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NTP Technical Report on the Toxicity Studies of Dimethylaminopropyl Chloride, Hydrochloride (CAS No. 5407-04-5) Administered by Gavage to F344/N Rats and B6C3F$_1$ Mice

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Administered by Gavage
to F344/N Rats and B6C3F$_1$ Mice

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PEER REVIEW

The draft report on the toxicity studies of dimethylaminopropyl chloride, hydrochloride was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that this Toxicity Study Report presents the experimental results and conclusions fully and clearly.

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CONTENTS

SUMMARY

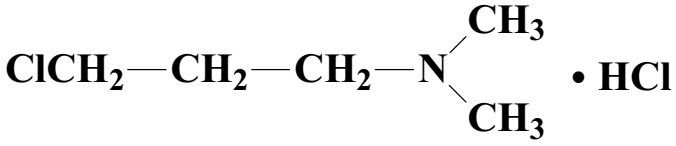
Background
Dimethylaminopropyl chloride, hydrochloride is used mainly to make drugs, agricultural chemicals, and photographic chemicals. The NTP conducted short-term (3-month) tests to determine the effects of dimethylaminopropyl chloride, hydrochloride on rats and mice.

Methods
We gave rats and mice dimethylaminopropyl chloride, hydrochloride by depositing the chemical dissolved in water directly into the animals' stomachs through a tube. Groups of 10 male and 10 female rats and mice were given doses of 6.25, 12.5, 25, 50, or 100 milligrams (mg) of dimethylaminopropyl chloride, hydrochloride per kilogram (kg) of body weight five days per week for 3 months. Similar groups of animals received just water on the same schedule and served as the controls. Tissues from approximately 40 sites were examined for each animal in the control and high-dose groups; tissues for the other animals were examined to a no-observed-effect level.

Results
Two female mice receiving 100 mg/kg and one male rat receiving 50 mg/kg died before the end of the study. Male rats and female mice receiving 50 mg/kg weighed less than the controls. Lung weights of female mice receiving 25, 50, or 100 mg/kg were significantly less than the lung weights of the controls. Serum bile acid concentrations for male rats receiving 50 mg/kg and for male and female rats receiving 100 mg/kg were greater than those of the controls. Male rats receiving 100 mg/kg had significantly increased rates of goblet cell hypertrophy of the nose.

Conclusions
Exposure to dimethylaminopropyl chloride, hydrochloride for 3 months caused goblet cell hypertrophy of the nose in male rats and increased serum bile acid concentrations in male and female rats. The highest doses of dimethylaminopropyl chloride, hydrochloride that did not result in any observable effects were 50 mg/kg per day for male rats and female mice, 100 to 200 mg/kg per day for female rats, and greater than 100 mg/kg per day for male mice.

ABSTRACT



DIMETHYLAMINOPROPYL CHLORIDE, HYDROCHLORIDE

CAS No. 5407-04-5

Chemical Formula: $C_5H_{12}ClN \cdot HCl$ Molecular Weight: 158.1

Synonyms: 3-Chloropropyldimethyl-ammonium chloride; (3-chloropropyl)dimethylamine, hydrochloride; *N*-(3-chloropropyl)-*N,N*-dimethylammonium chloride; 3-dimethylamino-1-propyl chloride hydrochloride; 3-dimethylaminopropyl chloride hydrochloride; DMPC; 1-propylamine, 3-chloro-*N,N*-dimethyl-, hydrochloride

Dimethylaminopropyl chloride, hydrochloride is used primarily as an industrial and research organic chemical intermediate acting as an alkylating reagent in Grignard and other types of reactions. It is also used as a pharmaceutical intermediate for the synthesis of many types of drugs, as an agricultural chemical intermediate, as a photographic chemical intermediate, and as a biochemical reagent for enzyme and other studies. Human occupational or other accidental exposure can occur by inhalation, ingestion, or skin absorption.

Male and female F344/N rats and B6C3F$_1$ mice received dimethylaminopropyl chloride, hydrochloride (greater than 99% pure) in water by gavage for 2 weeks or 3 months. Genetic toxicology studies were conducted in *Salmonella typhimurium* and mouse peripheral blood erythrocytes.

In the 2-week toxicity studies, groups of five male and five female F344/N rats and B6C3F$_1$ mice were administered doses of 0, 6.25, 12.5, 25, 50, or 100 mg dimethylaminopropyl chloride, hydrochloride/kg body weight in deionized water by gavage, 5 days per week for 16 days. All dosed male and female rats and mice survived until the end of the 2-week study; one vehicle control female mouse died early. Mean body weights of all dosed groups of rats and mice were similar to those of the vehicle control groups. No gross or microscopic lesions were considered related to dimethylaminopropyl chloride, hydrochloride administration.

In the 3-month toxicity studies, groups of 10 male and 10 female F344/N rats and B6C3F$_1$ mice were administered doses of 0, 6.25, 12.5, 25, 50, or 100 mg/kg in deionized water by gavage, 5 days per week for 3 months. One male rat in the 50 mg/kg group died during week 12 of the study, and one female mouse in the 100 mg/kg group died during week 9 and another during week 13. The final mean body weights of 50 mg/kg male rats and 50 mg/kg female mice were significantly less than those of the vehicle controls. Possible chemical-related clinical findings in rats included lethargy in one 50 mg/kg male and one 100 mg/kg male, tremors in one 100 mg/kg male, and ataxia in one 50 mg/kg male and two 100 mg/kg males. Absolute lung weights in the 25, 50, and 100 mg/kg groups of female mice were significantly less than those of the vehicle controls. Total serum bile acid concentrations were increased in 50 mg/kg male rats and 100 mg/kg male and female rats. The incidence of goblet cell hypertrophy of the nose was significantly increased in 100 mg/kg male rats compared to the vehicle controls. There were no significant histopathologic findings in mice.

Dimethylaminopropyl chloride, hydrochloride was mutagenic in the *Salmonella typhimurium* base substitution strains TA100 and TA1535, with and without hamster or rat liver S9 activation enzymes; no mutagenic activity was seen in TA97 or TA98. No increase in the frequency of micronucleated erythrocytes was seen in peripheral blood of male or female mice administered dimethylaminopropyl chloride, hydrochloride for 3 months by gavage.

In summary, dimethylaminopropyl chloride, hydrochloride caused increased incidences of goblet cell hypertrophy in the nose of male rats and increased serum bile acid concentrations in male and female rats. In mice, dimethylaminopropyl chloride, hydrochloride caused deaths in females administered 100 mg/kg. The estimated no-observed-effect levels were 50 mg/kg per day for male rats and female mice, 100 to 200 mg/kg per day for female rats, and greater than 100 mg/kg per day for male mice.

INTRODUCTION

CHEMICAL AND PHYSICAL PROPERTIES

Dimethylaminopropyl chloride, hydrochloride is a hygroscopic, white to off-white crystalline solid with a melting point of 141° to 144° C (MSDS, 1992; *Aldrich*, 1994). It is soluble in water (400 mg in 4 mL) giving a colorless to faintly yellowish clear solution (*Aldrich*, 1994).

PRODUCTION, USE, AND HUMAN EXPOSURE

Chloroalkylamine hydrochlorides, including dimethylaminopropyl chloride, hydrochloride, can be prepared by chlorination of the corresponding aminoalkyl ether hydrochloride with hydrochloric acid with the removal of water (STN, 1994). Further purification of this chemical may be accomplished by treating aqueous solutions with activated carbon (Woodrum and Barnett, 1975).

Dimethylaminopropyl chloride, hydrochloride has been listed in U.S. International Trade Commission publications (USITC, 1990, 1993). The USITC reporting guidelines specify that each company report quantities greater than 10,000 pounds or sales of $10,000 or more. In 1994, 31 companies were identified as suppliers of this chemical (STN, 1994).

