | | | | | | |
| Absolute (g) | 1.557 | 1.493 | 1.519 | 1.419 | 1.616 | 1.553 |
| Relative (mg/g) | 61.53 | 59.25 | 60.38 | 57.91 | 63.13 | 62.62 |
| **FEMALE** | | | | | | |
| Necropsy body weight (g) | 20.9 | 20.1 | 20.4 | 19.4 | 20.6 | 19.7 |
| Liver | | | | | | |
| Absolute (g) | 1.300 | 1.237 | 1.177 | 1.101 | 1.212 | 1.125 |
| Relative (mg/g) | 62.27 | 61.58 | 57.81 | 56.69** | 58.76 | 57.00 |

[1] n=5. Liver weights and body weights are given in grams; relative liver weights (liver-weight-to-body-weight ratios) are given as mg liver weight/g body weight.

** Significantly different (P≤0.01) from the control group by Dunnett's test.

## 2-Week Inhalation Study in F344/N Rats

The exposure concentrations used in the 2-week inhalation study in rats (0, 10, 30, 89, 267, and 800 mg HDDC/$m^3$) were chosen based on the reported inhalation $LC_{LO}$ of 750 mg/$m^3$ in mice and because of the lack of information on inhalation toxicity of HDDC in rats. Survival and mean body weight data for rats in the 2-week inhalation study of HDDC are presented in Table 6. On Day 8 of the study (after 6 days of exposure to HDDC), the body weights of female rats exposed to the highest concentration of HDDC (800 mg/$m^3$) were notably less than those of the controls. One of the 5 female rats in the 800 mg/$m^3$ exposure group died on Day 3; the remaining 4 females in this exposure group died on Day11. Although the body weights of male rats in the highest exposure group were not reduced when the rats were weighed on Day 8, 4 of the 5 males in this group died on Day11, and the remaining male rat died on Day 15. The body weights of male and female rats exposed to 267 mg HDDC/$m^3$ were also less than those of controls, although this difference was not statistically significant. No deaths occurred in any other exposure group.

**TABLE 6 Survival and Weight Gain of F344/N Rats in the 2-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride**

| HDDC (mg/$m^3$) | Survival[1] | Mean Body Weight (grams) | | | | Final Weight Relative to Controls (%)[3] |
|---|---|---|---|---|---|---|
| | | Initial | Day 8 | Day 17 | Change[2] | |
| **MALE** | | | | | | |
| 0 | 5/5 | 107 | 141 | 192 | 85 | |
| 10 | 5/5 | 104 | 151 | 197 | 93 | 103 |
| 30 | 5/5 | 106 | 138 | 198 | 92 | 103 |
| 89 | 5/5 | 106 | 144 | 189 | 83 | 99 |
| 267 | 5/5 | 105 | 134 | 183 | 78 | 95 |
| 800 | 0/5[4] | 106 | 134 | — | — | — |
| **FEMALE** | | | | | | |
| 0 | 5/5 | 88 | 110 | 136 | 48 | |
| 10 | 5/5 | 88 | 113 | 138 | 50 | 101 |
| 30 | 5/5 | 88 | 111 | 134 | 47 | 99 |
| 89 | 5/5 | 89 | 111 | 138 | 49 | 101 |
| 267 | 5/5 | 89 | 103 | 128 | 39 | 94 |
| 800 | 0/5[5] | 89 | 90[6] | — | — | — |

1 Number surviving at 2 weeks/number of animals per exposure group. For groups with no survivors, no Day 17 body

2 Mean weight change.

3 (Exposure group mean/control group mean) x 100.

4 Day of death: 11, 11, 11, 11, 15.

5 Day of death: 3, 11, 11, 11, 11.

6 n=4.

Clinical signs related to exposure included nasal discharge, rales and dyspnea, diarrhea, ocular discharge, and hypoactivity. These signs were seen only in male and female rats exposed to the highest concentration of HDDC. At necropsy, there were no exposure-related gross lesions.

There were no statistically significant chemical-related changes in the liver, thymus, heart, right testis, kidney, lung, or brain weights of male or female rats in any group.

Exposure-related histopathologic lesions were present in the nasal passages and larynx. In the larynx, there were focal areas of inflammation and necrosis with ulceration of laryngeal epithelium at exposure levels as low as 10 $mg/m^3$ in males and 89 $mg/m^3$ in females. Nasal lesions also were present in both males and females at exposure levels of 89 $mg/m^3$ and greater. Microscopic changes included focal areas of inflammation and ulceration in both respiratory and olfactory portions of the nasal mucosa. Degeneration characterized by thinning (atrophy) and necrosis of the olfactory and respiratory epithelium was frequently associated with areas of inflammation and ulceration.

A number of histopathologic lesions were present in rats that died or were killed moribund. These included lymphoid depletion and necrosis in the thymus, lymph nodes, and spleen as well as depletion of pancreatic zymogen granules and arrest of ovarian follicular development. None of these lesions resulted from a direct toxic effect of HDDC exposure.

## 13-Week Inhalation Study in F344/N Rats

Concentrations of HDDC used in the 13-week study in rats were selected based on the weight gain depression and the inflammation and ulceration of the nasal cavity and larynx seen in rats at higher concentrations in the 2-week study. Concentrations chosen for the 13-week study were 0, 1.6, 5, 16, 50, and 160 mg HDDC/$m^3$. All rats exposed to HDDC by inhalation for 13 weeks survived to the end of the study. The final mean body weights of most groups of rats exposed to HDDC were slightly lower than the mean body weights of the controls (Table 7; Figure 1); these differences, however, were not statistically significant.

**TABLE 7 Survival and Weight Gain of F344/N Rats in the 13-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride**

| HDDC ($mg/m^3$) | Survival[1] | Mean Body Weight (grams) | | | Final Weight Relative to Controls (%)[3] |
|---|---|---|---|---|---|
| | | Initial | Final | Change[2] | |
| **MALE** | | | | | |
| 0 | 10/10 | 147 | 352 | 206 | |
| 1.6 | 10/10 | 143 | 337 | 193 | 96 |
| 5 | 10/10 | 142 | 338 | 196 | 96 |
| 16 | 10/10 | 142 | 341 | 200 | 97 |
| 50 | 10/10 | 150 | 339 | 189 | 96 |
| 160 | 10/10 | 142 | 326 | 184 | 93 |
| **FEMALE** | | | | | |
| 0 | 10/10 | 114 | 203 | 89 | |
| 1.6 | 10/10 | 114 | 195 | 81 | 96 |
| 5 | 10/10 | 114 | 203 | 89 | 100 |
| 16 | 10/10 | 112 | 198 | 86 | 97 |
| 50 | 10/10 | 112 | 201 | 90 | 99 |
| 160 | 10/10 | 114 | 202 | 88 | 99 |

1 Number of animals surviving at end of study/number of animals per exposure group.
2 Mean weight change.
3 (Exposure group mean/control group mean) x 100.

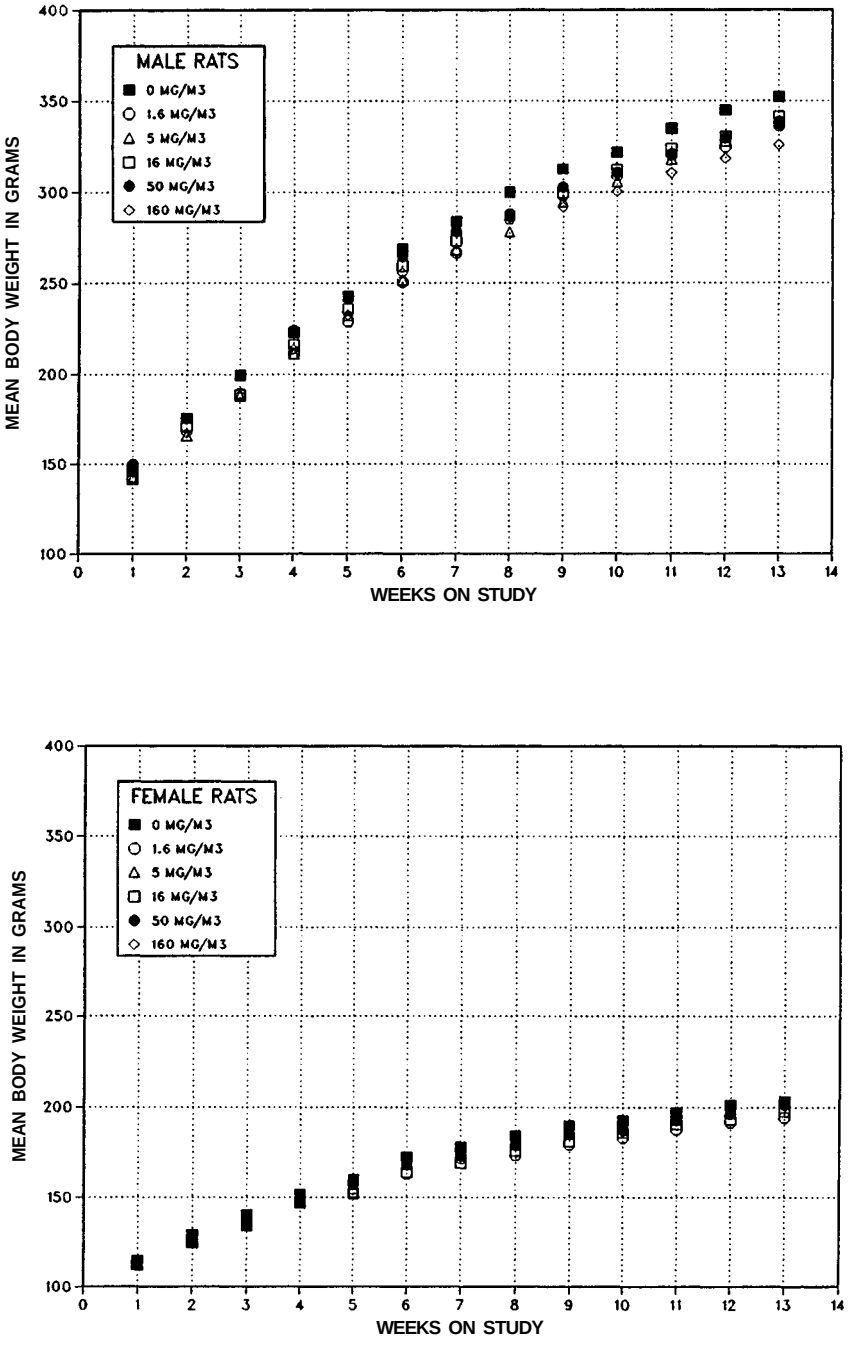


**FIGURE 1 Body Weights of F344/N Rats Exposed to 1,6-Hexanediamine Dihydrochloride by Inhalation for 13 Weeks**

No clinical signs of toxicity related to HDDC exposure were seen in the 13-week rat study. Nasal discharge occurred in male rats in the 5 and 16 mg/m$^3$ exposure groups and in female rats in all exposure groups (including the control group) except those in the 160mg/m$^3$ group. Similarly, rales occurred in all female groups but not in exposed males. However, because these signs appeared late in the study and because the incidence was not dose related, the signs were not considered to be the result of specific HDDC toxicity.

The only consistent changes in organ weights seen in rats were decreases in absolute and relative lung weights compared to those of the controls. However, all control male and female rats had inflammatory lesions in the lungs and had lung weights that were greater than those of historical controls (NTP, 1990a). Similar inflammatory lesions have been seen in other inhalation studies and are of unknown etiology (NTP, 1990b, 1991b). Sporadic changes in other absolute and relative organ weights were seen but did not appear to be chemical related (Appendix A).

At Day 4, the only change noted in the hematology parameters of rats exposed by inhalation to HDDC was a slight decrease in the mean platelet count in female rats in the lowest exposure group (Table B1). At Day 18, hematocrit values were increased in female rats in the 2 highest exposure groups and segmented neutrophil counts were decreased minimally in male rats in the highest exposure group. By Day 94, there was a significant decrease in leukocyte and lymphocyte counts in females in the highest exposure groups, and in segmented neutrophil counts in females in the 3 highest exposure groups (16, 50, and 160 mg/m$^3$). Female rats in the 2 lowest exposure groups had increased hematocrit values. A slight decrease in erythrocyte count was noted in male rats in the 16mg/m$^3$ exposure group, and a minor increase in mean cell hemoglobin values occurred in female rats in the 160 mg/m$^3$ exposure group and male rats in the 50 mg/m$^3$ exposure group.

Clinical chemistry changes on Day 4 included a small increase in alanine aminotransferase activity in male rats in the lowest exposure group (1.6 mg/m$^3$) and a slight increase in the urea nitrogen level in female rats in the 5 mg/m$^3$ exposure group (Table B1). By Day18, concentrations of urea nitrogen increased in male rats in the 2-highest exposure groups (50 and 160 mg/m$^3$) and female rats in the 4 highest exposure groups (5, 16, 50, and 160 mg/m$^3$). Sorbitol dehydrogenase (SDH) activity was slightly elevated in female rats in the highest exposure group. At Day 94, alkaline phosphatase activity was slightly increased in male rats in several exposure groups (1.6, 50, and

160mg/$m^3$), and SDH activity was elevated in males in the 50 mg/$m^3$ exposure group. No other significant clinical chemistry changes occurred in male or female rats at Day 94.

There were no gross lesions attributed to HDDC exposure. Chemical-related microscopic lesions were limited to the upper respiratory tract (larynx and nasal passages) of male and female rats in the 2 highest exposure groups (Table 8). The morphology, incidence, and severity of microscopic lesions were similar for males and females, and there was a dose-related increase in the incidence and severity of these lesions. In the larynx, minimal to mild focal erosion/ulceration was present in rats at the 160 mg/$m^3$ exposure level as a result of necrosis of the laryngeal epithelium (Plate 1). A minimal to mild inflammatory infiltrate in the mucosa was associated with these erosions; sometimes the infiltrate extended into the lumen of the larynx. In 2 rats with laryngeal erosion/ulceration and inflammation, there was also hyperplasia of the laryngeal epithelium. In the nasal passages, chemical-related lesions were present in the olfactory and respiratory regions, primarily in Levels I and II of the anterior and mid portions of the nasal passages. Degeneration of the olfactory epithelium was generally limited to the 160 mg/$m^3$ exposure animals. The olfactory epithelium in the dorsal meatus of Level II was more commonly affected, but in some rats lesions were also present on the ethmoid turbinates of Level III. Degeneration was characterized by focal areas with thinning of the olfactory epithelial layer (Plate 2). This normally pseudostratified columnar epithelium was sometimes reduced to only 1 cell layer in thickness; in some areas a respiratory or nonkeratinizing squamous epithelium replaced the olfactory epithelium (metaplasia). Frequently a vacuolar change in the olfactory epithelium was a part of the degenerative lesion, and in the more severely affected areas there was degeneration of the underlying olfactory nerve bundles (Plate 3). Chemical-related lesions in the respiratory epithelium of the nasal passages included degeneration and focal erosion/ulceration of the mucosa in Levels I and II. Degeneration was characterized by loss of cilia and decreased height of the columnar epithelium; squamous metaplasia (nonkeratinizing) was present in 160 mg/$m^3$ exposure animals. In Levels I and II, the incidence of inflammation in the respiratory mucosa was increased slightly in the higher exposure groups but the severity (minimal to mild) was not dose related.

Administration of HDDC to rats by inhalation caused no changes in any of the sperm morphology or vaginal cytology parameters evaluated (Appendix C).