Dimethylaminopropyl chloride, hydrochloride is a member of the class of nitrogen mustard-type compounds. It is used primarily as an industrial and research organic chemical intermediate acting as an alkylating reagent in Grignard and other types of reactions. It is also used as a pharmaceutical intermediate for the synthesis of many types of drugs including analgesics, antiarrythmics, antibiotics, anticholesteremics, antidepressants (anxiolytics, tranquilizers), antiischemics, and antineoplastics. Dimethylaminopropyl chloride, hydrochloride is also used as an agricultural chemical intermediate, a photographic chemical intermediate, and a biochemical reagent for enzyme and other studies (STN, 1994).

Human occupational or other accidental exposure could occur by inhalation, ingestion, or skin absorption. According to Soper *et al.* (1979), dimethylaminopropyl chloride, hydrochloride and its analogs are stable compounds and, when used as pharmaceutical intermediates, may persist through drug syntheses and remain as trace contaminants in the final products.

No standards or guidelines have been set for occupational exposure to or workplace maximum allowable levels of dimethylaminopropyl chloride, hydrochloride. The American Conference of Governmental Industrial Hygienists (2004) has not adopted a Threshold Limit Value (TLV) for this compound.

ABSORPTION, DISTRIBUTION, AND METABOLISM

No information on the metabolism of dimethylaminopropyl chloride, hydrochloride was found in the available literature. Nitrogen mustards are biotransformed to reactive electrophilic aziridinium ions (Williams and Weisburger, 1991); however, while dialkylaminoethyl chlorides probably exist in equilibrium with cyclic aziridinium ion intermediates at near neutral pH, dialkylaminopropyl chlorides do not (Thompson *et al.,* 1981).

Dimethylaminopropyl chloride, hydrochloride, a monofunctional nitrogen mustard-type chemical, has been shown to be a DNA alkylating agent, but weaker than bifunctional nitrogen mustards and without DNA-interstrand crosslinking ability (Bodell, 1990). While higher concentrations of monofunctional nitrogen mustard-type compounds are required for DNA cross-linking than related polyfunctional alkylating agents, exposure of cells to these compounds interferes with the progression of cells from S phase to metaphase (Wheeler *et al.*, 1970).

Dimethylaminopropyl chloride, hydrochloride is one of a group of aliphatic and aromatic amine compounds, which act as competitive inhibitors of human kidney histamine N-methyltransferase (Boudikova-Girard *et al.,* 1993). Dimethylaminopropyl chloride, hydrochloride, as the free base, inhibited this enzyme by 50% at a concentration of 0.32 mM.

Dimethylaminopropyl chloride, hydrochloride has been studied as one of a group of tertiary amine analogs of choline useful as substrates for investigations of mechanisms of membrane transport (Deves and Krupka, 1990). Dimethylaminopropyl chloride, hydrochloride migrates rapidly across the cell membrane of human erythrocytes (characteristic of lipid-soluble molecules) and, in the protonated form, is a strong inhibitor of choline uptake at the transport carrier site (61% inhibition). An observed decrease in flux ratio (the ratio of infinite-trans to zero-trans flux) indicates a preferential binding by dimethylaminopropyl chloride, hydrochloride at the inner carrier site. Dimethylaminopropyl chloride, hydrochloride has also been demonstrated to have high affinity as an inhibitor of choline uptake (55% inhibition; Porter *et al.,* 1992).

TOXICITY AND CARCINOGENICITY

Experimental Animals

No toxicity or carcinogenicity studies of dimethylaminopropyl chloride, hydrochloride in experimental animals were identified in the available literature.

Humans

Dimethylaminopropyl chloride, hydrochloride is an irritant to skin, eyes, and mucous membranes (MSDS, 1992). No epidemiology studies or case reports investigating the association of exposure to dimethylaminopropyl chloride, hydrochloride and a cancer risk in humans were identified in the available literature. According to the SmithKline Beecham Material Safety Data Sheet, no determination of carcinogen status has been made by the company or reported by the National Toxicology Program, the International Association for Research on Cancer, or the Occupational Safety and Health Administration (MSDS, 1992). Thompson *et al*. (1981) postulated that, based on results in a battery of short-term tests, some dialkylaminoalkyl chlorides are likely to be carcinogenic but the carcinogenic potential of the dialkylaminopropyl chlorides, including dimethylaminopropyl chloride, hydrochloride, is probably lower than that of the dialkylaminoethyl chloride analogs.

GENETIC TOXICITY

Thompson *et al*. (1981) conducted a comparative study of the mutagenicities of dialkylaminoethyl and dialkylaminopropyl compounds, including dimethylaminopropyl chloride, hydrochloride, in several strains of *Salmonella typhimurium* and under a variety of different testing protocols. Dimethylaminopropyl chloride, hydrochloride was found to be weakly mutagenic in *S. typhimurium* strains TA100 and TA1535 in a gradient plate test, but in a standard *Salmonella* mutagenicity assay, positive results were only seen in TA1535 with concentrations up to 1,800 µM. The dialkylaminoethyl compounds were markedly more mutagenic than the dialkylaminopropyl compounds, both in the number of *Salmonella* strains they mutated and in the lowest dose at which mutation was induced. Dimethylaminopropyl chloride, hydrochloride was also negative in tests for induction of unscheduled DNA synthesis in primary hepatocyte cultures derived from male F344 rats (Thompson *et al*., 1981).

Dean *et al*. (1985) reported that dimethylaminopropyl chloride, hydrochloride was mutagenic in *S. typhimurium* strains TA1535 and TA1537, with and without exogenous metabolic activation, and in TA100 in the presence of activating enzymes only. Dimethylaminopropyl chloride, hydrochloride was not mutagenic in *Escherichia coli* WP2 or WP2 uvrA, and it did not induce mitotic gene conversion in *S. cerevisiae* JD1 or chromosomal aberrations in rat liver epithelial cell lines RL_1 and RL_4.

In a chemical class study, Bodell (1990) reported mixed results in a variety of short-term tests with various structural analogs of dimethylaminopropyl chloride, hydrochloride. As expected, monofunctional analogs demonstrated DNA alkylating ability and induction of sister chromosome exchanges in a rat 9L transformed cell line, while bifunctional nitrogen mustards showed increased genotoxicity due to their ability to crosslink as well as alkylate.

STUDY RATIONALE

Dimethylaminopropyl chloride, hydrochloride was nominated for study based on the potential for human exposure through its use as an intermediate for the synthesis of a variety of drugs and agricultural chemicals and because there was no existing information on its toxicity.

Based on the use of dimethylaminopropyl chloride, hydrochloride as an intermediate in synthesis of drugs and agricultural chemicals, the oral route of administration is the primary route of widespread human exposure. Thus, the NTP conducted 2-week and 3-month toxicity studies in F344/N rats and B6C3F$_1$ mice using gavage as the administration route.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF DIMETHYLAMINOPROPYL CHLORIDE, HYDROCHLORIDE

Dimethylaminopropyl chloride, hydrochloride was obtained from Aldrich Chemical Company (Milwaukee, WI) in one lot (07530KG) that was used in the 2-week and 3-month studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and the study laboratory, BioReliance Corporation (Rockville, MD). Reports on analyses performed in support of the study of dimethylaminopropyl chloride, hydrochloride are on file at the National Institute of Environmental Health Sciences.

The chemical, a hygroscopic, white powder, was identified as dimethylaminopropyl chloride, hydrochloride by the analytical chemistry laboratory using Fourier transform infrared (FT-IR) and proton nuclear magnetic resonance (NMR) spectroscopy and by the study laboratory using IR spectroscopy and comparison against a reference standard from the same lot.

The purity of lot 07530KG was determined by the analytical chemistry laboratory and the study laboratory using gas chromatography. The analytical chemistry laboratory detected one major peak and one impurity peak; the impurity had an area of 0.53% relative to the total integrated peak area. Purity was determined by the study laboratory on two shipments of lot 07530KG using gas chromatography. For the first shipment, purity was determined to be 99.25% (at receipt) and 98.76% (within 30 days prior to the start of the 2-week study). A bulk purity reanalysis was performed (within 30 days prior to the start of the 3-month study) on a second shipment of lot 07530KG, and the relative purity of this bulk dimethylaminopropyl chloride, hydrochloride to its bulk reference standard sample (taken from the first shipment and stored at less than or equal to –20° C) was 100%. Two significant impurities were detected, and they constituted 0.46% and 0.57% of the bulk dimethylaminopropyl chloride, hydrochloride. The overall purity of lot 07530KG was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored at ambient temperature under a headspace of inert gas and protected from light and moisture. Stability of the bulk chemical was monitored during the 3-month study by the study laboratory using gas chromatography. No degradation of the bulk chemical was observed.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Dose formulations were prepared once for the 2-week studies and four times for the 3-month studies. The dose formulations were prepared by adding the appropriate amount of chemical, diluting to the specified volume with deionized water, and sonicating and/or mixing on a magnetic stirrer until dissolved. Aliquots of the dose formulations sufficient for daily dosing were stored at ambient temperature under a headspace of inert gas in amber glass vials sealed with Teflon®-lined butyl rubber stoppers and crimped aluminum caps for up to 35 days.

Homogeneity of the dose formulations was not confirmed as they were in the form of solutions. Stability studies of dose formulations of approximately 0.3 mg/mL were performed by the analytical chemistry laboratory using gas chromatography. Stability was confirmed for up to 35 days for dose formulations stored in amber glass vials sealed with Teflon®-lined septa and aluminum caps, at refrigerated (5° C) and ambient (approximately 25° C) temperatures, and for up to 7 days under simulated animal room conditions, exposed to air and light at room temperature.

Analyses of the dose formulations of dimethylaminopropyl chloride, hydrochloride were conducted by the study laboratory using gas chromatography. The aqueous solution of the test chemical was first neutralized with 1 N sodium hydroxide solution followed by extraction of the chemical with methylene chloride and quantification using gas chromatography. During the 2-week studies, the dose formulations were analyzed once; all six of the dose formulations for rats and mice were within 10% of the target concentrations (Table F2). All five animal room samples of these dose formulations for rats and mice were within 10% of the target. During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; animal room samples of these dose formulations were also analyzed (Table F3). All 18 dose formulations analyzed were within 10% of the target concentrations; 14 of 15 animal room samples for rats and all 15 animal room samples for mice were within 10% of target.

2-WEEK STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Quality Laboratory Animals and Services for Research (Germantown, NY). On receipt, the rats and mice were 4 to 5 weeks old. Animals were quarantined for 12 (rats) or 13 (mice) days and were 6 to 7 weeks old on the first day of the studies. Groups of five male and five female rats and mice were administered doses of 6.25, 12.5, 25, 50, or 100 mg dimethylaminopropyl chloride, hydrochloride/kg body weight, in deionized water by gavage, 5 days per week for 16 days; vehicle control animals received deionized water alone. The dosing volume was 5 mL/kg body weight for rats and 10 mL/kg for mice. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded daily for rats and mice. The animals were weighed

initially, on day 8, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Necropsies were performed on all rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Histopathologic examinations were performed on all gross lesions found in rats and mice.

3-MONTH STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Quality Laboratory Animals and Services for Research (Germantown, NY). On receipt, the rats and mice were 4 to 5 weeks old. Rats were quarantined for 12 (males) or 13 (females) days and mice were quarantined for 15 (males) or 16 (females) days; animals were 5 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Blood was collected from five male and five female control rats and sentinel mice at the end of the 3-month studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). All results were negative.

Groups of 10 male and 10 female core study rats and mice and 10 male and 10 female clinical pathology study rats were administered doses of 6.25, 12.5, 25, 50, or 100 mg dimethylaminopropyl chloride, hydrochloride/kg body weight, in deionized water by gavage, 5 days per week for 3 months; vehicle control animals received deionized water alone. The dosing volume was 5 mL/kg body weight for rats and 10 mL/kg for mice. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded weekly for rats and mice. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Blood was collected from the retroorbital sinus of clinical pathology rats on days 4 and 24 and from core study animals at the end of the studies for hematology (rats and mice) and clinical chemistry (rats); animals were anesthetized with a carbon dioxide:oxygen mixture. Hematology and clinical chemistry measurements were conducted by Laboratory Corporation of America (Burlington, NC). A Roche Hitachi 717^{TM} Chemistry Analyzer was used for clinical chemistry and an ABX Pentra 60^{TM} Analyzer was used for hematology measurements using the procedures described in the respective instrument manuals. The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm morphology/motility and vaginal cytology evaluations on vehicle control and 25, 50, and 100 mg/kg core study rats and mice. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were

moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Complete histopathologic examinations were performed on core study vehicle controls, 100 mg/kg core study animals, and animals that died early. For all remaining core study groups, tissues were examined to a no-observed-effect level. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on vehicle control and 100 mg/kg core study rats and mice and animals that died early. Table 1 lists the tissues and organs routinely examined.

Upon completion of the laboratory pathologist's histopathologic evaluation, 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 where quality assessment was performed. 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).

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Dimethylaminopropyl Chloride, Hydrochloride

2-Week Studies	3-Month Studies
Study Laboratory	
BioReliance Corporation (Rockville, MD)	BioReliance Corporation (Rockville, MD)
Strain and Species	
F344/N rats $B6C3F_1$ mice	F344/N rats $B6C3F_1$ mice
Animal Source	
Taconic Quality Laboratory Animals and Services for Research (Germantown, NY)	Taconic Quality Laboratory Animals and Services for Research (Germantown, NY)
Time Held Before Studies	
Rats: 12 days Mice: 13 days	Rats: 12 (males) or 13 (females) days Mice: 15 (males) or 16 (females) days
Average Age When Studies Began	
6 to 7 weeks	5 to 6 weeks
Date of First Dose	
Rats: August 31, 1999 Mice: September 1, 1999	Rats: June 12 (males) or 13 (females), 2000 Mice: June 15 (males) or 16 (females), 2000
Duration of Dosing	
16 days	13 to 14 weeks
Date of Last Dose	
Rats: September 15, 1999 Mice: September 16, 1999	Rats: September 11 (males) or 12 (females), 2000 Mice: September 13 (males) or 14 (females), 2000
Necropsy Dates	
Rats: September 16, 1999 Mice: September 17, 1999	Rats: September 12 (males) or 13 (females), 2000 Mice: September 14 (males) or 15 (females), 2000
Average Age at Necropsy	
8 to 9 weeks	19 to 20 weeks
Size of Study Groups	
5 males and 5 females	10 males and 10 females (core study rats and mice); 10 males and 10 females (clinical pathology rats)
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies
Animals per Cage	
Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 5 Mice: 1 (males) or 5 (females)

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Dimethylaminopropyl Chloride, Hydrochloride

2-Week Studies	3-Month Studies
Method of Animal Identification Tail tattoo	Tail tattoo
Diet Irradiated NTP-2000 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as 2-week studies
Water Tap water (Washington Suburban Sanitary Commission Potomac Plant) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 2-week studies
Cages Polycarbonate (Lab Products, Inc., Seaford, DE), changed twice per week	Same as 2-week studies, except changed once weekly for male mice
Bedding Irradiated heat-treated Sani-Chips® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed twice per week	Same as 2-week studies, except changed once weekly for male mice
Cage Filters Remay 2016 (Snow Filtration Co., West Chester, OH), changed once	Same as 2-week studies, except changed every 2 weeks
Racks Stainless steel (Lab Products, Inc., Seaford, DE), changed once	Same as 2-week studies, except changed every 2 weeks
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses 0, 6.25, 12.5, 25, 50, or 100 mg/kg in deionized water by gavage [dosing volume 5 mL/kg (rats) or 10 mL/kg (mice)]	0, 6.25, 12.5, 25, 50, or 100 mg/kg in deionized water by gavage [dosing volume 5 mL/kg (rats) or 10 mL/kg (mice)]
Type and Frequency of Observation Observed twice daily; animals were weighed initially, on day 8, and at the end of the studies; clinical findings were recorded daily.	Observed twice daily; animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded initially and weekly thereafter.
Method of Sacrifice Carbon dioxide asphyxiation	Same as 2-week studies
Necropsy Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Dimethylaminopropyl Chloride, Hydrochloride