**TABLE 8 Incidence and Severity of Histopathologic Lesions in F344/N Rats in the 13-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride[1]**

| | HDDC ($mg/m^3$) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1.6 | 5.0 | 16 | 50 | 160 |
| **MALE** | | | | | | |
| **Larynx** | | | | | | |
| Inflammation | 1 (2.0) | 0 | 0 | 0 | 2 (1.0) | 7 (1.4) |
| Erosion/Ulcer | 0 | 0 | 0 | 0 | 0 | 2 (2.0) |
| Hyperplasia | 0 | 0 | 0 | 0 | 0 | 1 (1.0) |
| **Nose/Nasal Passages** | | | | | | |
| Respiratory Epithelium | | | | | | |
| Degeneration | 0 | 0 | 0 | 0 | 3 (1.0) | 10 (2.0) |
| Erosion/Ulcer | 0 | 0 | 0 | 0 | 0 | 2 (1.0) |
| Inflammation | 2 (1.0) | 0 | 0 | 2 (1.0) | 2 (1.0) | 5 (1.4) |
| Squamous metaplasia | 0 | 0 | 0 | 0 | 0 | 4 (1.2) |
| Olfactory epithelium | | | | | | |
| Degeneration | 0 | 0 | 0 | 0 | 1 (1.0) | 10 (2.1) |
| Erosion/Ulcer | 0 | 0 | 0 | 0 | 0 | 0 |
| Inflammation | 0 | 0 | 0 | 0 | 0 | 1 (1.0) |
| **FEMALE** | | | | | | |
| **Larynx** | | | | | | |
| Inflammation | 2 (2.0) | 0 | 0 | 0 | 0 | 5 (2.6) |
| Erosion/Ulcer | 0 | 0 | 0 | 0 | 0 | 4 (2.0) |
| Hyperplasia | 0 | 0 | 0 | 0 | 0 | 1 (3.0) |
| **Nose/Nasal Passages** | | | | | | |
| Respiratory Epithelium | | | | | | |
| Degeneration | 0 | 0 | 0 | 1 (1.0) | 4 (1.2) | 8 (1.8) |
| Erosion/Ulcer | 0 | 0 | 0 | 0 | 1 (1.0) | 4 (1.5) |
| Inflammation | 4 (1.5) | 0 | 0 | 6 (1.7) | 8 (1.5) | 8 (1.6) |
| Squamous metaplasia | 0 | 0 | 0 | 0 | 1 (1.0) | 4 (1.0) |
| Olfactory epithelium | | | | | | |
| Degeneration | 1 (1.0) | 0 | 0 | 0 | 0 | 9 (2.2) |
| Erosion/Ulcer | 0 | 0 | 0 | 0 | 0 | 0 |
| Inflammation | 1 (1.0) | 0 | 0 | 0 | 0 | 0 |

[1] n=10 for all groups. The average severity score ( ) was based on the number of animals with lesions from each group; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

## 2-Week Inhalation Study in B6C3F$_1$ Mice

Survival and mean body weight changes of male and female mice exposed to HDDC by inhalation for 2 weeks are shown in Table 9. Body weights of all male and female mice exposed to 800mg/m$^3$ of HDDC decreased during the course of the study; in addition, all female mice and 2 male mice at this exposure level died before completion of the study. Although the mean body weight recorded for female mice in the 10 mg/m$^3$ exposure group was notably lower than that of controls on Day 8, the mean body weight of this group was similar to that of the control group on the last day of the study. Female mice exposed at 30, 89, or 267 mg/m$^3$ and male mice exposed at 30 or 800 mg/m$^3$ showed a slight depression in body weight gain relative to controls.

**TABLE 9 Survival and Weight Gain of B6C3F$_1$ Mice in the 2-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride**

| HDDC (mg/m$^3$) | Survival[1] | Mean Body Weight (grams) | | | | Final Weight Relative to Controls (%)[3] |
|---|---|---|---|---|---|---|
| | | Initial | Day 8 | Day 17 | Change[2] | |
| **MALE** | | | | | | |
| 0 | 5/5 | 21.2 | 21.4 | 25.1 | 3.9 | |
| 10 | 5/5 | 21.1 | 21.6 | 24.9 | 3.8 | 99 |
| 30 | 5/5 | 21.2 | 22.0 | 24.3 | 3.1 | 97 |
| 89 | 5/5 | 21.3 | 23.4 | 26.2 | 4.9 | 104 |
| 267 | 5/5 | 21.4 | 22.9 | 25.3 | 3.9 | 101 |
| 800 | 3/5[4] | 21.3 | 19.1[5] | 18.4[6] | -2.9 | 73 |
| **FEMALE** | | | | | | |
| 0 | 5/5 | 17.7 | 18.1 | 21.3 | 3.6 | |
| 10 | 5/5 | 17.6 | 16.0 | 22.1 | 4.5 | 104 |
| 30 | 5/5 | 17.7 | 18.3 | 20.2 | 2.5 | 95 |
| 89 | 5/5 | 17.5 | 17.9 | 20.6 | 3.1 | 97 |
| 267 | 5/5 | 17.6 | 18.6 | 19.1 | 1.5 | 90 |
| 800 | 0/5[7] | 17.6 | 15.6 | — | — | — |

1 Number surviving at end of study/number of animals per group. For groups with no survivors, no final mean body weights or body weight changes are given.
2 Mean weight change.
3 (Exposure group mean/control group mean) x 100.
4 Day of death: 3,9.
5 n=4.
6 n=3.
7 Day of death: 9, 10, 10, 10, 13.

Clinical signs of HDDC toxicity in male and female mice were related to irritation of the upper respiratory tract and included dyspnea and nasal discharge. Other abnormal signs noted were typical of those seen in moribund animals. These were seen primarily in the highest exposure group (800 mg/$m^3$) and included ruffled fur, abnormal posture, hypoactivity, tremors, prostration, and decrease in body mass compared to controls. At necropsy, the only chemical-related gross finding was a small spleen in male and female mice exposed to the highest concentration of HDDC (800 mg/$m^3$).

Exposure-related changes in absolute and relative organ weights of mice in the 2-week inhalation study are shown in Table10. For surviving male mice in the highest exposure group (800 mg/$m^3$), absolute and relative liver weights were significantly reduced. There were no surviving females in the 800 mg/$m^3$ exposure group. Absolute liver weight of male mice in the 30 mg/$m^3$ exposure group and relative liver weights of male mice in the 30 and 10 mg/$m^3$ exposure groups also were reduced. Thymus weights of male and female mice were significantly reduced in the 800 mg/$m^3$ and 267 mg/$m^3$ exposure groups respectively. Relative lung weights were increased in male mice in the highest exposure group. No other statistically significant differences in absolute or relative organ weights were seen.

Microscopic lesions attributed to HDDC exposure were present in the nasal passages, larynx, testis, and trachea. In the larynx and trachea, focal areas of inflammation and necrosis with ulceration of the respiratory mucosa were present at the 2 highest exposure levels (267 and 800 mg/$m^3$). The nasal lesions included degeneration characterized by a thinning (atrophy) and necrosis of the olfactory and respiratory epithelium. This was sometimes associated with focal inflammation and ulceration. Mild testicular degeneration was present in 2 mice from the highest exposure group (800 mg/$m^3$); 1 of these mice died on Day 9 of the study. Other microscopic lesions were considered secondary to stress or moribund condition.

**TABLE 10 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios of B6C3F$_1$ Mice in the 2-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride[1]**

| | HDDC (mg/m$^3$) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 10 | 30 | 89 | 267 | 800 |
| **MALE** | | | | | | |
| Necropsy body wt (g) | 25.6 | 24.9 | 24.3 | 26.6 | 25.3 | 19.1 |
| Liver | | | | | | |
| Absolute (g) | 1.57 | 1.40 | 1.35* | 1.64 | 1.50 | 0.89** |
| Relative (mg/g) | 62.25 | 56.54* | 55.60** | 62.40 | 59.25 | 47.88** |
| Lung | | | | | | |
| Absolute (g) | 0.18 | 0.18 | 0.18 | 0.22 | 0.20 | 0.20 |
| Relative (mg/g) | 7.19 | 7.25 | 7.53 | 8.23 | 7.82 | 11.00** |
| Thymus | | | | | | |
| Absolute (g) | 0.04 | 0.05 | 0.06 | 0.04 | 0.04 | 0.01* |
| Relative (mg/g) | 1.57 | 2.12 | 2.48 | 1.65 | 1.57 | 0.61 |
| **FEMALE** | | | | | | |
| Necropsy body weight (g) | 21.3 | 22.1 | 20.2 | 20.6 | 19.1 | |
| Liver | | | | | | |
| Absolute (g) | 1.33 | 1.28 | 1.20 | 1.26 | 1.19 | |
| Relative (mg/g) | 62.38 | 57.80 | 59.37 | 61.40 | 62.16 | |
| Lung | | | | | | |
| Absolute (g) | 0.19 | 0.19 | 0.17 | 0.17 | 0.18 | |
| Relative (mg/g) | 8.98 | 8.41 | 8.61 | 8.28 | 9.35 | |
| Thymus | | | | | | |
| Absolute (g) | 0.07 | 0.07 | 0.06 | 0.06 | 0.04** | |
| Relative (mg/g) | 3.38 | 3.12 | 2.96 | 2.87 | 1.87 | |

[1] n=5 for all groups except the 800 mg/m$^3$ exposure group. For male mice in the 800 mg/m$^3$ exposure group, n=3; for female mice in this exposure group, n=0. Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

[2] No data; all animals in this exposure group died before scheduled termination.

* Significantly different (P≤0.05) from the control group by Dunnett's test.

** Significantly different (P≤0.01) from the control group by Dunnett's test.

## 13-Week Inhalation Study in B6C3F$_1$ Mice

The survival and mean body weights of base-study mice exposed to HDDC by inhalation for 13 weeks are shown in Table 11 and Figure 2. All mice survived to the end of the study, and there were no exposure-related changes in body weight.

**TABLE 11 Survival and Weight Gain of B6C3F$_1$ Mice in the 13-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride**

| HDDC (mg/m$^3$) | Survival[1] | Mean Body Weight (grams) Initial | Final | Change[2] | Final Weight Relative to Controls (%)[3] |
|---|---|---|---|---|---|
| **MALE** | | | | | |
| 0 | 10/10 | 24.4 | 32.1 | 7.7 | |
| 1.6 | 10/10 | 20.6 | 33.1 | 12.6 | 103 |
| 5 | 10/10 | 24.7 | 31.8 | 7.1 | 99 |
| 16 | 10/10 | 23.6 | 31.3 | 7.7 | 97 |
| 50 | 10/10 | 24.8 | 32.9 | 8.1 | 102 |
| 160 | 10/10 | 23.8 | 32.0 | 8.1 | 100 |
| **FEMALE** | | | | | |
| 0 | 10/10 | 19.5 | 26.7 | 7.1 | |
| 1.6 | 10/10 | 17.9 | 26.2 | 8.4 | 98 |
| 5 | 10/10 | 19.9 | 26.8 | 6.9 | 100 |
| 16 | 10/10 | 19.4 | 26.0 | 6.6 | 98 |
| 50 | 10/10 | 19.8 | 27.6 | 7.9 | 104 |
| 160 | 10/10 | 19.4 | 26.8 | 7.4 | 101 |

1 Number of animals surviving at end of study/number of animals per exposure group.
2 Mean weight change.
3 (Exposure group mean/control group mean) x 100.

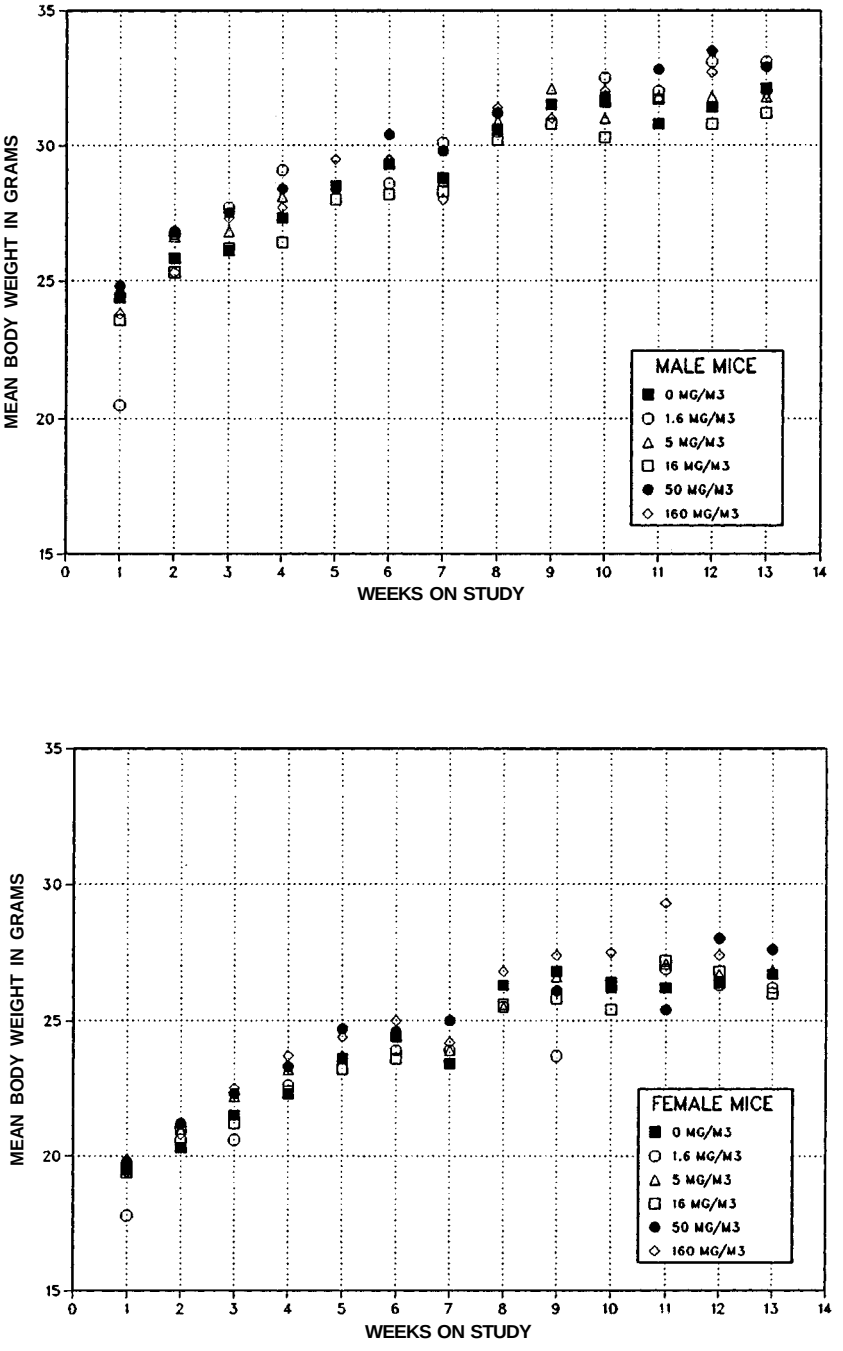


**FIGURE 2 Body Weights of B6C3F$_1$ Mice Exposed to 1,6-Hexanediamine Dihydrochloride by Inhalation for 13 Weeks**

Changes in organ weights and organ-weight-to-body-weight ratios in mice treated with HDDC for 13 weeks are shown in Table 12. A statistically significant increase occurred in the absolute and relative lung weights of female mice in the highest exposure group (160mg/$m^3$). Absolute and relative liver weights were significantly increased in male mice in the 2 highest exposure groups (50 and 160 mg/$m^3$); liver-weight-to-body-weight ratios were also increased in male mice in the 5 and 16 mg/$m^3$ exposure groups. Other changes in organ weights were not considered to be specifically related to HDDC toxicity (AppendixA).

**TABLE 12 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios of B6C3F$_1$ Mice in the 13-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride[1]**

| | HDDC (mg/$m^3$) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1.6 | 5 | 16 | 50 | 160 * |
| **MALE** | | | | | | |
| Necropsy body weight (g) | 32.4 | 33.1 | 32.0 | 31.6 | 33.1 | 32.5 |
| Liver | | | | | | |
| Absolute (g) | 1.61 | 1.73 | 1.69 | 1.64 | 1.80** | 1.80** |
| Relative (mg/g) | 49.75 | 52.27 | 52.91* | 52.04* | 54.37** | 55.33** |
| Lung | | | | | | |
| Absolute (g) | 0.27 | 0.27 | 0.25 | 0.27 | 0.28 | 0.28 |
| Relative (mg/g) | 8.45 | 8.24 | 7.85 | 8.49 | 8.62 | 8.58 |
| **FEMALE** | | | | | | |
| Necropsy body weight (g) | 26.9 | 27.5 | 28.1 | 27.0 | 27.8 | 27.6 |
| Liver | | | | | | |
| Absolute (g) | 1.34 | 1.40 | 1.50 | 1.41 | 1.45 | 1.44 |
| Relative (mg/g) | 50.05 | 51.07 | 53.33 | 52.14 | 52.20 | 52.28 |
| Lung | | | | | | |
| Absolute (g) | 0.24 | 0.25 | 0.24 | 0.26 | 0.26 | 0.29** |
| Relative (mg/g) | 8.98 | 9.06 | 8.64 | 9.55 | 9.31 | 10.30** |

[1] n=10. Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test.

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test.

No exposure-related clinical signs were observed in male or female mice. In addition, no chemical-related gross lesions were seen at necropsy.

Exposure-related microscopic lesions were limited to the upper respiratory tract (larynx and nasal passages) of male and female mice in the 2 highest exposure groups (50 and 160mg/$m^3$). The morphology, incidence, and severity of microscopic lesions were similar for males and females; there was a dose-related increase in the incidence and severity of these lesions (Table 13).