2-Week Studies	3-Month Studies
Clinical Pathology	
None	Blood was collected from the retroorbital sinus of clinical pathology rats on days 4 and 24 and from core study animals at the end of the studies for hematology (rats and mice) and clinical chemistry (rats); animals were anesthetized with a carbon dioxide:oxygen mixture. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, platelets, and nucleated erythrocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, bile acids, triglycerides, cholesterol, and lipoprotein
Histopathology	
All gross lesions were examined.	Complete histopathology was performed on vehicle control and 100 mg/kg core study rats and mice and animals that died early. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone and marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice), heart, kidney, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina. Tissues were examined in the remaining core study groups to a no-observed-effect level.
Sperm Motility and Vaginal Cytology	
None	At the end of the studies, sperm samples were collected from male animals in the vehicle control, 25, 50, and 100 mg/kg groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid heads per cauda and per gram cauda, and epididymal spermatozoal motility. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from core study females administered 0, 25, 50, or 100 mg/kg for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.

STATISTICAL METHODS

Calculation and Analysis of Lesion Incidences

The incidences of lesions are presented in Appendix A as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test (Gart *et al.,* 1979), a procedure based on the overall proportion of affected animals, was used to determine significance.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across doses.

QUALITY ASSURANCE METHODS

The 3-month studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The Quality Assurance Unit of BioReliance Corporation performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al.* (1992). Dimethylaminopropyl chloride, hydrochloride was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA1535 either in buffer or S9 mix (metabolic activation enzymes

and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of dimethylaminopropyl chloride, hydrochloride. In the absence of toxicity, 10,000 µg/plate was selected as the high dose. All positive trials were repeated under the conditions that elicited the positive response.

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

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in up to 10 animals per dose group.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dose group and the vehicle control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month study were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call

is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The results presented in the Abstract of this Toxicity Report represent a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

RATS

2-WEEK STUDY

All male and female rats survived until the end of the study (Table 2). Mean body weights and body weight gains of all dosed groups of males and females were similar to those of the vehicle control groups. Abnormal breathing was observed in one 100 mg/kg female on days 11 to 14. On day 13, diarrhea was observed in the same female. The biological significance of these observations is not known.

Organ weights were similar to those of the vehicle controls in all dosed groups of males and females (Table C1). No gross or microscopic lesions were considered related to dimethylaminopropyl chloride, hydrochloride administration.

TABLE 2
Survival and Body Weights of Rats in the 2-Week Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	94 ± 5	164 ± 4	70 ± 4	
6.25	5/5	93 ± 4	166 ± 4	73 ± 2	101
12.5	5/5	96 ± 5	177 ± 9	81 ± 4	108
25	5/5	94 ± 3	169 ± 5	75 ± 5	103
50	5/5	95 ± 3	169 ± 5	74 ± 3	103
100	5/5	93 ± 4	165 ± 9	71 ± 4	100
Female					
0	5/5	90 ± 4	134 ± 2	44 ± 2	
6.25	5/5	91 ± 3	132 ± 3	41 ± 2	99
12.5	5/5	91 ± 4	132 ± 3	40 ± 2	98
25	5/5	89 ± 4	135 ± 2	46 ± 4	101
50	5/5	92 ± 4	134 ± 4	42 ± 3	100
100	5/5	89 ± 3	131 ± 3	42 ± 2	98

[a] Number of animals surviving at 17 days per number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test

Dose selection rationale: Based on the lack of toxicity in rats at the doses used in this study, doses of 0, 100, 200, 400, 800, or 1,000 mg of dimethylaminopropyl chloride, hydrochloride/kg body weight per day were selected for the 3-month study. Because all rats dosed with 200 mg/kg or more died in the first week of the 3-month study and the study was aborted, a new 3-month study was conducted with doses of 0, 6.25, 12.5, 25, 50, or 100 mg dimethylaminopropyl chloride, hydrochloride/kg body weight per day and the results are reported here.

3-Month Study

One male in the 50 mg/kg group died during week 12 of the study. The final mean body weight of 50 mg/kg males and the mean body weight gains of 50 and 100 mg/kg males were significantly less than those of the vehicle controls (Table 3; Figure 1). Possible chemical-related clinical findings included lethargy in one 50 mg/kg male and one 100 mg/kg male, tremors in one 100 mg/kg male, and ataxia in one 50 mg/kg male and two 100 mg/kg males.

The hematology and clinical chemistry data for rats in the 3-month toxicity study of dimethylaminopropyl chloride, hydrochloride are listed in Table B1. At week 14, there were small increases in serum total bile acid concentrations in the 50 mg/kg male and 100 mg/kg male and female rats. Other changes in the hematology and clinical chemistry data were sporadic and, at most, minimal and were not considered toxicologically relevant.

Differences in absolute and relative organ weights in males were not considered to be chemical related and were generally attributed to reduced body weights; no differences were observed in females (Table C2). No biologically significant changes in reproductive parameters were noted in males or females (Tables D1 and D2).

The incidence of goblet cell hypertrophy of the nose was significantly increased in 100 mg/kg males compared to that in the vehicle controls (vehicle control, 1/10; 6.25 mg/kg, 2/10; 12.5 mg/kg, 2/10; 25 mg/kg, 1/10; 50 mg/kg, 3/10; 100 mg/kg, 7/10; Table A1). Of the three sections of the nasal passages that are routinely examined (Levels I, II, and III), goblet cell hypertrophy was present in Level II. The respiratory epithelium lining the septum was minimally thickened and had an undulating surface (Plates 1 and 2). The cells were taller, and the goblet cells were larger and more prominent. Goblet cell hypertrophy is a common response to mucosal irritation; however, the mechanism for this response is unknown.

TABLE 3
Survival and Body Weights of Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	70 ± 4	331 ± 6	261 ± 5	
6.25	10/10	74 ± 2	329 ± 5	255 ± 5	99
12.5	10/10	73 ± 3	335 ± 7	262 ± 6	101
25	10/10	74 ± 2	333 ± 5	259 ± 7	101
50	9/10[c]	73 ± 2	306 ± 6*	232 ± 7**	92
100	10/10	77 ± 3	313 ± 6	236 ± 4**	95
Female					
0	10/10	82 ± 3	189 ± 4	107 ± 3	
6.25	10/10	83 ± 2	183 ± 3	100 ± 3	97
12.5	10/10	83 ± 2	188 ± 4	105 ± 4	99
25	10/10	73 ± 5	191 ± 4	118 ± 5	101
50	10/10	82 ± 2	192 ± 2	110 ± 3	101
100	10/10	83 ± 3	190 ± 4	107 ± 2	101

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's or Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 14 weeks per number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Week of death: 12