**TABLE 13 Incidence and Severity of Histopathologic Lesions in B6C3F$_1$ Mice in the 13-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride[1]**

| | HDDC (mg/$m^3$) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1.6 | 5.0 | 16 | 50 | 160 |
| **MALE** | | | | | | |
| **Larynx** | | | | | | |
| Inflammation | 5 (1.0) | 0 | 0 | 2 (1.0) | 4 (1.0) | 3 (1.0) |
| Erosion/Ulcer | 0 | 0 | 0 | 0 | 0 | 4 (1.0) |
| Hyperplasia | 0 | 0 | 0 | 0 | 0 | 1 (1.0) |
| **Nose/Nasal Passages** | | | | | | |
| Respiratory epithelium | | | | | | |
| Hyaline degeneration | 0 | 0 | 0 | 1 (1.0) | 8 (1.0) | 10 (1.8) |
| Erosion/Ulcer | 0 | 0 | 0 | 0 | 1 (1.0) | 6 (1.3) |
| Inflammation | 0 | 0 | 0 | 0 | 0 | 3 (1.0) |
| Olfactory epithelium | | | | | | |
| Hyaline degeneration | 0 | 0 | 0 | 2 (1.0) | 8 (1.0) | 10 (2.3) |
| Inflammation | 0 | 0 | 0 | 0 | 0 | 3 (1.7) |
| **FEMALE** | | | | | | |
| **Larynx** | | | | | | |
| Inflammation | 2 (1.0) | 3 (1.0) | 5 (1.0) | 9 (1.2) | 3 (1.0) | 2 (1.0) |
| Erosion/Ulcer | 0 | 0 | 0 | 0 | 0 | 3 (1.7) |
| Necrosis | 0 | 0 | 0 | 0 | 0 | 4 (1.5) |
| **Nose/Nasal Passages** | | | | | | |
| Respiratory epithelium | | | | | | |
| Hyaline degeneration | 0 | 0 | 0 | 0 | 10 (1.0) | 10 (2.0) |
| Erosion/Ulcer | 0 | 0 | 0 | 0 | 0 | 4 (1.2) |
| Inflammation | 0 | 0 | 0 | 0 | 0 | 2 (1.0) |
| Olfactory epithelium | | | | | | |
| Hyaline degeneration | 0 | 0 | 0 | 1 (2.0) | 10 (1.0) | 10 (2.1) |
| Inflammation | 0 | 0 | 0 | 0 | 0 | 2 (1.0) |

[1] n=10 for all groups. The average severity score ( ) was based on the number of animals with lesions from each group; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Necrosis of the laryngeal epithelium and minimal to mild focal erosion/ulceration were present in the larynx of mice exposed to 160 mg/m$^3$. Focal necrosis or hyperplasia of the adjacent epithelium was associated with some erosions. In the nasal passages, exposure-related lesions were present in the respiratory and olfactory regions, primarily in Levels II and III of the mid-portion of the nasal passages. In the respiratory epithelium, the primary change was hyaline degeneration characterized by the accumulation of an eosinophilic proteinaceous material. Minimal to mild focal areas of erosion/ulceration and inflammation were present in the 160mg/m$^3$ exposure group (Plate 4). Hyaline degeneration of the olfactory epithelium was generally limited to the 50 and 160 mg/m$^3$ exposure levels. This was characterized by the accumulation of eosinophilic proteinaceous material in the olfactory sustentacular cells and resulted in a loss of olfactory sensory cells (Plates 5 and 6).

Administration of HDDC to mice by inhalation caused no changes in the sperm morphology parameters evaluated (Table C3), with the exception of an increase in sperm motility in the 16 and 160 mg/m$^3$ exposure groups. However, this change was not dose related, and the values for sperm motility were all well within the range for historical controls for NTP studies. Consequently, the increase in sperm motility was not interpreted as an adverse effect.

## Mating Trials

The results of mating trials conducted on rats and mice exposed to HDDC by inhalation for 13 weeks appear in Appendix C. In rats, HDDC demonstrated no reproductive toxicity. There was no effect on male or female fertility, body weights or body weight gains, gestation length, litter size, neonatal survival, pup weights, sex ratios of pups, or pup morphology in rats exposed to HDDC. Similarly, reproductive effects of HDDC on mice were minimal. There was no effect on male or female body weights or body weight gains, and no effect on male or female fertility. Three female mice exposed to 16 mg/m$^3$ and 1female and 1 male mouse exposed to 50 mg/m$^3$ died before scheduled termination; however, these deaths were not considered compound related. A statistically significant increase in the mean gestation length of mice in the 50 and 160 mg/m$^3$ exposure groups was noted; however, in the absence of other reproductive toxicity, this effect was not considered biologically significant. HDDC had no effect on litter size, neonatal survival, sex ratio of pups, or pup morphology in mice. Pups in the 160 mg/m$^3$ exposure group had mean weights similar to that of controls at birth and on lactation Day 5; however, mean

weights for pups in this exposure group were lower than that of controls on lactation Days 14 and21.

## Genetic Toxicity Studies

1,6-Hexanediamine (33 to 10,000 µg/plate) was tested in 2 independent studies for induction of mutations in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98, with and without Aroclor 1254-induced male Sprague-Dawley rat and Syrian hamster liver S9 (Mortelmans *et al.*, 1986; Table D1). In the first study, distilled water was the solvent for 1,6-hexanediamine; in the second study, dimethylsulfoxide was used as the solvent. The chemical was toxic at doses of 3333 µg/plate and greater in both studies. No increases in mutations were observed in any of the 4 *S. typhimurium* tester strains after incubation with 1,6-hexanediamine.

In cytogenetics tests with cultured Chinese hamster ovary (CHO) cells, no significant increases in sister chromatid exchanges or chromosomal aberrations (Abs) were observed after exposure to 1,6-hexanediamine, with or without S9 (Tables D2 and D3). In the Abs test with S9, both trials showed an increase in total aberrations at the highest concentration tested (500 µg/ml). In both of these trials, however, these aberrations were concentrated in fewer than 5% of the total cells scored. Hence, the percentage of cells with aberrations (the endpoint that is evaluated in this test) was not sufficiently elevated for a positive response.

The frequency of micronucleated erythrocytes was measured in peripheral blood smears of male and female mice exposed to 1,6-hexanediamine dihydrochloride in the 13-week inhalation studies (Table D4). No significant increases were seen in the frequencies of micronucleated normochromatic erythrocytes (NCEs) or polychromatic erythrocytes (PCEs) in male or female mice. The percentage of PCEs among the total erythrocyte population was increased at the highest exposure levels for male and female mice.

**PLATE 1**

Focal ulceration (arrows) of laryngeal epithelium in a male rat exposed to 1,6-hexanediamine dihydrochloride at a concentration of 160 mg/$m^3$. Mild inflammation is present in the underlying mucosa with inflammatory exudate in the lumen (L) of the larynx. 90x.

**PLATE 2**

Degeneration of olfactory mucosa in the dorsal meatus of the nasal passage in a male rat exposed to 1,6-hexanediamine dihydrochloride at a concentration of 160mg/$m^3$. There is thinning of the olfactory epithelium (arrow) with the focal accumulation of a serum exudate and a few inflammatory cells on the mucosal surface. 160x.

**PLATE 3**

Degeneration of the olfactory mucosa in a female rat exposed to 1,6-hexane-diamine dihydrochloride at a concen-tration of 160 mg/$m^3$. Compared to the less affected mucosa on the left, there is marked thinning of the epithelial layer (arrow). On the opposite side of the turbinate there is a vacuolar degener-ation and loss of olfactory epithelium. Note the degeneration of the nerve fiber bundles (N) in the underlying mucosa. 160x.

**PLATE 4**

Focal ulceration (arrows) of the nasal respiratory epithelium in a female mouse exposed to 1,6-hexanediamine dihydro-chloride at a concentration of 160mg/$m^3$. A mild inflammatory infiltrate is present in the underlying mucosa. 400x.

**PLATE 5**

Olfactory mucosa from a control male mouse for comparison with Plate 6, in which the mouse was exposed to 1,6-hexanediamine dihydrochloride. 160x.

**PLATE 6**

Hyaline degeneration in the olfactory mucosa of a male mouse exposed to 1,6-hexanediamine dihydrochloride at a concentration of 160 mg/$m^3$. Eosinophilic protein droplets (arrows) distort the normal nuclear arrangement of the olfactory layer. 160x.

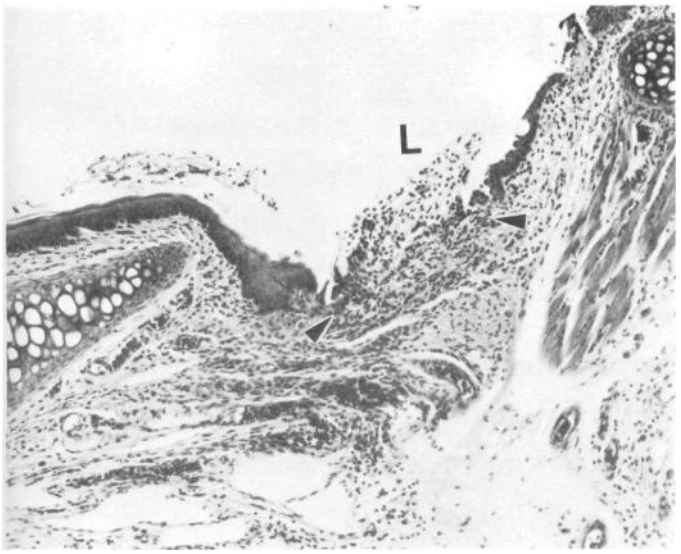


PLATE 1

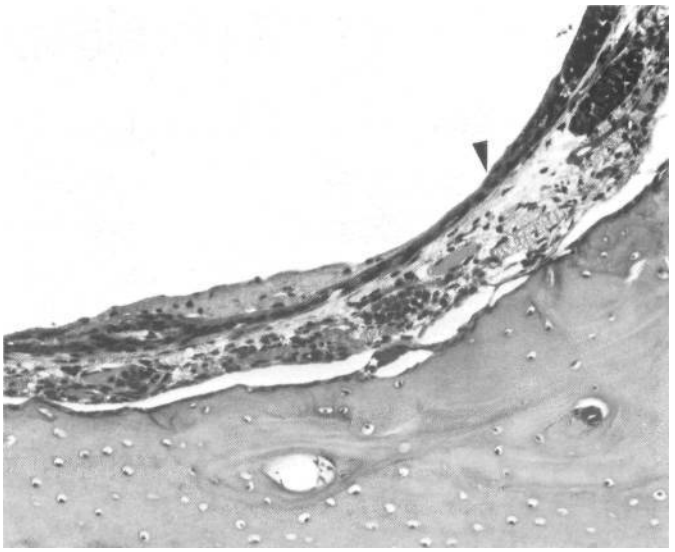

PLATE 2

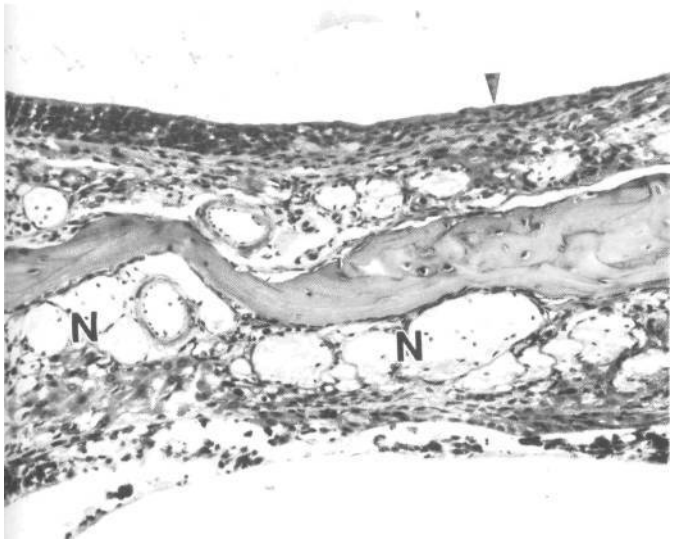


PLATE 3

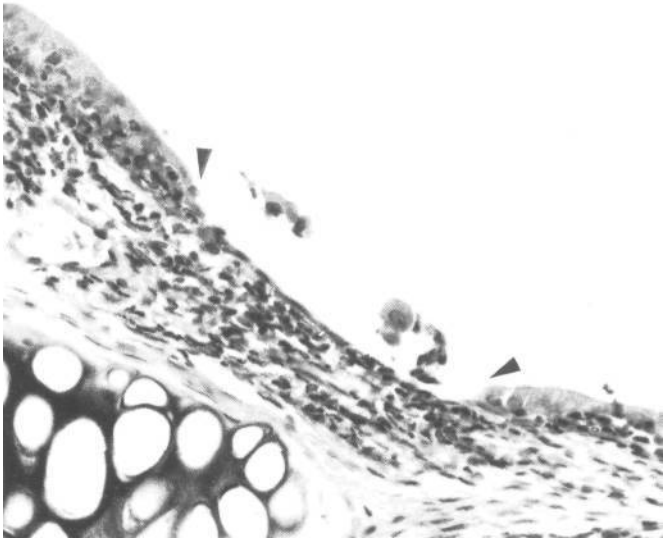

PLATE 4

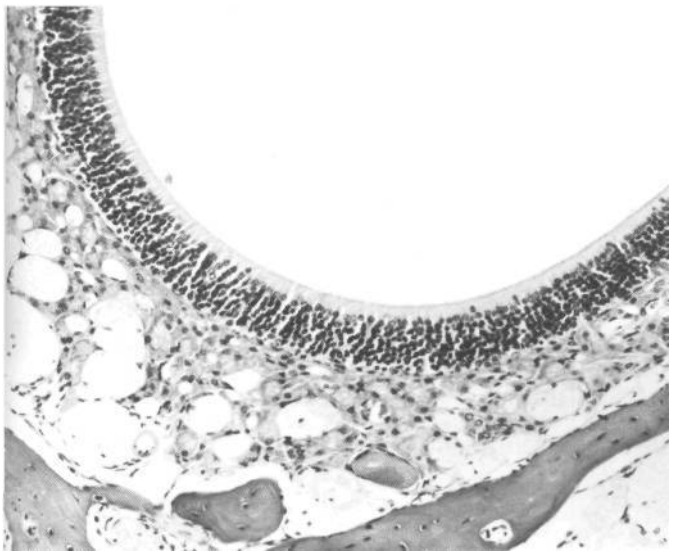

PLATE 5

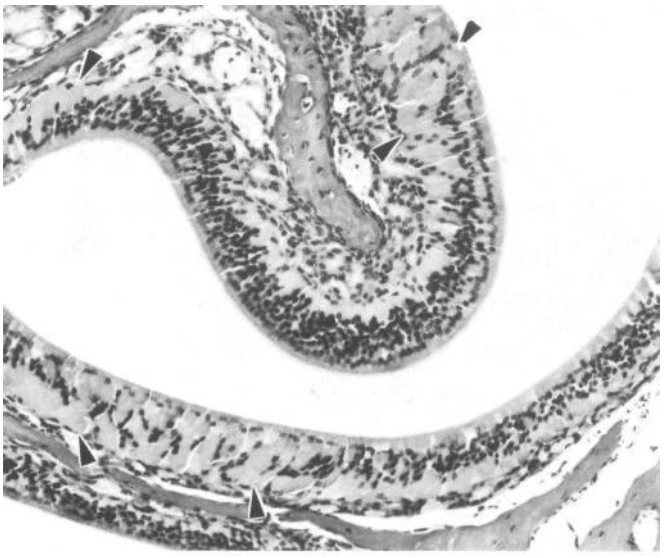

PLATE 6

# DISCUSSION

The toxic effects of 1,6-hexanediamine dihydrochloride (HDDC) in F344/N rats and B6C3F$_1$ mice after inhalation exposure were restricted almost exclusively to the upper airways and resulted from the strong irritant properties of the compound. At HDDC concentrations of 16 mg/m$^3$ and greater, inflammation and necrosis were present in the nasal and laryngeal epithelium. These lesions were sometimes accompanied by ulceration and compensatory hyperplasia or metaplasia of the epithelial lining. The distribution of lesions was similar in rats and mice. Aside from the respiratory tract lesions described above, no specific target organ for HDDC toxicity was identified in these studies.

In the 2-week drinking water studies, no changes in survival or body weight, no gross or microscopic pathologic changes, and no clinical abnormalities related to HDDC consumption were seen. At the highest HDDC concentrations, water intake was reduced due to the poor palatability of the dosed water. Because no significant toxic effects were noted in these studies and because higher concentrations of HDDC would probably have caused ill effects resulting from decreased water consumption, further drinking water studies were not conducted.

In the 2-week inhalation studies, ulceration, necrosis, and inflammation were present in the larynx and nasal passages of rats and mice; both respiratory and olfactory epithelium in the nasal mucosa were affected. Because of mortality and clinical signs of respiratory distress in the highest exposure group and severity of respiratory tract lesions in animals administered HDDC at levels of 89 mg/m$^3$ and greater, an upper exposure concentration of 160 mg/m$^3$ was selected for the 13-week studies in each species. Following 13 weeks of exposure to HDDC, essentially the same lesions seen in the 2-week studies were present in the larynx and nose/nasal passages of each species.