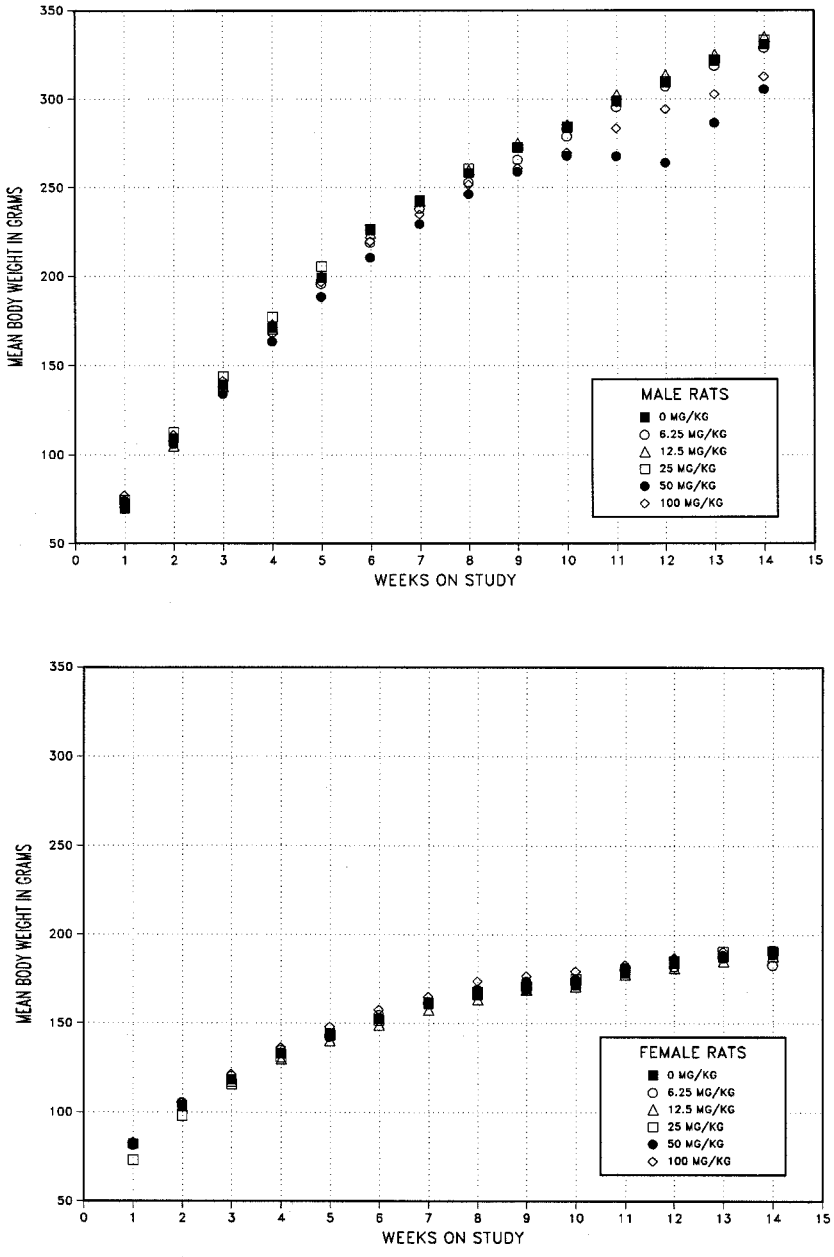


FIGURE 1
Body Weights of F344/N Rats Administered Dimethylaminopropyl Chloride, Hydrochloride by Gavage for 3 Months

MICE

2-WEEK STUDY

All dosed male and female mice survived until the end of the study; one vehicle control female died early (Table 4). Mean body weights and body weight gains of all dosed groups of males and females were similar to those of the vehicle control groups. There were no clinical findings related to chemical administration.

There were no biologically significant differences in organ weights between the dosed and vehicle control groups (Table C3). A single gross lesion in the seminal vesicle of a 100 mg/kg male was identified histopathologically as mild dilation. The lesion was not considered related to dimethylaminopropyl chloride, hydrochloride administration.

TABLE 4
Survival and Body Weights of Mice in the 2-Week Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	20.6 ± 1.2	25.6 ± 0.6	5.0 ± 0.8	
6.25	5/5	22.9 ± 1.0	26.1 ± 0.5	3.1 ± 0.7	102
12.5	5/5	22.1 ± 1.0	25.7 ± 0.7	3.6 ± 0.3	100
25	5/5	22.6 ± 1.4	25.0 ± 0.9	2.4 ± 1.0	98
50	5/5	21.9 ± 1.4	25.0 ± 1.0	3.1 ± 1.3	98
100	5/5	22.7 ± 1.0	24.9 ± 0.6	2.2 ± 0.7	97
Female					
0	4/5[c]	19.8 ± 0.7	20.9 ± 0.6	1.1 ± 0.3	
6.25	5/5	19.6 ± 0.5	20.8 ± 0.2	1.2 ± 0.4	99
12.5	5/5	19.7 ± 0.3	21.5 ± 0.2	1.8 ± 0.4	103
25	5/5	20.0 ± 0.7	21.4 ± 0.6	1.4 ± 0.2	102
50	5/5	20.1 ± 0.7	21.7 ± 0.7	1.6 ± 0.6	104
100	5/5	19.4 ± 0.6	19.8 ± 0.7	0.5 ± 0.3	95

[a] Number of animals surviving at 17 days per number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. Differences from the vehicle control group are not significant by Dunnett's test.
[c] Day of death: 13

Dose selection rationale: Based on the lack of toxicity in mice at the doses used in this study, doses of 0, 100, 200, 400, 800, or 1,000 mg of dimethylaminopropyl chloride, hydrochloride/kg body weight per day were selected for the 3-month study. Because all mice receiving 800 mg/kg and five males and all females receiving 400 mg/kg died during the first 2 days of the study and the study was aborted, a new 3-month study was conducted with doses of 0, 6.25, 12.5, 25, 50, or 100 mg dimethylaminopropyl chloride, hydrochloride/kg body weight per day and the results are reported here.

3-Month Study

Two females in the 100 mg/kg group died during the study, one during week 9 and the other during week 13 (Table 5). The final mean body weight of 50 mg/kg females and the mean body weight gains of all dosed groups of females were significantly less than those of the vehicle controls (Table 5; Figure 2). The final mean body weights of the remaining dosed groups of males and females were similar to those of the vehicle controls. The significantly lower mean body weight gains of dosed females were probably due to the fact that the initial mean body weight of the female vehicle control group was significantly lower than that of the dosed female groups. Clinical findings were limited to the thin appearance of one 50 mg/kg male.

The hematology data for mice in the 3-month toxicity study of dimethylaminopropyl chloride, hydrochloride are listed in Table B2. Minimal (approximately 5%) decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts occurred in the 100 mg/kg female mice; the males were unaffected. This minimal change in the erythron was characterized as normocytic and normochromic and, while this may suggest a treatment effect, would be of questionable clinical significance. There also were minimal increases in the platelet counts in the 100 mg/kg male (11%) and female (18%) mice. The mechanism for this apparent change was unknown, but the minimal nature would suggest that the clinical significance was questionable.

Absolute lung weights in the 25, 50, and 100 mg/kg female groups were significantly less than those of the vehicle controls (Table C4). There were no significant differences in reproductive parameters in males or females (Tables D3 and D4).

There were no significant histopathologic findings in males or females.

TABLE 5
Survival and Body Weights of Mice in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	24.3 ± 0.8	39.1 ± 1.6	14.8 ± 1.3	
6.25	10/10	24.0 ± 0.8	37.1 ± 1.7	13.0 ± 1.1	95
12.5	10/10	24.6 ± 0.6	38.6 ± 1.1	14.1 ± 0.8	99
25	10/10	24.0 ± 0.7	35.3 ± 1.2	11.3 ± 1.1	90
50	10/10	23.4 ± 0.9	38.8 ± 1.4	15.4 ± 1.0	99
100	10/10	24.8 ± 0.7	39.2 ± 1.6	14.4 ± 1.1	100
Female					
0	10/10	16.9 ± 0.7	30.9 ± 0.8	14.0 ± 1.3	
6.25	10/10	18.8 ± 0.3*	30.1 ± 1.0	11.3 ± 0.8*	97
12.5	10/10	19.2 ± 0.3**	29.0 ± 1.0	9.8 ± 0.8**	94
25	10/10	19.1 ± 0.4**	28.3 ± 0.7	9.2 ± 0.6**	92
50	10/10	19.2 ± 0.4**	28.0 ± 0.9*	8.9 ± 0.7**	91
100	8/10[c]	19.2 ± 0.4**	28.4 ± 1.2	8.9 ± 0.8**	92

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 14 weeks per number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Week of death: 9, 13

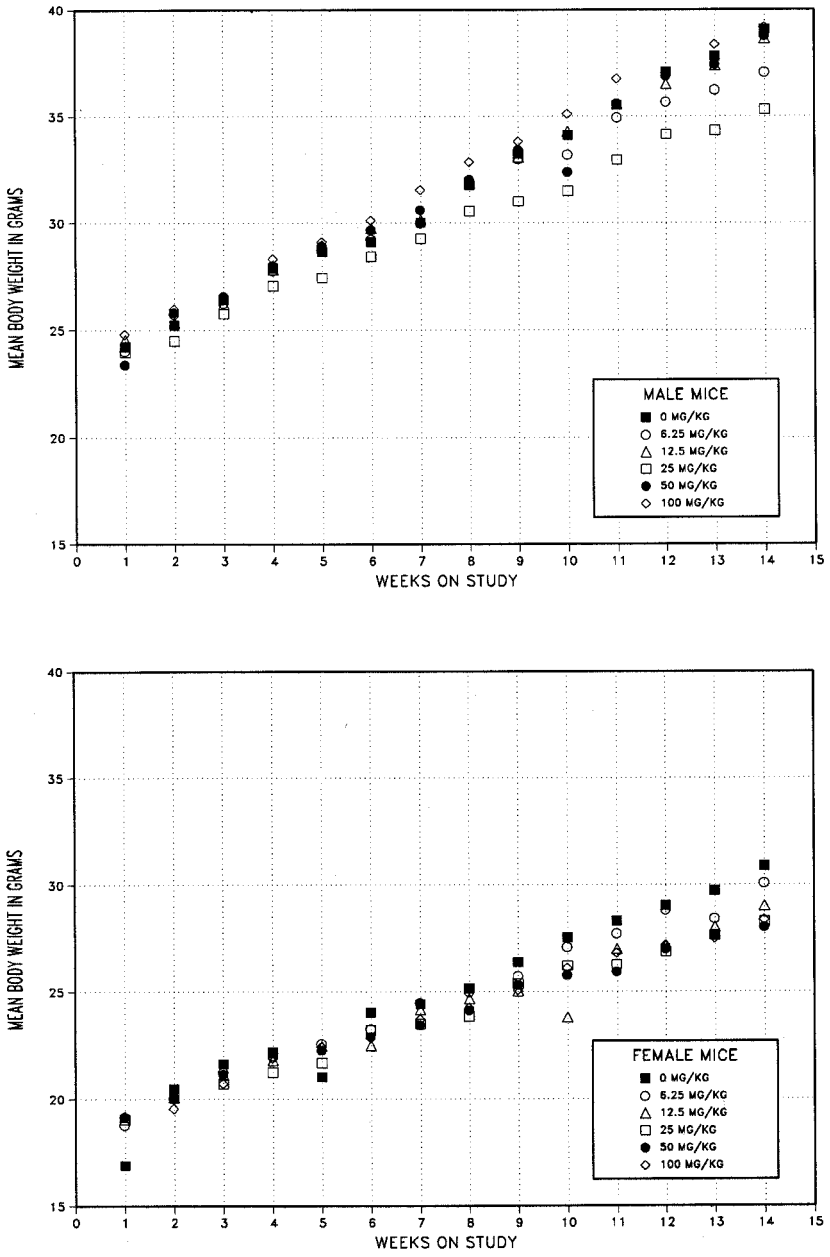


FIGURE 2
Body Weights of B6C3F$_1$ Mice Administered Dimethylaminopropyl Chloride, Hydrochloride by Gavage for 3 Months

GENETIC TOXICOLOGY

Dimethylaminopropyl chloride, hydrochloride (100 to 10,000 µg/plate) was mutagenic in *Salmonella typhimurium* strains TA100 and TA1535, with and without 30% hamster or rat liver S9 activation enzymes; these two strains mutate via base substitution (Table E1). No mutagenic activity was seen in the frameshift strains TA97 or TA98, with or without S9 (Table E1). No significant increases were seen in the frequencies of micronucleated erythrocytes in peripheral blood samples obtained from male and female mice in the 3-month study (Table E2). All dosed female mice showed mean values of micronucleated normochromatic erythrocytes (NCEs) higher than the vehicle control group frequency, but the differences were not significant. In male mice, all but one dosed group had higher mean frequencies of micronucleated NCEs compared to the vehicle controls, but again, none differed significantly.

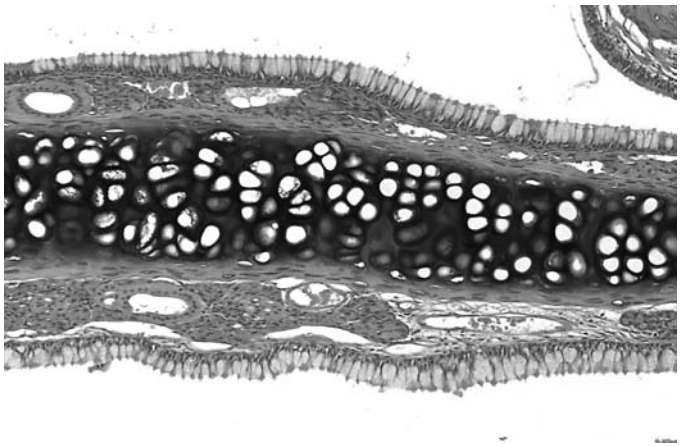


PLATE 1
Respiratory epithelium lining the nasal septum from a vehicle control male F344/N rat in the 3-month gavage study of dimethylaminopropyl chloride, hydrochloride. H&E; 40×

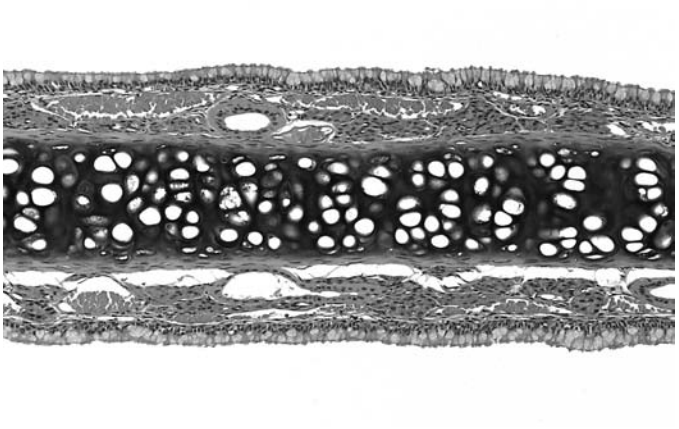


PLATE 2
Compared to the vehicle control (Plate 1), note the prominent goblet cells that are increased in size in a male F344/N rat administered 100 mg/kg dimethylaminopropyl chloride, hydrochloride by gavage for 3 months. H&E; 40×

DISCUSSION

Dimethylaminopropyl chloride, hydrochloride, a nitrogen mustard-type compound, was nominated for toxicological testing by the National Toxicology Program. Reasons for its nomination included high potential for human exposure resulting from its use as a chemical intermediate for the synthesis of a variety of chemicals and the fact that nitrogen mustard-type compounds are known to be genotoxic. Short-term toxicity studies were conducted by administering dimethylaminopropyl chloride, hydrochloride in deionized water by gavage at doses of 0, 6.25, 12.5, 25, 50, or 100 mg/kg body weight to F344/N rats and B6C3F$_1$ mice, five days per week for 2 weeks or 3 months.

In the 3-month rat study, no chemical-related deaths occurred. Lethargy, tremors, and ataxia were seen in two of the 10 males in the 50 mg/kg group and in three of the 10 males in the 100 mg/kg group. These clinical findings may be related to chemical administration because it has been reported that dimethylaminopropyl chloride, hydrochloride is a strong inhibitor of choline uptake (Porter *et al*., 1992), and this in turn may affect the synthesis and availability of the neurotransmitter acetylcholine. A significant negative dose-related trend in mean body weight gain occurred in males, and the body weight decrease in the 50 and 100 mg/kg groups was statistically significant compared to the vehicle control group. No statistically significant difference in body weight gain was observed in females. There was a significant dose-related decrease in the absolute thymus weight of males. However, pairwise comparison between dosed and vehicle control males did not reveal any significant results. In females, only the absolute and relative weights of the thymus in the 6.25 mg/kg group were significantly lower than those of the vehicle controls. Because the changes observed in thymus weights were not accompanied by histopathogic changes, they were not considered related to chemical administration.