Almost all treatment-related lesions in male and female rats in the 13-week study were limited to the 160 mg/m$^3$ exposure group. No apparent sex-related differences in the incidence, severity, or character of these lesions were observed. The site-specific and morphological effects of HDDC on the upper respiratory tract (larynx and nasal cavity) were consistent with those produced by other irritant chemicals administered by inhalation (Giddens and Fairchild, 1972; Jiang *et al.*, 1983; Buckley *et al.*, 1984; Boorman *et al.*, 1987; Morgan and Monticello, 1990; NTP, 1991b). In a report on the toxicity of

HDDC in rats treated by inhalation for 13 weeks (Johannsen *et al.*, 1987), the authors described lesions similar to those seen in the present NTP study. Inflammation and squamous metaplasia of the nasal respiratory epithelium, trachea, and lungs were seen. These lesions were generally more severe than those seen in the present study and occurred only at the highest HDDC concentration tested (215 mg/$m^3$). No gross or microscopic lesions were seen in rats administered 12.8 or 51 mg/$m^3$ HDDC. High mortality in the 215 mg/$m^3$ exposure group of the Johannsen study resulted in discontinuation of this group after 7weeks of exposure. As in the NTP study, the cause of early mortality in the highest exposure group of the Johannsen study could not be determined.

In mice, the character of the nasal lesions was slightly different than in rats. At the highest exposure concentrations in the 2-week study, laryngeal lesions and degeneration with necrosis and ulceration of the respiratory and olfactory epithelium resembled those in rats. However, in the 13-week study, inflammation was less prominent in mice and degenerative changes in the olfactory and respiratory epithelium were characterized by the accumulation of an eosinophilic hyaline material in the cytoplasm of respiratory and olfactory cells. These intracytoplasmic hyaline droplets, believed to represent a proteinaceous secretory material, have been described in both treated and control rats and mice (Monticello *et al.*, 1990). The amount and distribution of these droplets are often increased in chemical-exposed animals, and this change has been described as an adaptive response to certain toxicants (Buckley *et al.*, 1985; Monticello *et al.*, 1990). In mice, the olfactory mucosa in Level III was frequently affected by this hyaline degeneration, while in rats the olfactory degeneration occurred primarily in the dorsal meatus of LevelII. Mice appeared to be somewhat more sensitive to the irritant properties of HDDC; degenerative lesions in mice generally occurred at lower doses and with higher incidence than in rats. The severity of lesions, however, was similar in both species. Similar results have been seen in previous NTP studies (NTP, 1991b).

No chemical-related lesions were seen in the lungs of rats or mice exposed to HDDC. The absolute and relative lung weights of HDDC-exposed rats in this study appeared to be reduced. However, the lung weights of exposed animals were well within the normal range, and the apparent reduction is considered to be an artifact resulting from the greater lung weights of the controls. Inflammation was present in the lungs of control male and female rats, resulting in increased lung weights in control rats compared to historical controls

(NTP, 1990a). This inflammation was identical to that observed in several previous inhalation studies and is of unknown etiology (NTP, 1990b, 1991b). Such inflammatory lesions have not been seen in mice.

Overall, hematologic changes in rats were minor, sporadic, and not accompanied by related clinical pathology findings. At the end of the study, female rats showed an exposure-related decrease in lymphocyte and segmented neutrophil counts, which contributed to an overall decrease in leukocyte count. In the highest exposure group (160 mg/m$^3$), leukocytes decreased approximately 29%, lymphocytes 26%, and segmented neutrophils44%. These findings differ from those of previous inhalation studies with 1,6-hexanediamine (HDA), in which leukocyte numbers either increased (Kulakov, 1965; Tkachenko, 1976) or showed no change (Izrailet and Laivina, 1980; Johannsen *et al.*, 1987). In the studies showing increases in leukocyte count, exposure concentrations were considerably lower (1.25 mg/m$^3$ maximum) than the concentrations used in the present study; the concentrations used by Johannsen *et al.*, on the other hand, were in the same range as those used in the present study. In male rats, no changes in the leukocyte parameters were noted at study termination; however, there was a decrease in the segmented neutrophil count in males in the highest exposure group at Day 18. Although no mechanism for the observed leukocyte changes was evident, decreases can result from a number of causes, including decreased or ineffective production (bone marrow or lymphoid), increased margination to tissues or the marginal pool, decreased release to the circulation, or increased destruction. The observed decrease in circulating leukocytes is consistent with the margination of cells during the inflammatory changes in the larynx and nasal passages. In addition, HDA and HDDC have been shown to inhibit the *in vitro* proliferation of mouse lymphocytes (Luebke *et al.*, 1989). This inhibition is at least partly due to inhibition of ornithine decarboxylase activity. Although no microscopic evidence of bone marrow toxicity was seen in the present study, it is possible that such activity may be responsible for the observed decreases in leukocyte numbers.

On Day 18, but not at study termination, slight increases in serum urea nitrogen concentrations were noted in male and female rats. Urea nitrogen and creatinine are used as indicators of renal dysfunction. In this study, creatinine levels were unchanged and there was no histologic evidence of nephropathy. The urea nitrogen concentration can be increased by several nonrenal causes (*e.g.*, dehydration, processes causing increased protein catabolism, and diet), and it is likely that the change in urea nitrogen

concentration was related to 1 or more of these causes. Slight increases in urea nitrogen concentration, which were also noted in the highest exposure group of the study by Johannsen *et al.* (1987), were attributed to a marked decrease in food intake with a resulting increase in endogenous protein catabolism.

In summary, under the exposure conditions employed in these studies, inhalation of HDDC by rats and mice produced lesions that could be attributed to the irritant effects of the compound. The observed NOAEL for respiratory damage was 5 mg/$m^3$ for rats and mice. No significant systemic toxicity was observed, and no specific target organs were identified. HDDC and HDA were not genotoxic, and inhalation of HDDC did not cause any adverse reproductive effects.

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# APPENDIX A

# Organ Weights and Organ-Weight-to-Body-Weight Ratios

## TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride[1]

| | 0 mg/m$^3$ | 1.6mg/m$^3$ | 5mg/m$^3$ | 16mg/m$^3$ | 50mg/m$^3$ | 160mg/m$^3$ |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Necropsy body wt | 338 ± 8 | 324 ± 8 | 323 ± 8 | 329 ± 4 | 326 ± 6 | 316 ± 8* |
| Brain | | | | | | |
| Absolute | 2.132 ± 0.174 | 1.943 ± 0.020 | 1.913 ± 0.015 | 1.933 ± 0.015[2] | 1.950 ± 0.014 | 1.974 ± 0.016 |
| Relative | 6.34 ± 0.55 | 6.01 ± 0.10 | 5.95 ± 0.11 | 5.89 ± 0.08[2] | 6.00 ± 0.10 | 6.28 ± 0.15 |
| Heart | | | | | | |
| Absolute | 1.061 ± 0.040 | 1.021 ± 0.029 | 1.037 ± 0.029 | 1.053 ± 0.017 | 1.065 ± 0.021 | 1.116 ± 0.050 |
| Relative | 3.14 ± 0.07 | 3.15 ± 0.06 | 3.22 ± 0.07 | 3.21 ± 0.06 | 3.27 ± 0.06 | 3.53 ± 0.13** |
| Right kidney | | | | | | |
| Absolute | 1.275 ± 0.034 | 1.236 ± 0.031 | 1.275 ± 0.076 | 1.238 ± 0.019 | 1.245 ± 0.027 | 1.242 ± 0.034 |
| Relative | 3.77 ± 0.06 | 3.81 ± 0.05 | 3.97 ± 0.26 | 3.77 ± 0.04 | 3.82 ± 0.03 | 3.93 ± 0.05 |
| Liver | | | | | | |
| Absolute | 12.010 ± 0.563 | 10.852 ± 0.338 | 11.057 ± 0.430 | 11.622 ± 0.310 | 11.143 ± 0.353 | 11.278 ± 0.329 |
| Relative | 35.47 ± 1.18 | 33.45 ± 0.64 | 34.23 ± 0.76 | 35.41 ± 0.95 | 34.21 ± 0.77 | 35.74 ± 0.94 |
| Lungs | | | | | | |
| Absolute | 2.162 ± 0.071 | 1.979 ± 0.093* | 1.709 ± 0.025** | 1.953 ± 0.023** | 1.764 ± 0.046** | 1.807 ± 0.061** |
| Relative | 6.39 ± 0.13 | 6.09 ± 0.21 | 5.32 ± 0.10** | 5.95 ± 0.06** | 5.42 ± 0.10** | 5.71 ± 0.12** |
| Epididymis | | | | | | |
| Absolute | 0.453 ± 0.007 | 0.524 ± 0.013 | 0.506 ± 0.021 | 0.457 ± 0.011 | 0.451 ± 0.007 | 0.461 ± 0.010 |
| Relative | 1.35 ± 0.03 | 1.62 ± 0.04** | 1.57 ± 0.04** | 1.39 ± 0.03 | 1.39 ± 0.02 | 1.46 ± 0.04 |
| Right testis | | | | | | |
| Absolute | 1.459 ± 0.021 | 1.436 ± 0.024 | 1.450 ± 0.024 | 1.473 ± 0.018 | 1.456 ± 0.017 | 1.479 ± 0.033 |
| Relative | 4.33 ± 0.09 | 4.44 ± 0.09 | 4.51 ± 0.06 | 4.48 ± 0.05 | 4.49 ± 0.10 | 4.70 ± 0.12** |
| Thymus | | | | | | |
| Absolute | 0.364 ± 0.017 | 0.353 ± 0.014 | 0.335 ± 0.016 | 0.324 ± 0.011 | 0.344 ± 0.018 | 0.337 ± 0.012 |
| Relative | 1.08 ± 0.04 | 1.09 ± 0.05 | 1.03 ± 0.03 | 0.99 ± 0.03 | 1.06 ± 0.06 | 1.07 ± 0.04 |
| **Female** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Necropsy body wt | 193 ± 3 | 182 ± 4 | 190 ± 3 | 186 ± 3 | 190 ± 3 | 189 ± 4 |
| Brain | | | | | | |
| Absolute | 1.800 ± 0.017 | 1.782 ± 0.019 | 1.821 ± 0.021 | 1.778 ± 0.021 | 1.790 ± 0.012 | 1.803 ± 0.015 |
| Relative | 9.36 ± 0.13 | 9.81 ± 0.17 | 9.60 ± 0.17 | 9.59 ± 0.15 | 9.45 ± 0.16 | 9.56 ± 0.19 |
| Heart | | | | | | |
| Absolute | 0.723 ± 0.016 | 0.708 ± 0.025 | 0.733 ± 0.021 | 0.689 ± 0.013 | 0.705 ± 0.012 | 0.715 ± 0.016 |
| Relative | 3.77 ± 0.11 | 3.88 ± 0.08 | 3.86 ± 0.11 | 3.71 ± 0.06 | 3.72 ± 0.10 | 3.79 ± 0.07 |
| Right kidney | | | | | | |
| Absolute | 0.804 ± 0.014 | 0.744 ± 0.016* | 0.788 ± 0.020 | 0.773 ± 0.013 | 0.788 ± 0.014 | 0.787 ± 0.016 |
| Relative | 4.18 ± 0.06 | 4.09 ± 0.04 | 4.14 ± 0.08 | 4.17 ± 0.07 | 4.16 ± 0.06 | 4.17 ± 0.09 |

**TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride (continued)**

| | | 0 mg/m$^3$ | 1.6mg/m$^3$ | 5mg/m$^3$ | 16mg/m$^3$ | 50mg/m$^3$ | 160mg/m$^3$ |
|---|---|---|---|---|---|---|---|
| **Female (continued)** | | | | | | | |
| Liver | | | | | | | |
| | Absolute | 6.770 ± 0.165 | 6.291 ± 0.229 | 6.757 ± 0.256 | 6.715 ± 0.138 | 6.589 ± 0.182 | 6.661 ± 0.161 |
| | Relative | 35.17 ± 0.68 | 34.54 ± 0.98 | 35.50 ± 1.11 | 36.25 ± 0.91 | 34.76 ± 1.02 | 35.29 ± 0.92 |
| Lungs | | | | | | | |
| | Absolute | 1.476 ± 0.039 | 1.314 ± 0.061* | 1.265 ± 0.032** | 1.302 ± 0.052* | 1.297 ± 0.041* | 1.402 ± 0.025 |
| | Relative | 7.66 ± 0.15 | 7.22 ± 0.29 | 6.66 ± 0.14** | 7.03 ± 0.30 | 6.83 ± 0.15* | 7.43 ± 0.19 |
| Thymus | | | | | | | |
| | Absolute | 0.292 ± 0.010 | 0.272 ± 0.013 | 0.301 ± 0.010 | 0.257 ± 0.011* | 0.250 ± 0.011* | 0.260 ± 0.012* |
| | Relative | 1.52 ± 0.06 | 1.49 ± 0.06 | 1.59 ± 0.06 | 1.38 ± 0.05 | 1.32 ± 0.06* | 1.37 ± 0.05* |

1 Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

2 n=9.

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test.

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test.

**TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride[1]**

| | 0 mg/m$^3$ | 1.6mg/m$^3$ | 5mg/m$^3$ | 16mg/m$^3$ | 50mg/m$^3$ | 160mg/m$^3$ |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Necropsy body wt | 32.4 ± 0.7 | 33.1 ± 1.0 | 32.0 ± 0.3 | 31.6 ± 0.5 | 33.1 ± 1.1 | 32.5 ± 0.7 |
| Brain | | | | | | |
| Absolute | 0.460 ± 0.006 | 0.461 ± 0.013 | 0.464 ± 0.007 | 0.449 ± 0.006 | 0.466 ± 0.005 | 0.472 ± 0.007 |
| Relative | 14.22 ± 0.30 | 13.95 ± 0.31 | 14.51 ± 0.21 | 14.25 ± 0.27 | 14.22 ± 0.47 | 14.55 ± 0.27 |
| Heart | | | | | | |
| Absolute | 0.178 ± 0.006 | 0.188 ± 0.010 | 0.168 ± 0.004 | 0.184 ± 0.006 | 0.184 ± 0.007 | 0.182 ± 0.011 |
| Relative | 5.51 ± 0.22 | 5.68 ± 0.25 | 5.25 ± 0.12 | 5.84 ± 0.18 | 5.59 ± 0.21 | 5.57 ± 0.24 |
| Right kidney | | | | | | |
| Absolute | 0.304 ± 0.007 | 0.353 ± 0.015 | 0.307 ± 0.004 | 0.324 ± 0.010 | 0.330 ± 0.015 | 0.342 ± 0.012* |
| Relative | 9.37 ± 0.17 | 10.62 ± 0.25 | 9.59 ± 0.11 | 10.23 ± 0.23 | 10.04 ± 0.49 | 10.50 ± 0.29** |
| Liver | | | | | | |
| Absolute | 1.612 ± 0.033 | 1.730 ± 0.054 | 1.694 ± 0.031 | 1.642 ± 0.032 | 1.800 ± 0.064** | 1.801 ± 0.057** |
| Relative | 49.75 ± 0.77 | 52.27 ± 1.07 | 52.91 ± 0.67* | 52.04 ± 0.91* | 54.37 ± 0.80** | 55.33 ± 1.14** |
| Lungs | | | | | | |
| Absolute | 0.272 ± 0.014 | 0.273 ± 0.010 | 0.251 ± 0.007 | 0.267 ± 0.009 | 0.283 ± 0.010 | 0.279 ± 0.016 |
| Relative | 8.45 ± 0.53 | 8.24 ± 0.19 | 7.85 ± 0.20 | 8.49 ± 0.33 | 8.62 ± 0.35 | 8.58 ± 0.44 |
| Epididymis | | | | | | |
| Absolute | 0.044 ± 0.002 | 0.053 ± 0.003* | 0.055 ± 0.002** | 0.043 ± 0.001 | 0.044 ± 0.001 | 0.043 ± 0.003 |
| Relative | 1.36 ± 0.06 | 1.58 ± 0.06 | 1.70 ± 0.05 | 1.38 ± 0.04 | 1.35 ± 0.04 | 1.31 ± 0.08 |
| Right testis | | | | | | |
| Absolute | 0.116 ± 0.003 | 0.115 ± 0.007 | 0.124 ± 0.003 | 0.111 ± 0.005 | 0.119 ± 0.002 | 0.120 ± 0.005 |
| Relative | 3.60 ± 0.10 | 3.45 ± 0.14 | 3.89 ± 0.07 | 3.51 ± 0.16 | 3.60 ± 0.08 | 3.68 ± 0.12 |
| Thymus | | | | | | |
| Absolute | 0.045 ± 0.003 | 0.044 ± 0.001 | 0.053 ± 0.004 | 0.048 ± 0.002 | 0.048 ± 0.002 | 0.050 ± 0.002 |
| Relative | 1.40 ± 0.08 | 1.33 ± 0.06 | 1.65 ± 0.12 | 1.51 ± 0.06 | 1.47 ± 0.07 | 1.54 ± 0.07 |
| **Female** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Necropsy body wt | 26.9 ± 0.7 | 27.5 ± 0.8 | 28.1 ± 0.7 | 27.0 ± 0.5 | 27.8 ± 0.9 | 27.6 ± 0.5 |
| Brain | | | | | | |
| Absolute | 0.463 ± 0.008 | 0.478 ± 0.008 | 0.464 ± 0.007 | 0.466 ± 0.006 | 0.474 ± 0.009 | 0.474 ± 0.009 |
| Relative | 17.33 ± 0.46 | 17.50 ± 0.45 | 16.57 ± 0.30 | 17.36 ± 0.45 | 17.15 ± 0.46 | 17.21 ± 0.42 |
| Heart | | | | | | |
| Absolute | 0.145 ± 0.005 | 0.152 ± 0.005 | 0.141 ± 0.003 | 0.144 ± 0.005 | 0.145 ± 0.004 | 0.152 ± 0.007 |
| Relative | 5.43 ± 0.17 | 5.54 ± 0.13 | 5.02 ± 0.13 | 5.36 ± 0.18 | 5.22 ± 0.13 | 5.49 ± 0.19 |
| Right kidney | | | | | | |
| Absolute | 0.199 ± 0.005 | 0.212 ± 0.007 | 0.205 ± 0.006 | 0.210 ± 0.006 | 0.212 ± 0.005 | 0.197 ± 0.004 |
| Relative | 7.43 ± 0.12 | 7.71 ± 0.17 | 7.30 ± 0.11 | 7.79 ± 0.17 | 7.66 ± 0.22 | 7.14 ± 0.12 |

**TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride (continued)**

| | | 0 mg/m$^3$ | 1.6mg/m$^3$ | 5mg/m$^3$ | 16mg/m$^3$ | 50mg/m$^3$ | 160mg/m$^3$ |
|---|---|---|---|---|---|---|---|
| **Female (continued)** | | | | | | | |
| Liver | | | | | | | |
| | Absolute | 1.342 ± 0.033 | 1.401 ± 0.037 | 1.500 ± 0.044 | 1.405 ± 0.031 | 1.450 ± 0.041 | 1.444 ± 0.041 |
| | Relative | 50.05 ± 1.22 | 51.07 ± 0.93 | 53.33 ± 0.40 | 52.14 ± 0.62 | 52.20 ± 0.87 | 52.28 ± 0.91 |
| Lungs | | | | | | | |
| | Absolute | 0.241 ± 0.012 | 0.248 ± 0.007 | 0.242 ± 0.009 | 0.257 ± 0.009 | 0.258 ± 0.007 | 0.285 ± 0.013** |
| | Relative | 8.98 ± 0.41 | 9.06 ± 0.24 | 8.64 ± 0.35 | 9.55 ± 0.31 | 9.31 ± 0.23 | 10.30 ± 0.41** |
| Thymus | | | | | | | |
| | Absolute | 0.053 ± 0.003 | 0.060 ± 0.003 | 0.061 ± 0.002 | 0.057 ± 0.002 | 0.064 ± 0.004* | 0.064 ± 0.004* |
| | Relative | 1.96 ± 0.08 | 2.19 ± 0.08 | 2.16 ± 0.06 | 2.12 ± 0.09 | 2.32 ± 0.17* | 2.33 ± 0.15* |

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test.

** Significantly different (P≤0.01) from the control group by Williams' or Dunnet's test.

# APPENDIX B

# Hematology and Clinical Chemistry Results

## TABLE B1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Studies of 1,6-Hexanediamine Dihydrochloride[1]

| Analysis | 0 mg/m$^3$ | 1.6 mg/m$^3$ | 5 mg/m$^3$ | 16 mg/m$^3$ | 50 mg/m$^3$ | 160 mg/m$^3$ |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| **Hematology** | | | | | | |
| Hematocrit (%) | | | | | | |
| Day 4 | 48.8 ± 0.7 | 47.8 ± 0.4 | 48.6 ± 0.7 | 49.2 ± 0.5 | 49.0 ± 0.5 | 48.1 ± 0.4 |
| Day 18 | 51.6 ± 0.3 | 52.3 ± 0.4[2] | 52.3 ± 0.6 | 52.1 ± 0.4 | 52.3 ± 0.4 | 52.6 ± 0.5 |
| Day 94 | 52.3 ± 0.6 | 51.3 ± 0.4 | 51.9 ± 0.6[2] | 50.0 ± 0.7 | 52.4 ± 0.5[2] | 51.4 ± 0.6 |
| Hemoglobin (g/dL) | | | | | | |
| Day 4 | 15.5 ± 0.2 | 15.3 ± 0.1 | 15.5 ± 0.2 | 15.6 ± 0.1 | 15.6 ± 0.1 | 15.3 ± 0.1 |
| Day 18 | 15.9 ± 0.1 | 16.2 ± 0.1[2] | 16.1 ± 0.2 | 16.1 ± 0.1 | 16.1 ± 0.1 | 16.2 ± 0.2 |
| Day 94 | 15.8 ± 0.2 | 15.5 ± 0.1 | 15.8 ± 0.2[2] | 15.2 ± 0.2 | 15.9 ± 0.1[2] | 15.7 ± 0.2 |
| Erythrocytes (10$^6$/µL) | | | | | | |
| Day 4 | 8.22 ± 0.16 | 8.05 ± 0.11 | 8.11 ± 0.16 | 8.22 ± 0.12 | 8.05 ± 0.12 | 8.07 ± 0.10 |
| Day 18 | 8.84 ± 0.09 | 8.94 ± 0.11[2] | 8.83 ± 0.16 | 8.84 ± 0.11 | 8.78 ± 0.11 | 8.93 ± 0.15 |
| Day 94 | 9.41 ± 0.10 | 9.24 ± 0.06 | 9.28 ± 0.12[2] | 8.94 ± 0.13* | 9.28 ± 0.09[2] | 9.26 ± 0.11 |
| Reticulocytes (10$^6$/µL) | | | | | | |
| Day 4 | 0.27 ± 0.02 | 0.25 ± 0.04 | 0.25 ± 0.03 | 0.22 ± 0.03 | 0.22 ± 0.02 | 0.23 ± 0.03 |
| Day 18 | 0.15 ± 0.02 | 0.13 ± 0.02[2] | 0.16 ± 0.01 | 0.15 ± 0.01 | 0.16 ± 0.02 | 0.14 ± 0.01 |
| Day 94 | 0.17 ± 0.02 | 0.16 ± 0.02 | 0.17 ± 0.02[2] | 0.19 ± 0.01[2] | 0.19 ± 0.01[2] | 0.16 ± 0.01 |
| Mean cell volume (fL) | | | | | | |
| Day 4 | 59.4 ± 0.8 | 59.4 ± 0.5 | 60.0 ± 0.6 | 60.0 ± 0.6 | 60.9 ± 0.8 | 59.6 ± 0.6 |
| Day 18 | 58.7 ± 0.6 | 58.4 ± 0.5[2] | 59.1 ± 0.7 | 59.1 ± 0.5 | 59.7 ± 0.6 | 58.9 ± 0.6 |
| Day 94 | 55.7 ± 0.2 | 55.6 ± 0.2 | 56.0 ± 0.2[2] | 55.9 ± 0.3 | 56.4 ± 0.2[2] | 55.5 ± 0.2 |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 4 | 18.8 ± 0.3 | 19.0 ± 0.2 | 19.1 ± 0.2 | 19.0 ± 0.2 | 19.4 ± 0.3 | 19.0 ± 0.2 |
| Day 18 | 18.0 ± 0.2 | 18.1 ± 0.1[2] | 18.2 ± 0.2 | 18.2 ± 0.2 | 18.3 ± 0.2 | 18.2 ± 0.2 |
| Day 94 | 16.8 ± 0.1 | 16.8 ± 0.1 | 17.0 ± 0.1[2] | 17.1 ± 0.1 | 17.1 ± 0.1*[2] | 17.0 ± 0.1 |
| Mean cell hemoglobin concentration (g/dL) | | | | | | |
| Day 4 | 31.7 ± 0.1 | 32.0 ± 0.2 | 31.8 ± 0.1 | 31.7 ± 0.1 | 31.8 ± 0.2 | 31.9 ± 0.1 |
| Day 18 | 30.9 ± 0.2 | 30.9 ± 0.2[2] | 30.7 ± 0.2 | 30.9 ± 0.2 | 30.7 ± 0.2 | 30.9 ± 0.2 |
| Day 94 | 30.2 ± 0.1 | 30.2 ± 0.1 | 30.5 ± 0.1[2] | 30.5 ± 0.2 | 30.3 ± 0.1[2] | 30.6 ± 0.2 |
| Nucleated erythrocytes (10$^3$/µL) | | | | | | |
| Day 4 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Day 18 | 0.01 ± 0.01 | 0.00 ± 0.00[2] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Day 94 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00[2] | 0.00 ± 0.00 | 0.00 ± 0.00[2] | 0.01 ± 0.01 |
| Methemoglobin (g/dL) | | | | | | |
| Day 4 | 0.04 ± 0.02 | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 |
| Day 18 | 0.04 ± 0.02 | 0.07 ± 0.02 | 0.13 ± 0.05 | 0.06 ± 0.04 | 0.02 ± 0.01 | 0.03 ± 0.01 |
| Day 94 | 0.10 ± 0.02 | 0.06 ± 0.02 | 0.12 ± 0.02[2] | 0.08 ± 0.02 | 0.07 ± 0.01[2] | 0.07 ± 0.02 |
| Platelets (10$^3$/µL) | | | | | | |
| Day 4 | 843.7 ± 20.2 | 851.5 ± 23.1 | 872.2 ± 20.8 | 839.1 ± 19.0 | 808.9 ± 18.8 | 770.2 ± 43.8 |
| Day 18 | 697.0 ± 13.4 | 690.4 ± 13.2[2] | 673.5 ± 19.9 | 686.7 ± 13.8 | 702.9 ± 16.3 | 681.8 ± 15.8 |
| Day 94 | 613.6 ± 30.1 | 573.0 ± 9.6 | 565.1 ± 11.3[2] | 613.1 ± 28.4[2] | 567.9 ± 19.4[2] | 586.5 ± 13.1 |
| Leukocytes (10$^3$/µL) | | | | | | |
| Day 4 | 6.36 ± 0.56 | 6.23 ± 0.63 | 6.44 ± 0.58 | 6.29 ± 0.74 | 6.79 ± 0.43 | 5.10 ± 0.41 |
| Day 18 | 4.88 ± 0.41 | 5.63 ± 0.59[2] | 4.57 ± 0.59 | 4.80 ± 0.47 | 4.99 ± 0.34 | 4.05 ± 0.35 |
| Day 94 | 8.63 ± 0.42 | 7.83 ± 0.29 | 8.79 ± 0.53[2] | 9.32 ± 0.60 | 9.18 ± 0.32[2] | 8.41 ± 0.50 |

**TABLE B1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Studies of 1,6-Hexanediamine Dihydrochloride (continued)**

| Analysis | 0 mg/m$^3$ | 1.6 mg/m$^3$ | 5 mg/m$^3$ | 16 mg/m$^3$ | 50 mg/m$^3$ | 160 mg/m$^3$ |
|---|---|---|---|---|---|---|
| **Male (continued)** | | | | | | |
| **Hematology (continued)** | | | | | | |
| Segmented neutrophils ($10^3$/μL) | | | | | | |
| Day 4 | 0.62 ± 0.08 | 0.55 ± 0.09 | 0.60 ± 0.10 | 0.75 ± 0.13 | 0.68 ± 0.10 | 0.49 ± 0.08 |
| Day 18 | 0.62 ± 0.05 | 0.57 ± 0.09[2] | 0.59 ± 0.11 | 0.52 ± 0.06 | 0.62 ± 0.11 | 0.37 ± 0.06** |
| Day 94 | 1.71 ± 0.23 | 1.51 ± 0.09 | 1.75 ± 0.17[2] | 1.88 ± 0.12 | 1.87 ± 0.05 | 1.54 ± 0.13 |
| Lymphocytes ($10^3$/μL) | | | | | | |
| Day 4 | 5.71 ± 0.50 | 5.64 ± 0.58 | 5.81 ± 0.52 | 5.49 ± 0.62 | 6.07 ± 0.45 | 4.58 ± 0.37 |
| Day 18 | 4.23 ± 0.37 | 5.06 ± 0.53[2] | 3.95 ± 0.52 | 4.24 ± 0.46 | 4.31 ± 0.27 | 3.64 ± 0.31 |
| Day 94 | 6.77 ± 0.32 | 6.23 ± 0.29 | 6.92 ± 0.55[2] | 7.29 ± 0.62 | 7.17 ± 0.33[2] | 6.73 ± 0.51 |
| Monocytes ($10^3$/μL) | | | | | | |
| Day 4 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Day 18 | 0.00 ± 0.00 | 0.00 ± 0.00[2] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Day 94 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00[2] | 0.00 ± 0.00 |
| Eosinophils ($10^3$/μL) | | | | | | |
| Day 4 | 0.03 ± 0.01 | 0.04 ± 0.02 | 0.03 ± 0.02 | 0.06 ± 0.02 | 0.03 ± 0.01 | 0.02 ± 0.01 |
| Day 18 | 0.04 ± 0.01 | 0.01 ± 0.01[2] | 0.04 ± 0.02 | 0.04 ± 0.01 | 0.06 ± 0.02 | 0.04 ± 0.02 |
| Day 94 | 0.15 ± 0.03 | 0.09 ± 0.02 | 0.12 ± 0.03[2] | 0.15 ± 0.03 | 0.11 ± 0.03[2] | 0.14 ± 0.03 |
| **Clinical Chemistry** | | | | | | |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 4 | 998 ± 44 | 1014 ± 25 | 1030 ± 60 | 1044 ± 49 | 1039 ± 34 | 999 ± 47 |
| Day 18 | 824 ± 39 | 844 ± 37[2] | 820 ± 33 | 845 ± 31 | 890 ± 37 | 871 ± 45 |
| Day 94 | 265 ± 8 | 285 ± 5* | 276 ± 8[2] | 275 ± 7 | 289 ± 6*[2] | 296 ± 7* |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 4 | 38 ± 1 | 44 ± 2* | 43 ± 2 | 39 ± 2 | 37 ± 1 | 40 ± 1 |
| Day 18 | 35 ± 2 | 36 ± 2 | 34 ± 1 | 30 ± 2 | 36 ± 1 | 34 ± 2 |
| Day 94 | 49 ± 2 | 47 ± 2 | 48 ± 3[2] | 52 ± 3 | 54 ± 5[2] | 52 ± 2 |
| Creatinine (mg/dL) | | | | | | |
| Day 4 | 0.70 ± 0.06 | 0.70 ± 0.06 | 0.62 ± 0.05 | 0.62 ± 0.04 | 0.65 ± 0.06 | 0.63 ± 0.05 |
| Day 18 | 0.59 ± 0.02[2] | 0.59 ± 0.02[2] | 0.61 ± 0.02 | 0.58 ± 0.02[2] | 0.58 ± 0.03 | 0.61 ± 0.01[2] |
| Day 94 | 0.62 ± 0.01 | 0.65 ± 0.02 | 0.63 ± 0.03[3] | 0.65 ± 0.02 | 0.63 ± 0.02[2] | 0.65 ± 0.02 |
| Glucose (mg/dL) | | | | | | |
| Day 4 | 81 ± 2 | 76 ± 5 | 80 ± 3 | 76 ± 2 | 76 ± 3 | 80 ± 1 |
| Day 18 | 149 ± 4[2] | 146 ± 3[2] | 146 ± 5 | 155 ± 4[2] | 150 ± 2 | 150 ± 3[2] |
| Day 94 | 87 ± 4 | 88 ± 8 | 82 ± 4[3] | 100 ± 9 | 80 ± 5[2] | 86 ± 8 |
| Sorbitol dehydrogenase (IU/L) | | | | | | |
| Day 4 | 23 ± 2 | 21 ± 1 | 21 ± 2 | 21 ± 2 | 22 ± 2 | 20 ± 2 |
| Day 18 | 23 ± 1 | 22 ± 1 | 24 ± 1 | 23 ± 1 | 24 ± 2 | 24 ± 1 |
| Day 94 | 26 ± 2 | 26 ± 1 | 25 ± 3[2] | 26 ± 1 | 33 ± 2*[2] | 30 ± 2 |
| Urea nitrogen (mg/dL) | | | | | | |
| Day 4 | 17.0 ± 0.5 | 16.7 ± 0.7 | 16.5 ± 0.4 | 16.5 ± 0.5 | 16.2 ± 0.6 | 16.3 ± 0.5 |
| Day 18 | 15.6 ± 0.7[2] | 17.3 ± 1.4[2] | 16.5 ± 0.6 | 17.1 ± 0.9[2] | 17.3 ± 0.3* | 18.2 ± 0.5**[2] |
| Day 94 | 15.3 ± 0.5 | 16.2 ± 0.6 | 15.0 ± 0.4[3] | 15.1 ± 0.4 | 14.9 ± 0.5[2] | 15.3 ± 0.5 |