No chemical-related changes occurred in hematology or clinical chemistry parameters measured except for the dose-related increase in total bile acids in male and female rats. While evaluation of bile acid concentration is often used as a marker of cholestasis, serum concentrations can be affected by mechanisms other than cholestasis. For example, altered enterohepatic circulation, impaired hepatic function, and noncholestatic liver injury can elevate circulating bile acid concentrations (Hofmann, 1988; Bai *et al*., 1992). In the current rat study, serum alkaline phosphatase activity, another marker of cholestasis, was unaffected, suggesting that the increase in serum bile concentration was probably not related to a cholestatic event. In addition, the serum alanine aminotransferase and sorbitol dehydrogenase activities, markers of hepatocellular injury, were unaffected in the current study. Thus, the mechanism for the increased bile acids may be related to some alteration of bile acid uptake or metabolism by the liver.

The only potential chemical-related lesion was minimal nasal goblet cell hypertrophy in male rats. The incidence in the 100 mg/kg males was significantly greater than that observed in the vehicle control group. The function of goblet cells is to secrete mucus in the nasal passages in response to various stimuli such as allergens. Mucous hypersecretion from surface epithelial cells may be stimulated by mucosal irritation. The cause for the goblet cell hypertrophy in this study was uncertain since there was no microscopic evidence of direct injury to the nasal epithelium. Goblet cell hypertrophy and hyperplasia are often seen in inhalation studies. For example, an increased rate of amount of mucous secretion is a common response to inhaled toxicants such as sulfur dioxide, ozone, nitrous oxide, and ammonia. The no-observed-effect level (NOEL) for male rats was estimated at 50 mg/kg based on the increased incidence of nasal lesions observed in male rats at the highest dose (100 mg/kg). The NOEL for female rats was estimated to be between 100 and 200 mg/kg per day.

In the 3-month mouse study, females appeared to be more sensitive to the chemical than males. Chemical-related mortality occurred in 100 mg/kg females (2 out of 10). No males died during the study. No chemical-related lesions were seen in mice. The NOEL for female mice was estimated at 50 mg/kg based on mortality observed at the higher dose. The estimated NOEL for male mice was estimated to be greater than 100 mg/kg per day.

In summary, dimethylaminopropyl chloride, hydrochloride caused increased incidences of goblet cell hypertrophy in the nose of male rats and increased serum bile acid concentrations in male and female rats. In mice, dimethylaminopropyl chloride, hydrochloride caused deaths in females administered 100 mg/kg. The estimated NOEL values were 50 mg/kg per day for male rats and female mice, 100 to 200 mg/kg per day for female rats, and greater than 100 mg/kg per day for male mice.

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APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS IN RATS AND MICE

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Natural death					1	
Survivors						
Terminal sacrifice	10	10	10	10	9	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule		1 (10%)				
Pancreas	(10)					(10)
Infiltration cellular, focal, lymphocyte	4 (40%)					4 (40%)
Cardiovascular System						
Heart	(10)					(10)
Myocardium, infiltration cellular, focal, lymphocyte						2 (20%)
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Lymph node				(1)		
Mediastinal hyperplasia				1 (100%)		
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Respiratory System						
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Goblet cell, hypertrophy	1 (10%)	2 (20%)	2 (20%)	1 (10%)	3 (30%)	7 (70%)
Special Senses System						
None						
Urinary System						
None						

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Died last week of study						1
Terminal sacrifice	10	10	10	10	10	9
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)					(10)
Hepatodiaphragmatic nodule	1 (10%)					
Pancreas	(10)					(10)
Infiltration cellular, focal, lymphocyte	2 (20%)					1 (10%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
Ovary	(10)	(1)	(1)			(10)
Cyst			1 (100%)			
Bilateral, cyst		1 (100%)				
Uterus	(10)					(10)
Hydrometra	2 (20%)					
Hematopoietic System						
Lymph node	(1)					
Mediastinal hyperplasia	1 (100%)					
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
None						

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10					10
Alimentary System						
Stomach, forestomach	(10)					(10)
Epithelium, hyperplasia, diffuse						1 (10%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(10)					(10)
Subcapsular, hyperplasia, focal	1 (10%)					1 (10%)
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Spleen	(10)					(10)
Hematopoietic cell proliferation						1 (10%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Special Senses System						
None						
Urinary System						
Kidney	(10)					(10)
Renal tubule, vacuolization cytoplasmic, focal	9 (90%)					8 (80%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Natural deaths						2
Survivors						
Died last week of study						1
Terminal sacrifice	10	10	10	10	10	7
Animals examined microscopically	10	1	1			10
Alimentary System						
Salivary glands	(10)					(8)
Infiltration cellular, focal, lymphocyte	1 (10%)					
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(10)					(7)
Subcapsular, hyperplasia, focal	10 (100%)					7 (100%)
Thyroid gland	(10)					(8)
Ectopic thymus	2 (20%)					1 (13%)
General Body System						
None						
Genital System						
Uterus	(10)					(8)
Endometrium, hyperplasia, cystic	1 (10%)					1 (13%)
Hematopoietic System						
Spleen	(10)					(7)
Hematopoietic cell proliferation	3 (30%)					1 (14%)
Thymus	(10)					(7)
Atrophy						1 (14%)
Integumentary System						
None						
Musculoskeletal System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Nervous System						
None						
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
Kidney	(10)					(8)
Hydronephrosis	1 (10%)					