**TABLE B1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Studies of 1,6-Hexanediamine Dihydrochloride (continued)**

| Analysis | 0 mg/m$^3$ | 1.6 mg/m$^3$ | 5 mg/m$^3$ | 16 mg/m$^3$ | 50 mg/m$^3$ | 160 mg/m$^3$ |
|---|---|---|---|---|---|---|
| **Female** | | | | | | |
| **Hematology** | | | | | | |
| Hematocrit (%) | | | | | | |
| Day 4 | 50.4 ± 0.8 | 51.1 ± 0.8[2] | 47.4 ± 0.9 | 48.3 ± 1.8[2] | 50.6 ± 0.7 | 50.1 ± 1.0 |
| Day 18 | 53.9 ± 0.7 | 55.3 ± 0.7 | 54.9 ± 0.9 | 54.8 ± 0.6 | 56.3 ± 0.8*[2] | 56.0 ± 0.5* |
| Day 94 | 50.6 ± 0.6 | 52.9 ± 0.6*[2] | 52.1 ± 0.6* | 50.4 ± 0.8 | 49.7 ± 0.7 | 50.2 ± 0.7 |
| Hemoglobin (g/dL) | | | | | | |
| Day 4 | 15.5 ± 0.2 | 15.6 ± 0.2[2] | 14.7 ± 0.2 | 14.8 ± 0.4[2] | 15.6 ± 0.2 | 15.4 ± 0.3 |
| Day 18 | 16.2 ± 0.2 | 16.4 ± 0.2 | 16.4 ± 0.3 | 16.4 ± 0.1 | 16.7 ± 0.3[2] | 16.6 ± 0.2 |
| Day 94 | 15.0 ± 0.2 | 15.7 ± 0.2[2] | 15.5 ± 0.1 | 15.0 ± 0.2 | 15.7 ± 0.8 | 15.1 ± 0.2 |
| Erythrocytes (10$^6$/µL) | | | | | | |
| Day 4 | 8.16 ± 0.18 | 8.39 ± 0.18[2] | 7.58 ± 0.17 | 7.88 ± 0.39[2] | 8.30 ± 0.13 | 8.24 ± 0.20 |
| Day 18 | 8.66 ± 0.14 | 8.96 ± 0.13 | 8.72 ± 0.16 | 8.83 ± 0.13 | 9.05 ± 0.13[2] | 8.99 ± 0.11 |
| Day 94 | 8.50 ± 0.11 | 8.87 ± 0.13[2] | 8.76 ± 0.09 | 8.41 ± 0.11 | 8.31 ± 0.11 | 8.42 ± 0.09 |
| Reticulocytes (10$^6$/µL) | | | | | | |
| Day 4 | 0.19 ± 0.03 | 0.19 ± 0.02[2] | 0.19 ± 0.02 | 0.24 ± 0.06[2] | 0.16 ± 0.02 | 0.17 ± 0.02 |
| Day 18 | 0.13 ± 0.02[2] | 0.14 ± 0.02 | 0.12 ± 0.01 | 0.13 ± 0.01 | 0.10 ± 0.01[2] | 0.11 ± 0.02 |
| Day 94 | 0.13 ± 0.02[2] | 0.16 ± 0.02[3] | 0.11 ± 0.02[3] | 0.14 ± 0.02 | 0.16 ± 0.02[3] | 0.13 ± 0.01 |
| Mean cell volume (fL) | | | | | | |
| Day 4 | 61.7 ± 0.7 | 60.9 ± 0.4[2] | 62.8 ± 0.6 | 61.6 ± 1.0[2] | 61.1 ± 0.4 | 60.9 ± 0.4 |
| Day 18 | 62.4 ± 0.5 | 61.8 ± 0.4 | 63.1 ± 0.3 | 62.2 ± 0.6 | 62.2 ± 0.3[2] | 62.3 ± 0.4 |
| Day 94 | 59.6 ± 0.5 | 59.8 ± 0.3[2] | 59.4 ± 0.5 | 59.9 ± 0.3 | 60.0 ± 0.3 | 59.7 ± 0.4 |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 4 | 19.0 ± 0.3 | 18.6 ± 0.1[2] | 19.5 ± 0.3 | 19.0 ± 0.4[2] | 18.8 ± 0.2 | 18.7 ± 0.1 |
| Day 18 | 18.7 ± 0.2 | 18.3 ± 0.2 | 18.8 ± 0.2 | 18.6 ± 0.2 | 18.5 ± 0.1[2] | 18.4 ± 0.2 |
| Day 94 | 17.7 ± 0.1 | 17.7 ± 0.1[2] | 17.7 ± 0.1 | 17.8 ± 0.1 | 19.0 ± 1.0 | 18.0 ± 0.1* |
| Mean cell hemoglobin concentration (g/dL) | | | | | | |
| Day 4 | 30.7 ± 0.1 | 30.5 ± 0.1[2] | 31.1 ± 0.2 | 30.8 ± 0.3[2] | 30.8 ± 0.2 | 30.7 ± 0.1 |
| Day 18 | 30.0 ± 0.3 | 29.6 ± 0.2 | 29.9 ± 0.3 | 30.0 ± 0.3 | 29.7 ± 0.2[2] | 29.6 ± 0.3 |
| Day 94 | 29.7 ± 0.4 | 29.7 ± 0.3[2] | 29.8 ± 0.3 | 29.7 ± 0.3 | 31.8 ± 1.8 | 30.2 ± 0.2 |
| Nucleated erythrocytes (10$^3$/µL) | | | | | | |
| Day 4 | 0.01 ± 0.01 | 0.01 ± 0.01[2] | 0.01 ± 0.01 | 0.01 ± 0.01[2] | 0.01 ± 0.01 | 0.02 ± 0.01 |
| Day 18 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00[2] | 0.00 ± 0.00 |
| Day 94 | 0.00 ± 0.00 | 0.00 ± 0.00[2] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Methemoglobin (g/dL) | | | | | | |
| Day 4 | 0.05 ± 0.02 | 0.05 ± 0.02[2] | 0.03 ± 0.01 | 0.05 ± 0.02[2] | 0.05 ± 0.02 | 0.03 ± 0.01 |
| Day 18 | 0.09 ± 0.04 | 0.13 ± 0.04 | 0.14 ± 0.05 | 0.23 ± 0.04 | 0.19 ± 0.05 | 0.18 ± 0.06 |
| Day 94 | 0.06 ± 0.03 | 0.11 ± 0.02[2] | 0.07 ± 0.02 | 0.08 ± 0.03 | 0.09 ± 0.02[2] | 0.10 ± 0.03 |
| Platelets (10$^3$/µL) | | | | | | |
| Day 4 | 871.6 ± 20.7 | 761.2 ± 26.6*[2] | 829.1 ± 32.4 | 823.0 ± 45.0[2] | 781.8 ± 28.2 | 828.7 ± 23.5 |
| Day 18 | 684.2 ± 41.9 | 620.1 ± 21.8[2] | 645.5 ± 15.0 | 634.8 ± 18.6[2] | 640.7 ± 16.1[2] | 660.6 ± 27.5 |
| Day 94 | 635.2 ± 19.7 | 580.0 ± 22.7[2] | 593.3 ± 16.4 | 613.5 ± 10.7 | 584.4 ± 19.6 | 589.4 ± 11.7 |
| Leukocytes (10$^3$/µL) | | | | | | |
| Day 4 | 5.04 ± 0.58 | 5.01 ± 0.42[2] | 5.00 ± 0.42 | 5.23 ± 0.49[2] | 5.54 ± 0.56 | 5.64 ± 0.76 |
| Day 18 | 4.64 ± 0.42 | 4.24 ± 0.19 | 5.37 ± 0.43 | 4.63 ± 0.26 | 5.17 ± 0.39[2] | 4.61 ± 0.32 |
| Day 94 | 9.04 ± 0.46 | 8.51 ± 0.31[2] | 8.81 ± 0.71 | 7.81 ± 0.46 | 7.04 ± 0.70* | 6.42 ± 0.34** |

**TABLE B1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Studies of 1,6-Hexanediamine Dihydrochloride (continued)**

| Analysis | 0 mg/m$^3$ | 1.6 mg/m$^3$ | 5 mg/m$^3$ | 16 mg/m$^3$ | 50 mg/m$^3$ | 160 mg/m$^3$ |
|---|---|---|---|---|---|---|
| **Female (continued)** | | | | | | |
| **Hematology (continued)** | | | | | | |
| Segmented neutrophils ($10^3$/µL) | | | | | | |
| Day 4 | 0.60 ± 0.15 | 0.56 ± 0.07[2] | 0.46 ± 0.11 | 0.58 ± 0.13[2] | 0.62 ± 0.10 | 0.53 ± 0.08 |
| Day 18 | 0.41 ± 0.08 | 0.50 ± 0.04 | 0.59 ± 0.08 | 0.55 ± 0.08 | 0.51 ± 0.10[2] | 0.50 ± 0.08 |
| Day 94 | 1.64 ± 0.09 | 1.48 ± 0.17[2] | 1.19 ± 0.19* | 1.15 ± 0.15* | 1.01 ± 0.15** | 0.92 ± 0.21** |
| Lymphocytes ($10^3$/µL) | | | | | | |
| Day 4 | 4.37 ± 0.53 | 4.39 ± 0.42[2] | 4.52 ± 0.33 | 4.60 ± 0.42[2] | 4.89 ± 0.47 | 5.04 ± 0.69 |
| Day 18 | 4.18 ± 0.40 | 3.73 ± 0.19 | 4.74 ± 0.38 | 4.07 ± 0.22 | 4.61 ± 0.31[2] | 4.09 ± 0.31 |
| Day 94 | 7.33 ± 0.42 | 6.97 ± 0.34[2] | 7.54 ± 0.62 | 6.60 ± 0.44 | 5.97 ± 0.61 | 5.43 ± 0.33** |
| Monocytes ($10^3$/µL) | | | | | | |
| Day 4 | 0.00 ± 0.00 | 0.00 ± 0.00[2] | 0.00 ± 0.00 | 0.00 ± 0.00[2] | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Day 18 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Day 94 | 0.00 ± 0.00 | 0.00 ± 0.00[2] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Eosinophils ($10^3$/µL) | | | | | | |
| Day 4 | 0.07 ± 0.03 | 0.06 ± 0.02[2] | 0.02 ± 0.01 | 0.03 ± 0.02[2] | 0.04 ± 0.02 | 0.06 ± 0.02 |
| Day 18 | 0.05 ± 0.02 | 0.01 ± 0.01 | 0.04 ± 0.01 | 0.01 ± 0.01 | 0.04 ± 0.02[2] | 0.03 ± 0.01 |
| Day 94 | 0.07 ± 0.02 | 0.07 ± 0.03[2] | 0.08 ± 0.02 | 0.06 ± 0.02 | 0.07 ± 0.03 | 0.07 ± 0.02 |
| **Clinical Chemistry** | | | | | | |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 4 | 952 ± 24 | 901 ± 42 | 965 ± 40 | 888 ± 41 | 926 ± 30 | 965 ± 41 |
| Day 18 | 710 ± 23 | 651 ± 26 | 714 ± 27 | 689 ± 27 | 695 ± 29 | 780 ± 38 |
| Day 94 | 225 ± 7 | 225 ± 13 | 231 ± 8[2] | 222 ± 6 | 242 ± 12 | 235 ± 8[2] |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 4 | 34 ± 2 | 31 ± 1 | 36 ± 2 | 32 ± 1 | 38 ± 2 | 39 ± 4 |
| Day 18 | 33 ± 2 | 31 ± 2 | 32 ± 2[2] | 34 ± 2[2] | 32 ± 2 | 35 ± 1 |
| Day 94 | 37 ± 1 | 36 ± 1 | 38 ± 1[2] | 36 ± 1 | 42 ± 4 | 33 ± 1[2] |
| Creatinine (mg/dL) | | | | | | |
| Day 4 | 0.56 ± 0.02 | 0.56 ± 0.02 | 0.61 ± 0.02 | 0.58 ± 0.02 | 0.60 ± 0.02 | 0.60 ± 0.00 |
| Day 18 | 0.51 ± 0.01 | 0.60 ± 0.07 | 0.50 ± 0.02 | 0.58 ± 0.05 | 0.51 ± 0.01[2] | 0.57 ± 0.02[2] |
| Day 94 | 0.63 ± 0.02 | 0.64 ± 0.02 | 0.69 ± 0.02 | 0.68 ± 0.03 | 0.67 ± 0.02 | 0.63 ± 0.02[2] |
| Glucose (mg/dL) | | | | | | |
| Day 4 | 151 ± 2 | 152 ± 3 | 147 ± 2 | 151 ± 2 | 148 ± 2 | 151 ± 3 |
| Day 18 | 152 ± 2[2] | 144 ± 3 | 152 ± 4 | 151 ± 3 | 149 ± 4 | 155 ± 2[2] |
| Day 94 | 82 ± 5 | 74 ± 4 | 78 ± 7 | 85 ± 4 | 91 ± 4 | 87 ± 6[2] |
| Sorbitol dehydrogenase (IU/L) | | | | | | |
| Day 4 | 25 ± 1 | 25 ± 1 | 27 ± 1 | 25 ± 1 | 28 ± 1 | 26 ± 1 |
| Day 18 | 18 ± 1 | 18 ± 1 | 18 ± 1 | 19 ± 0 | 18 ± 1 | 22 ± 1* |
| Day 94 | 24 ± 2 | 24 ± 3 | 32 ± 3[2] | 27 ± 3 | 29 ± 3 | 23 ± 2 |
| Urea nitrogen (mg/dL) | | | | | | |
| Day 4 | 21.7 ± 1.0 | 22.5 ± 0.9 | 25.6 ± 0.9* | 22.7 ± 1.2 | 23.8 ± 0.9 | 23.6 ± 1.3 |
| Day 18 | 17.6 ± 0.7 | 18.1 ± 0.7 | 20.2 ± 0.4** | 20.4 ± 0.8* | 19.0 ± 0.7* | 21.5 ± 0.7** |
| Day 94 | 15.9 ± 0.5 | 17.0 ± 0.6 | 16.9 ± 0.7 | 15.5 ± 0.5 | 15.4 ± 0.5 | 15.1 ± 0.7[2] |

1 Mean ± standard error for groups of 10 animals, unless otherwise specified.

2 n = 9.

3 n = 8.

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.

** Significantly different (P≤0.01) from the control group by Shirley's test.

# APPENDIX C

# Reproductive Tissue Evaluations and Results of Mating Trials

**TABLE C1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride**

| Study Parameters[1] | 0 mg/m$^3$ | 16 mg/m$^3$ | 50 mg/m$^3$ | 160 mg/m$^3$ |
|---|---|---|---|---|
| **Weights (g)** | | | | |
| | 338 ± 8 | 329 ± 4 | 326 ± 6 | 316 ± 8* |
| | 0.453 ± 0.007 | 0.457 ± 0.011 | 0.451 ± 0.007 | 0.461 ± 0.010 |
| | 0.167 ± 0.003 | 0.164 ± 0.006 | 0.168 ± 0.003 | 0.174 ± 0.005 |
| | 1.459 ± 0.021 | 1.473 ± 0.018 | 1.456 ± 0.017 | 1.479 ± 0.033 |
| **Spermatozoal measurements** | | | | |
| | 74.24 ± 2.22 | 73.02 ± 2.11 | 73.97 ± 1.14 | 72.54 ± 1.95 |
| | 484.73 ± 38.42 | 545.56 ± 40.13 | 503.27 ± 38.05 | 512.52 ± 41.25 |
| | 0.920 ± 0.085 | 0.780 ± 0.117 | 0.860 ± 0.155 | 0.760 ± 0.107 |

[1] Data presented as mean ± standard error; n=10, except where noted. Differences from the control group for testicular and epididymal weights are not significant by Dunnett's test or Williams' test; epididymal tail weights are not significant by Dunn's test; spermatozoal measurements are not significant by Dunn's test.

* Significantly different (P≤0.05) from the control group by Williams' test.

**TABLE C2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Inhalation Study of 1,6-HexanediamineDihydrochloride**

| Study Parameters[1] | 0 mg/m$^3$ | 16 mg/m$^3$ | 50 mg/m$^3$ | 160 mg/m$^3$ |
|---|---|---|---|---|
| **Necropsy body weight** | 193 ± 3 | 186 ± 3 | 190 ± 3 | 189 ± 4 |
| **Estrous cycle length (days)** | 4.90 ± 0.10 | 4.90 ± 0.10 | 4.90 ± 0.10 | 4.80 ± 0.13 |
| **Estrous stages as % of cycle** | | | | |
| | 41.4 | 38.6 | 45.7 | 41.4 |
| | 18.6 | 18.6 | 14.3 | 14.3 |
| | 18.6 | 21.4 | 18.6 | 24.3 |
| | 21.4 | 18.6 | 20.0 | 18.6 |
| | 0.0 | 2.9 | 1.4 | 1.4 |

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error; n=10, except where noted. Differences from the control group for necropsy body weights are not significant by Dunnett's test; estrous cycle lengths are not significant by Dunn's or Shirley's test. By multivariate analysis of variance (MANOVA), dosed groups do not differ significantly from controls in the relative length of time spent in the estrous stages.

**TABLE C3 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride**

| Study Parameters[1] | 0 mg/m$^3$ | 16 mg/m$^3$ | 50 mg/m$^3$ | 160 mg/m$^3$ |
|---|---|---|---|---|
| **Weights (g)** | | | | |
| | 32.4 ± 0.7 | 31.6 ± 0.5 | 33.1 ± 1.1 | 32.5 ± 0.7 |
| | 0.044 ± 0.002 | 0.043 ± 0.001 | 0.044 ± 0.001 | 0.043 ± 0.003 |
| | 0.016 ± 0.001 | 0.017 ± 0.001 | 0.016 ± 0.001 | 0.016 ± 0.001 |
| | 0.116 ± 0.003 | 0.111 ± 0.005 | 0.119 ± 0.002 | 0.120 ± 0.005 |
| **Spermatozoal measurements** | | | | |
| | 68.54 ± 2.95 | 77.28 ± 1.42* | 72.44 ± 1.69 | 76.23 ± 2.32** |
| | 893.43 ± 106.47 | 895.10 ± 149.45 | 985.97 ± 93.11 | 1156.07 ± 177.35 |
| | 1.360 ± 0.093 | 1.880 ± 0.605 | 1.620 ± 0.266 | 1.440 ± 0.160 |

1 Data presented as mean ± standard error; n=10, except where noted. Differences from the control group for necropsy body weights and testicular and epididymal weights are not significant by Dunnett's test; epididymal tail weights are not significant by Dunn's test.

* Significantly different (P≤0.05) from the control group by Shirley's test.

** Significantly different (P≤0.01) from the control group by Shirley's test.

**TABLE C4 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Inhalation Study of 1,6-Hexanediamine Dihydrochloride**

| Study Parameters[1] | 0 mg/m$^3$ | 16 mg/m$^3$ | 50 mg/m$^3$ | 160 mg/m$^3$ |
|---|---|---|---|---|
| **Necropsy body weight** | 26.9 ± 0.7 | 27.0 ± 0.5 | 27.8 ± 0.9 | 27.6 ± 0.5 |
| **Estrous cycle length (days)** | 4.00 ± 0.00 | 4.11 ± 0.11[2] | 4.30 ± 0.21 | 4.33 ± 0.017[2] |
| **Estrous stages as % of cycle** | | | | |
| | 28.6 | 27.1 | 17.1 | 28.6 |
| | 20.0 | 22.9 | 27.1 | 30.0 |
| | 27.1 | 27.1 | 31.4 | 31.4 |
| | 24.3 | 22.9 | 24.3 | 10.0 |

1 Necropsy body weight and estrous cycle length data are presented as mean ± standard error; n=10, except where noted. Differences from the control group for necropsy body weights are not significant by Dunnett's test; estrous cycle lengths are not significant by Dunn's or Shirley's test. Evidence by multivariate analysis of variance (MANOVA) suggests that there was a treatment-related difference in the relative length of time spent in the estrous stages (Wilk's criterion, P<0.01).

2 For 1/10 animals in the 16 mg/m$^3$ and 160 mg/m$^3$ dose groups, estrous cycle length was longer than 7 days or was unclear; data for these animals are not included in the mean.

**TABLE C5 Mean Body Weights and Length of Gestation for Female F344/N Rats in the Mating Trial Study of 1,6-Hexanediamine Dihydrochloride**

| | 0 mg/m$^3$ | 16 mg/m$^3$ | 50 mg/m$^3$ | 160 mg/m$^3$ |
|---|---|---|---|---|
| **Dam Weight During Gestation**[1] | | | | |
| | 27 | 30 | 33 | 34 |
| | 192 ± 2 | 189 ± 2 | 191 ± 2 | 184 ± 2** |
| | 259 ± 3 | 262 ± 3 | 264 ± 3 | 254 ± 4 |
| | 67 ± 2 | 73 ± 2 | 73 ± 3 | 70 ± 3 |
| | ± | 101 | 102 | 98 |
| **Length of Gestation**[1] | | | | |
| | 27 | 30 | 33 | 34 |
| | 21.81 ± 0.05 | 21.95 ± 0.06 | 22.00 ± 0.06 | 21.93 ± 0.04 |
| **Dam Weight During Lactation**[1] | | | | |
| Lactation day 0 | | | | |
| | 27 | 29 | 33 | 34 |
| | 214 ± 3 | 212 ± 2 | 209 ± 3 | 207 ± 2* |
| Lactation day 5 | | | | |
| | 27 | 28 | 32 | 33 |
| | 216 ± 2 | 212 ± 2 | 212 ± 3 | 209 ± 2* |
| Lactation day 14 | | | | |
| | 26 | 28 | 32 | 33 |
| | 232 ± 3 | 232 ± 2 | 231 ± 3 | 226 ± 4 |
| Lactation day 21 | | | | |
| | 26 | 28 | 32 | 33 |
| | 229 ± 2 | 231 ± 2 | 228 ± 2 | 226 ± 2 |

[1] Data presented as mean ± standard deviation. Gestation lengths are not significant by Dunn's test.

* Significantly different (P≤0.05) from the control group by Williams' test.

** Significantly different (P≤0.01) from the control group by Williams' test.

**TABLE C6 Survival, Sex Distribution, and Mean Body Weights of F344/N Rat Pups in the Mating Trial Study of 1,6-Hexanediamine Dihydrochloride**

| | 0 mg/m$^3$ | 16 mg/m$^3$ | 50 mg/m$^3$ | 160 mg/m$^3$ |
|---|---|---|---|---|
| **Day 0** | | | | |
| | 27 | 30 | 33 | 34 |
| | 8.63 ± 0.64 | 9.27 ± 0.55 | 9.42 ± 0.52 | 8.47 ± 0.55 |
| | 8.63 ± 0.64 | 9.13 ± 0.58 | 9.39 ± 0.52 | 8.41 ± 0.55 |
| | 48.94 ± 3.95 | 51.41 ± 3.14[2] | 45.94 ± 2.74 | 43.31 ± 3.13 |
| | 5.1 ± 0.1 | 5.1 ± 0.1[2] | 5.1 ± 0.1 | 5.1 ± 0.1 |
| **Day 5** | | | | |
| | 27 | 30 | 33 | 34 |
| | 8.56 ± 0.63 | 9.00 ± 0.58 | 8.82 ± 0.60 | 8.15 ± 0.54 |
| | 49.69 ± 3.99 | 49.60 ± 2.79[3] | 47.29 ± 2.86[4] | 43.13 ± 3.30[5] |
| | 9.3 ± 0.2 | 9.3 ± 0.2[3] | 9.4 ± 0.2[4] | 9.6 ± 0.2[5] |
| **Day 14** | | | | |
| | 27 | 30 | 33 | 34 |
| | 8.52 ± 0.65 | 8.97 ± 0.58 | 8.82 ± 0.60 | 8.12 ± 0.54 |
| | 51.95 ± 3.66[6] | 49.43 ± 2.85[3] | 47.21 ± 2.82[4] | 43.23 ± 3.28[5] |
| | 21.9 ± 0.6[6] | 22.1 ± 0.4[3] | 21.9 ± 0.4[4] | 22.4 ± 0.6[5] |
| **Day 21** | | | | |
| | 27 | 30 | 33 | 34 |
| | 8.26 ± 0.62 | 8.87 ± 0.58 | 8.73 ± 0.61 | 7.94 ± 0.52 |
| | 53.10 ± 3.59[6] | 49.85 ± 2.82[3] | 47.21 ± 2.92[4] | 43.90 ± 3.24[5] |
| | 33.3 ± 1.0[6] | 33.4 ± 0.6[3] | 33.6 ± 0.7[4] | 33.9 ± 0.9[5] |

[1] Data presented as mean ± standard deviation. Differences from the control group for percent of live male pups, litter size, and number of pups born alive are not significant by Dunn's test; pup weights are not significant by Dunnett's test.

[2] n = 29.

[3] n = 28.

[4] n = 32.

[5] n = 33.

[6] n = 26.

**TABLE C7 Mean Body Weights and Length of Gestation for Female B6C3F$_1$ Mice in the Mating Trial Study of 1,6-Hexanediamine Dihydrochloride**

| | 0 mg/m$^3$ | 16 mg/m$^3$ | 50 mg/m$^3$ | 160 mg/m$^3$ |
|---|---|---|---|---|
| **Dam Weight During Gestation**[1] | | | | |
| | 22 | 23 | 27 | 24 |
| | 25.6 ± 0.3 | 25.6 ± 0.4 | 27.2 ± 0.5* | 26.2 ± 0.3 |
| | 44.3 ± 0.8 | 44.4 ± 0.8 | 43.4 ± 0.8 | 44.2 ± 0.5 |
| | 18.7 ± 0.8 | 18.8 ± 0.8 | 16.2 ± 0.9 | 18.0 ± 0.5 |
| | – | 100 | 98 | 100 |
| **Length of Gestation**[1] | | | | |
| | 30 | 30 | 31 | 31 |
| | 17.68 ± 0.10 | 18.00 ± 0.12 | 18.11 ± 0.09** | 18.11 ± 0.08** |
| **Dam Weight During Lactation**[1] | | | | |
| Lactation day 0 | | | | |
| | 35 | 32 | 33 | 35 |
| | 31.4 ± 0.3 | 32.0 ± 0.4 | 32.5 ± 0.4 | 31.8 ± 0.3 |
| Lactation day 5 | | | | |
| | 34 | 32 | 33 | 35 |
| | 35.3 ± 0.4 | 35.7 ± 0.5 | 35.3 ± 0.4 | 34.9 ± 0.4 |
| Lactation day 14 | | | | |
| | 34 | 32 | 33 | 35 |
| | 39.7 ± 0.5 | 39.6 ± 0.6 | 38.9 ± 0.6 | 38.8 ± 0.6 |
| Lactation day 21 | | | | |
| | 34 | 32 | 33 | 35 |
| | 34.8 ± 0.5 | 34.2 ± 0.5 | 34.7 ± 0.6 | 33.9 ± 0.61 |

[1] Data presented as mean ± standard deviation. Differences from the control group were evaluated by Williams' or Dunnett's test (weight) and Shirley's test (gestation length).

* Significantly different (P≤0.05) from the control group by Williams' test.

** Significantly different (P≤0.01) from the control group by Shirley's test.

**TABLE C8 Survival, Sex Distribution, and Mean Body Weights of B6C3F$_1$ Mouse Pups in the Mating Trial Study of 1,6-Hexanediamine Dihydrochloride**

| | 0 mg/m$^3$ | 16 mg/m$^3$ | 50 mg/m$^3$ | 160 mg/m$^3$ |
|---|---|---|---|---|
| **Day 0** | | | | |
| | 35 | 34 | 33 | 35 |
| | 9.11 ± 0.38 | 9.00 ± 0.44 | 8.73 ± 0.46 | 9.57 ± 0.25 |
| | 8.86 ± 0.37 | 8.53 ± 0.53 | 8.61 ± 0.45 | 9.37 ± 0.24 |
| | 53.33 ± 3.51 | 50.56 ± 3.56[2] | 53.18 ± 2.11 | 50.56 ± 3.25 |
| | 1.37 ± 0.03 | 1.39 ± 0.03[2] | 1.41 ± 0.04 | 1.37 ± 0.02 |
| **Day 5** | | | | |
| | 35 | 34 | 33 | 35 |
| | 8.54 ± 0.45 | 8.44 ± 0.52 | 8.58 ± 0.46 | 9.37 ± 0.24 |
| | 55.94 ± 3.53[3] | 50.79 ± 3.58[2] | 53.28 ± 2.51 | 50.32 ± 3.29 |
| | 3.57 ± 0.08[3] | 3.56 ± 0.08[2] | 3.68 ± 0.11 | 3.55 ± 0.06 |
| **Day 14** | | | | |
| | 35 | 34 | 33 | 35 |
| | 8.51 ± 0.44 | 8.41 ± 0.52 | 8.58 ± 0.46 | 9.34 ± 0.24 |
| | 56.43 ± 3.51[3] | 50.80 ± 3.50[2] | 53.28 ± 2.51 | 50.08 ± 3.33 |
| | 8.19 ± 0.21[3] | 7.95 ± 0.15[2] | 8.25 ± 0.27 | 7.63 ± 0.16 |
| **Day 21** | | | | |
| | 35 | 34 | 33 | 35 |
| | 8.46 ± 0.43 | 8.41 ± 0.52 | 8.58 ± 0.46 | 9.31 ± 0.24 |
| | 56.15 ± 3.58[3] | 51.11 ± 3.57[2] | 53.28 ± 2.51 | 50.03 ± 3.26 |
| | 10.94 ± 0.24[3] | 10.68 ± 0.17[2] | 10.93 ± 0.29 | 10.21 ± 0.19* |

[1] Data presented as mean ± standard deviation. Differences from the control group for percent of live male pups, litter size, and number of pups born alive are not significant by Dunn's test. The significance of differences in pup weights between dosed and control groups was evaluated by Dunnett's or Williams' test.

[2] n = 32.

[3] n = 34.

* Significantly different (P≤0.05) from the control group by Williams' test.

# APPENDIX D

# Genetic Toxicology

**TABLE D1 Mutagenicity of 1,6-Hexanediamine in *Salmonella typhimurium*[1]**

| Strain | Dose (±g/plate) | Revertants/plate[2] | | | | | |
|---|---|---|---|---|---|---|---|
| | | -S9 | | +10% hamster S9 | | +10% rat S9 | |
| | | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| **Study 1** | | | | | | | |
| **TA100** | 0 | 120 ± 5.1 | 101 ± 4.9 | 143 ± 8.5 | 120 ± 8.4 | 116 ± 10.2 | 116 ± 10.2 |
| | 33 | | 108 ± 12.0 | | 100 ± 6.4 | | |
| | 100 | 104 ± 11.0 | 93 ± 2.3 | 128 ± 1.3 | 100 ± 4.6 | 114 ± 6.6 | 119 ± 4.6 |
| | 333 | 101 ± 3.6 | 92 ± 5.5 | 140 ± 4.7 | 92 ± 10.2 | 100 ± 11.5 | 99 ± 7.5 |
| | 1000 | 77 ± 6.0 | 77 ± 12.2 | 98 ± 4.0 | 104 ± 2.3 | 112 ± 10.3 | 99 ± 9.4 |
| | 3333 | 63 ± 2.4 | Toxic | 57 ± 12.2[3] | 13 ± 9.4[3] | 89 ± 10.7 | 115 ± 3.7 |
| | 10,000 | Toxic | | 51 ± 12.7[3] | | 50 ± 1.2[3] | 46 ± 29.5[3] |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control[4] | | 277 ± 18.4 | 237 ± 6.8 | 1100 ± 18.7 | 607 ± 48.0 | 688 ± 39.0 | 692 ± 31.1 |
| **TA1535** | 0 | 28 ± 0.7 | 19 ± 2.4 | 12 ± 2.3 | 8 ± 2.9 | 19 ± 0.6 | 18 ± 1.9 |
| | 33 | | 14 ± 2.6 | | 7 ± 1.0 | | |
| | 100 | 16 ± 2.3 | 18 ± 2.0 | 9 ± 1.7 | 6 ± 1.2 | 10 ± 1.9 | 21 ± 4.1 |
| | 333 | 16 ± 1.5 | 16 ± 1.3 | 11 ± 3.5 | 7 ± 1.5 | 10 ± 1.7 | 20 ± 2.9 |
| | 1000 | 14 ± 3.5 | 14 ± 2.9 | 9 ± 1.5 | 8 ± 0.9 | 9 ± 2.2 | 20 ± 2.8 |
| | 3333 | 5 ± 1.3[3] | Toxic | 1 ± 0.6[3] | Toxic | 9 ± 1.3 | 12 ± 6.2 |
| | 10,000 | 2 ± 0.9[3] | | 0 ± 0.0[3] | | 3 ± 0.7[3] | 5 ± 5.0[3] |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 315 ± 14.6 | 217 ± 2.4 | 357 ± 17.6 | 265 ± 27.4 | 260 ± 7.7 | 248 ± 11.7 |
| **TA1537** | 0 | 6 ± 1.2 | 5 ± 0.0 | 7 ± 2.3 | 6 ± 2.8 | 16 ± 2.6 | 23 ± 1.2 |
| | 33 | | 4 ± 1.5 | | 2 ± 0.3 | | |
| | 100 | 6 ± 0.3 | 5 ± 1.0 | 3 ± 0.6 | 4 ± 0.3 | 9 ± 1.5 | 16 ± 0.9 |
| | 333 | 6 ± 1.2 | 4 ± 1.2 | 3 ± 1.2 | 4 ± 0.9 | 6 ± 0.0 | 19 ± 0.9 |
| | 1000 | 6 ± 0.6 | 7 ± 1.0 | 5 ± 2.2 | 4 ± 1.0 | 7 ± 0.9 | 14 ± 5.3 |
| | 3333 | 4 ± 0.9 | 5 ± 0.5 | 2 ± 1.2 | Toxic | 7 ± 3.0 | 12 ± 2.3 |
| | 10,000 | 0 ± 0.0[3] | | 0 ± 0.3[3] | | 5 ± 0.3 | 9 ± 2.0 |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 110 ± 6.9 | 263 ± 16.0 | 446 ± 16.1 | 307 ± 13.6 | 217 ± 5.3 | 137 ± 8.1 |
| **TA98** | 0 | 18 ± 0.9 | 14 ± 0.7 | 29 ± 0.9 | 23 ± 3.3 | 24 ± 2.2 | 49 ± 3.7 |
| | 33 | | 12 ± 1.8 | | 26 ± 3.8 | | |
| | 100 | 13 ± 2.4 | 15 ± 5.0 | 26 ± 1.5 | 21 ± 2.8 | 34 ± 2.9 | 61 ± 6.8 |
| | 333 | 16 ± 1.7 | 13 ± 1.9 | 21 ± 2.7 | 25 ± 4.5 | 23 ± 4.2 | 53 ± 5.5 |
| | 1000 | 13 ± 2.4 | 8 ± 2.5 | 22 ± 3.8 | 25 ± 0.9 | 26 ± 3.0 | 61 ± 4.7 |
| | 3333 | 8 ± 3.9[3] | Toxic | 9 ± 3.8[3] | Toxic | 28 ± 1.2 | 34 ± 9.5 |
| | 10,000 | Toxic | | 5 ± 4.8[3] | | 31 ± 2.5 | 9 ± 5.9[3] |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 654 ± 54.9 | 565 ± 18.8 | 926 ± 12.5 | 634 ± 34.5 | 462 ± 37.8 | 468 ± 6.7 |

**TABLE D1 Mutagenicity of 1,6-Hexanediamine in *Salmonella typhimurium* (continued)**

| Strain | Dose (±g/plate) | Revertants/plate -S9 Trial 1 | -S9 Trial 2 | +10% hamster S9 Trial 1 | +10% hamster S9 Trial 2 | +10% rat S9 Trial 1 | +10% rat S9 Trial 2 |
|---|---|---|---|---|---|---|---|
| **Study 2** | | | | | | | |
| **TA100** | 0 | 97 ± 6.1 | 116 ± 4.3 | 112 ± 13.1 | 127 ± 3.6 | 85 ± 6.5 | 104 ± 2.8 |
| | 33 | | 120 ± 1.9 | | 117 ± 3.8 | | |
| | 100 | 101 ± 2.6 | 130 ± 4.1 | 107 ± 9.9 | 139 ± 4.4 | 98 ± 6.4 | 118 ± 2.0 |
| | 333 | 94 ± 2.3 | 116 ± 5.9 | 98 ± 6.9 | 106 ± 5.3 | 106 ± 6.5 | 110 ± 5.5 |
| | 1000 | 94 ± 4.7 | 113 ± 3.1 | 107 ± 5.0 | 111 ± 4.9 | 95 ± 9.2 | 116 ± 3.5 |
| | 3333 | Toxic | 0 ± 0.0[3] | 40 ± 21.2[3] | 76 ± 5.4 | 105 ± 10.5 | 110 ± 4.8 |
| | 6666 | | | | | | 0 ± 0.0[3] |
| | 10,000 | Toxic | | 19 ± 16.4[3] | | 27 ± 13.4[3] | |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 322 ± 9.3 | 397 ± 20.2 | 1506 ± 62.8 | 792 ± 20.3 | 504 ± 29.3 | 690 ± 18.0 |
| **TA1535** | 0 | 26 ± 0.7 | 25 ± 1.2 | 9 ± 3.2 | 7 ± 1.5 | 5 ± 1.3 | 8 ± 1.2 |
| | 33 | | 25 ± 4.7 | | 8 ± 2.3 | | |
| | 100 | 22 ± 2.9 | 27 ± 5.5 | 10 ± 1.2 | 9 ± 1.2 | 7 ± 2.4 | 5 ± 0.6 |
| | 333 | 21 ± 3.6 | 16 ± 1.8 | 9 ± 1.3 | 7 ± 1.5 | 8 ± 2.7 | 9 ± 0.7 |
| | 1000 | 20 ± 0.9 | 11 ± 1.7 | 12 ± 3.3 | 4 ± 1.5 | 12 ± 1.5 | 8 ± 1.7 |
| | 3333 | 18 ± 10.7 | 0 ± 0.0[3] | 7 ± 5.0[3] | 3 ± 2.0 | 18 ± 2.3 | 9 ± 2.3 |
| | 6666 | | | | | | 0 ± 0.0[3] |
| | 10,000 | 5 ± 4.5[3] | | 0 ± 0.0[3] | | 5 ± 0.9[3] | |
| Trial summary | | Negative | Negative | Negative | Negative | Equivocal | Negative |
| Positive control | | 334 ± 10.6 | 359 ± 15.0 | 396 ± 5.3 | 213 ± 8.8 | 185 ± 10.4 | 114 ± 14.3 |
| **TA1537** | 0 | 5 ± 0.0 | 7 ± 0.6 | 8 ± 1.3 | 7 ± 1.9 | 10 ± 3.2 | 5 ± 1.2 |
| | 33 | | 4 ± 0.7 | | 7 ± 1.7 | | |
| | 100 | 8 ± 2.0 | 4 ± 0.9 | 7 ± 1.2 | 5 ± 0.0 | 5 ± 1.2 | 11 ± 0.9 |
| | 333 | 5 ± 0.3 | 4 ± 2.4 | 5 ± 1.8 | 7 ± 0.7 | 6 ± 0.9 | 6 ± 1.2 |
| | 1000 | 4 ± 1.0 | 2 ± 0.3 | 9 ± 2.1 | 7 ± 1.0 | 4 ± 1.5 | 5 ± 2.8 |
| | 3333 | 3 ± 1.7 | 0 ± 0.0[3] | 1 ± 0.6[3] | 3 ± 2.0 | 4 ± 1.2 | 4 ± 0.6 |
| | 6666 | | | | | | 0 ± 0.0[3] |
| | 10,000 | 0 ± 0.0[3] | | Toxic | | 3 ± 2.7[3] | |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 143 ± 15.1 | 78 ± 3.8 | 400 ± 16.2 | 383 ± 15.5 | 142 ± 8.6 | 234 ± 15.6 |
| **TA98** | 0 | 15 ± 2.0 | 14 ± 2.0 | 21 ± 2.7 | 15 ± 1.5 | 24 ± 3.0 | 15 ± 1.5 |
| | 33 | | 13 ± 2.4 | | 26 ± 5.9 | | |
| | 100 | 16 ± 1.2 | 13 ± 0.3 | 27 ± 1.7 | 18 ± 4.2 | 23 ± 2.3 | 24 ± 5.7 |
| | 333 | 18 ± 1.9 | 18 ± 3.5 | 33 ± 2.6 | 24 ± 2.6 | 20 ± 4.9 | 16 ± 3.8 |
| | 1000 | 16 ± 2.0 | 11 ± 1.7 | 29 ± 6.9 | 24 ± 1.2 | 20 ± 2.7 | 19 ± 4.4 |
| | 3333 | 1 ± 1.0[3] | Toxic | 4 ± 1.2[3] | 2 ± 1.7[3] | 14 ± 3.2 | 14 ± 0.7 |
| | 6666 | | | | | | 0 ± 0.0[3] |
| | 10,000 | Toxic | | 7 ± 3.1[3] | | 4 ± 2.2[3] | |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 707 ± 19.1 | 373 ± 15.6 | 1126 ± 35.9 | 383 ± 22.4 | 194 ± 9.3 | 325 ± 23.3 |

**TABLE D1 Mutagenicity of 1,6-Hexanediamine in *Salmonella typhimurium* (continued)**

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[1] Studies performed at SRI, International. The detailed protocol and these data are presented in Mortelmans *et al* (1986). Cells and 1,6-hexanediamine or solvent (distilled water, study 1; dimethylsulfoxide, study 2) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver and male Sprague-Dawley rat liver. 0 µg/plate dose is the solvent control.

[2] Revertants are presented as mean ± the standard error from 3 plates.

[3] Slight toxicity

[4] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

## TABLE D2 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 1,6-Hexanediamine[1]

| Compound | Dose (µg/mL) | Total Cells | No. of Chromo-somes | No of SCEs | SCEs/ Chromo-some | SCEs/ Cell | Hrs in BrdU | Increase over Solvent (%)[2] |
|---|---|---|---|---|---|---|---|---|
| **-S9** | | | | | | | | |
| **Trial 1** | | | | | | | | |
| Summary: Negative | | | | | | | | |
| Solvent Control | | 50 | 1049 | 440 | 0.41 | 8.8 | 26.0 | |
| Mitomycin-C | | | | | | | | |
| | 0.0007 | 50 | 1050 | 568 | 0.54 | 11.4 | 26.0 | 28.97 |
| | 0.005 | 10 | 209 | 213 | 1.01 | 21.3 | 26.0 | 142.97 |
| 1,6-Hexanediamine | | | | | | | | |
| | 16 | 50 | 1050 | 402 | 0.38 | 8.0 | 26.0 | -8.72 |
| | 50 | 50 | 1048 | 396 | 0.37 | 7.9 | 26.0 | -9.91 |
| | 160 | 50 | 1049 | 394 | 0.37 | 7.9 | 26.0 | -10.46 |
| | 500 | 50 | 1047 | 490 | 0.46 | 9.8 | 26.0 | 11.58 |
| | | | | | | | | P=0.075[3] |
| **+S9** | | | | | | | | |
| **Trial 1** | | | | | | | | |
| Summary: Negative | | | | | | | | |
| Solvent Control | | 50 | 1053 | 355 | 0.33 | 7.1 | 26.0 | |
| Cyclophosphamide | | | | | | | | |
| | 0.1 | 50 | 1049 | 522 | 0.49 | 10.4 | 26.0 | 47.60 |
| | 0.6 | 10 | 210 | 269 | 1.28 | 26.9 | 26.0 | 279.96 |
| 1,6-Hexanediamine | | | | | | | | |
| | 5 | 50 | 1049 | 382 | 0.36 | 7.6 | 26.5 | 8.01 |
| | 160 | 13[4] | 270 | 99 | 0.36 | 7.6 | 26.5 | 8.76 |
| | 500 | 50 | 1047 | 402 | 0.38 | 8.0 | 26.5 | 13.89 |
| | | | | | | | | P=0.045 |
| **+S9** | | | | | | | | |
| **Trial 2** | | | | | | | | |
| Summary: Negative | | | | | | | | |
| Solvent Control | | 50 | 1048 | 416 | 0.39 | 8.3 | 26.0 | |
| Cyclophosphamide | | | | | | | | |
| | 0.1 | 50 | 1051 | 513 | 0.48 | 10.3 | 26.0 | 22.97 |
| | 0.6 | 10 | 210 | 236 | 1.12 | 23.6 | 26.0 | 183.12 |
| 1,6-Hexanediamine | | | | | | | | |
| | 50 | 50 | 1049 | 419 | 0.39 | 8.4 | 26.0 | 0.62 |
| | 160 | 50 | 1047 | 404 | 0.38 | 8.1 | 26.0 | -2.79 |
| | 500 | 50 | 1049 | 388 | 0.36 | 7.8 | 26.0 | -6.82 |
| | 1000 | 50 | 1042 | 423 | 0.40 | 8.5 | 26.0 | 2.27 |
| | | | | | | | | P=0.620 |

**TABLE D2 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 1,6-Hexanediamine (continued)**

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[1] Study performed at Environmental Health Research & Testing, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway *et al*. (1987).

[2] Percentage increase in SCEs/chromosome of culture exposed to 1,6-hexanediamine relative to those of culture exposed to solvent.

[3] Significance was tested by the linear regression trend test vs. log of the dose.

[4] Number of cells were decreased due to technical error, not toxicity.

## TABLE D3 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 1,6-Hexanediamine[1]

| -S9 | | | | | +S9 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dose (µg/mL) | Total Cells | No. of Abs | Abs/ Cell | Cells with Abs (%) | Dose (µg/mL) | Total Cells | No. of Abs | Abs/ Cell | Cells with Abs (%) |
| **Trial 1** – Harvest time: 12.0 hours | | | | | **Trial 1** – Harvest time: 13.0 hours | | | | |
| Summary: Negative | | | | | Summary: Negative | | | | |
| Solvent control | | | | | Solvent control | | | | |
| | 200 | 2 | 0.01 | 1.0 | | 200 | 2 | 0.01 | 1.0 |
| Mitomycin-C | | | | | Cyclophosphamide | | | | |
| 0.0625 | 200 | 35 | 0.18 | 16.5 | 2.5 | 200 | 39 | 0.20 | 17.5 |
| 0.25 | 50 | 26 | 0.52 | 32.0 | 7.5 | 50 | 24 | 0.48 | 38.0 |
| 1,6-Hexanediamine | | | | | 1,6-Hexanediamine | | | | |
| 160 | 200 | 4 | 0.02 | 2.0 | 160 | 200 | 3 | 0.02 | 1.5 |
| 300 | 200 | 3 | 0.02 | 1.5 | 300 | 200 | 0 | 0.00 | 0.0 |
| 500 | 200 | 5 | 0.03 | 2.5 | 500 | 200 | 28 | 0.14 | 4.0 |
| | | | | P=0.173[2] | | | | | P=0.038 |
| | | | | | **Trial 2** – Harvest time: 13.0 hours | | | | |
| | | | | | Summary: Negative | | | | |
| | | | | | Solvent control | | | | |
| | | | | | | 200 | 4 | 0.02 | 2.0 |
| | | | | | Cyclophosphamide | | | | |
| | | | | | 2.5 | 200 | 37 | 0.19 | 17.0 |
| | | | | | 7.5 | 50 | 19 | 0.38 | 34.0 |
| | | | | | 1,6-Hexanediamine | | | | |
| | | | | | 160 | 200 | 1 | 0.01 | 0.5 |
| | | | | | 300 | 200 | 1 | 0.01 | 0.5 |
| | | | | | 500 | 200 | 9 | 0.05 | 4.5 |
| | | | | | | | | | P=0.048 |

[1] Study performed at Environmental Health Research & Testing, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with 1,6-hexanediamine or solvent (distilled water), then arrested in first metaphase by addition of Colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.

[2] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose.

TABLE D4 Frequency of Micronuclei in Mouse Peripheral Blood Erythrocytes Following Inhalation of 1,6-Hexanediamine Dihydrochloride[1]

| Treatment (mg/m$^3$) | Micronucleated Cells/1000 Cells[2] PCEs | NCEs | PCEs (%) |
|---|---|---|---|
| **Males** | | | |
| 0 | 1.74 ± 0.42 | 1.93 ± 0.15 | 2.07 ± 0.21 |
| 16 | 1.61 ± 0.25 | 1.82 ± 0.15 | 2.10 ± 0.18 |
| 50 | 2.70 ± 0.47 | 2.19 ± 0.15 | 4.31 ± 1.09** |
| 160 | 2.26 ± 0.52 | 1.79 ± 0.12 | 2.59 ± 0.31* |
| | P=0.180[3] | P=0.972 | P=0.016 |
| **Females** | | | |
| 0 | 1.90 ± 0.42 | 1.38 ± 0.13 | 1.83 ± 0.11 |
| 16 | 1.17 ± 0.43 | 1.35 ± 0.19 | 2.07 ± 0.12 |
| 50 | 0.93 ± 0.27 | 1.14 ± 0.07 | 1.79 ± 0.06 |
| 160 | 2.31 ± 0.44 | 1.21 ± 0.11 | 2.76 ± 0.17** |
| | P=0.052 | P=0.218 | P<0.001 |

1 Inhalation exposure: 90 days

2 Values presented as mean ± standard error of the treatment group. PCE=polychromatic erythrocytes, NCE=normochromatic erythrocytes

3 Cochran-Armitage trend test for PCEs, analysis of variance using the SAS GLM procedure for NCEs, and analysis of variance on ranks for %PCE.

* P≤0.05; *t*-tests on ranks for %PCE.

** P≤0.01