APPENDIX B
CLINICAL PATHOLOGY RESULTS

TABLE B1
Clinical Pathology Data for Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male						
Hematology						
n						
Day 4	8	8	6	8	8	7
Day 24	10	9	10	10	10	10
Week 14	10	10	10	10	9	10
Hematocrit (%)						
Day 4	39.8 ± 0.8	39.5 ± 1.1	38.1 ± 0.9	40.4 ± 0.8	39.2 ± 0.6	41.2 ± 1.1
Day 24	46.8 ± 0.6	46.9 ± 0.8	47.3 ± 1.2	46.2 ± 0.5	46.6 ± 0.6	46.7 ± 0.5
Week 14	47.3 ± 0.5	47.6 ± 0.3	47.0 ± 0.4	48.4 ± 0.3	48.1 ± 0.7	48.5 ± 0.4
Hemoglobin (g/dL)						
Day 4	13.5 ± 0.3	13.3 ± 0.3	13.0 ± 0.3	13.6 ± 0.3	13.3 ± 0.2	13.9 ± 0.4
Day 24	15.7 ± 0.3	15.7 ± 0.2	15.7 ± 0.4	15.7 ± 0.1	15.5 ± 0.2	15.9 ± 0.2
Week 14	15.9 ± 0.2	15.9 ± 0.1	15.9 ± 0.1	16.1 ± 0.1	16.1 ± 0.2	16.4 ± 0.2*
Erythrocytes (10^6/µL)						
Day 4	6.22 ± 0.10	6.15 ± 0.16	5.93 ± 0.13	6.26 ± 0.15	6.10 ± 0.10	6.47 ± 0.15
Day 24	7.22 ± 0.12	7.24 ± 0.11	7.24 ± 0.18	7.07 ± 0.07	7.15 ± 0.12	7.13 ± 0.06
Week 14	8.36 ± 0.10	8.38 ± 0.05	8.32 ± 0.06	8.48 ± 0.06	8.33 ± 0.14	8.53 ± 0.06
Reticulocytes (10^6/µL)						
Day 4	0.48 ± 0.03	0.48 ± 0.02	0.46 ± 0.02	0.46 ± 0.02	0.49 ± 0.03	0.44 ± 0.02
Day 24	0.40 ± 0.02	0.37 ± 0.02	0.39 ± 0.03	0.37 ± 0.02	0.38 ± 0.02	0.34 ± 0.02
Week 14	0.22 ± 0.02	0.23 ± 0.01	0.23 ± 0.01	0.28 ± 0.02	0.31 ± 0.02	0.22 ± 0.01
Nucleated erythrocytes/100 leukocytes						
Day 4	0.50 ± 0.19	1.63 ± 0.32	0.83 ± 0.40	1.13 ± 0.40	0.75 ± 0.25	0.86 ± 0.40
Day 24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.10 ± 0.10	0.10 ± 0.10	0.30 ± 0.21	0.10 ± 0.10	0.33 ± 0.17	0.30 ± 0.15
Mean cell volume (fL)						
Day 4	64.0 ± 0.3	64.1 ± 0.3	64.5 ± 0.2	64.8 ± 0.3	64.1 ± 0.2	63.7 ± 0.3
Day 24	64.9 ± 0.3	64.8 ± 0.3	65.2 ± 0.3	65.2 ± 0.4	65.2 ± 0.4	65.7 ± 0.3
Week 14	56.8 ± 0.2	56.9 ± 0.2	56.5 ± 0.2	57.0 ± 0.1	57.8 ± 0.3	56.8 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	21.7 ± 0.2	21.6 ± 0.2	21.9 ± 0.1	21.6 ± 0.1	21.8 ± 0.2	21.5 ± 0.1
Day 24	21.8 ± 0.1	21.6 ± 0.1	21.7 ± 0.2	22.2 ± 0.2	21.8 ± 0.1	22.3 ± 0.1*
Week 14	19.0 ± 0.1	19.0 ± 0.1	19.1 ± 0.1	18.9 ± 0.1	19.3 ± 0.1	19.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.9 ± 0.3	33.6 ± 0.2	34.1 ± 0.1	33.5 ± 0.1	33.9 ± 0.2	33.8 ± 0.3
Day 24	33.6 ± 0.1	33.4 ± 0.2	33.2 ± 0.3	34.0 ± 0.3	33.4 ± 0.1	34.1 ± 0.2
Week 14	33.5 ± 0.1	33.4 ± 0.2	33.8 ± 0.1	33.2 ± 0.2	33.4 ± 0.2	33.81 ± 0.1
Platelets (10^3/µL)						
Day 4	784.50 ± 31.15	775.25 ± 18.21	723.83 ± 44.01	708.00 ± 56.98	767.13 ± 33.76	755.29 ± 44.59
Day 24	641.90 ± 28.67	692.89 ± 18.81	583.90 ± 22.69	565.40 ± 34.94	652.10 ± 32.55	623.10 ± 23.40
Week 14	490.20 ± 13.04	552.90 ± 22.42	527.70 ± 19.21	553.90 ± 12.12*	556.56 ± 10.79**	538.10 ± 13.96
Leukocytes (10^3/µL)						
Day 4	8.11 ± 0.29	7.78 ± 0.62	7.25 ± 0.33	8.06 ± 0.51	8.45 ± 0.46	8.69 ± 0.39
Day 24	8.74 ± 0.34	8.99 ± 0.68	8.50 ± 0.39	8.24 ± 0.56	8.68 ± 0.43	8.14 ± 0.54
Week 14	8.68 ± 0.52	9.99 ± 0.54	9.31 ± 0.36	9.89 ± 0.36	10.43 ± 0.35	9.53 ± 0.43
Segmented neutrophils (10^3/µL)						
Day 4	1.40 ± 0.14	1.07 ± 0.16	1.43 ± 0.16	1.45 ± 0.16	1.41 ± 0.11	1.38 ± 0.19
Day 24	1.30 ± 0.13	1.49 ± 0.27	1.01 ± 0.15	1.15 ± 0.11	1.23 ± 0.10	1.21 ± 0.15
Week 14	1.17 ± 0.11	1.79 ± 0.10**	1.47 ± 0.12	1.44 ± 0.16	1.63 ± 0.19	1.69 ± 0.15

TABLE B1
Clinical Pathology Data for Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	8	8	6	8	8	7
Day 24	10	9	10	10	10	10
Week 14	10	10	10	10	9	10
Lymphocytes (10^3/μL)						
Day 4	6.70 ± 0.29	6.69 ± 0.63	5.81 ± 0.27	6.56 ± 0.43	7.02 ± 0.45	7.24 ± 0.38
Day 24	7.05 ± 0.36	7.10 ± 0.48	6.99 ± 0.37	6.73 ± 0.49	6.99 ± 0.36	6.66 ± 0.40
Week 14	7.46 ± 0.51	8.12 ± 0.55	7.77 ± 0.29	8.37 ± 0.38	8.78 ± 0.40	7.72 ± 0.37
Atypical lymphocytes (10^3/μL)						
Day 4	0.00 ± 0.00	0.00 ± 0.000	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 24	0.00 ± 0.00	0.00 ± 0.000	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.000	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes (10^3/μL)						
Day 4	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.03	0.01 ± 0.01	0.05 ± 0.03
Day 24	0.37 ± 0.07	0.38 ± 0.06	0.46 ± 0.05	0.30 ± 0.06	0.39 ± 0.06	0.25 ± 0.06
Week 14	0.02 ± 0.01	0.03 ± 0.02	0.06 ± 0.02	0.04 ± 0.02	0.01 ± 0.01	0.06 ± 0.05
Basophils (10^3/μL)						
Day 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 24	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)						
Day 4	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
Day 24	0.03 ± 0.01	0.02 ± 0.02	0.04 ± 0.02	0.06 ± 0.02	0.07 ± 0.03	0.02 ± 0.01
Week 14	0.03 ± 0.01	0.05 ± 0.02	0.01 ± 0.01	0.05 ± 0.02	0.01 ± 0.01	0.06 ± 0.02
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 24	10	9	10	10	10	10
Week 14	10	10	10	10	9	10
Urea nitrogen (mg/dL)						
Day 4	12.9 ± 0.7	13.1 ± 0.6	12.8 ± 0.5	14.1 ± 0.4	13.3 ± 0.3	12.7 ± 0.4
Day 24	12.3 ± 0.3	13.0 ± 0.5	12.4 ± 0.5	12.6 ± 0.4	12.9 ± 0.5	12.9 ± 0.6
Week 14	15.9 ± 0.4	16.5 ± 0.3	16.1 ± 0.5	14.5 ± 0.6	15.0 ± 0.5	16.6 ± 0.5
Creatinine (mg/dL)						
Day 4	0.30 ± 0.02	0.31 ± 0.03	0.31 ± 0.02	0.31 ± 0.02	0.31 ± 0.02	0.29 ± 0.02
Day 24	0.35 ± 0.02	0.38 ± 0.02	0.37 ± 0.02	0.34 ± 0.02	0.36 ± 0.02	0.38 ± 0.01
Week 14	0.49 ± 0.01	0.49 ± 0.02	0.48 ± 0.01	0.46 ± 0.02	0.42 ± 0.02**	0.46 ± 0.02*
Total protein (g/dL)						
Day 4	5.4 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.4 ± 0.1
Day 24	6.3 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.3 ± 0.1	6.3 ± 0.1
Week 14	7.0 ± 0.1	7.0 ± 0.1	7.1 ± 0.0	7.2 ± 0.1	6.9 ± 0.1	7.2 ± 0.1
Albumin (g/dL)						
Day 4	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.1 ± 0.1
Day 24	4.4 ± 0.1	4.5 ± 0.0	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0	4.3 ± 0.0
Week 14	4.7 ± 0.1	4.7 ± 0.0	4.7 ± 0.0	4.8 ± 0.0	4.7 ± 0.0	4.8 ± 0.0*

TABLE B1
Clinical Pathology Data for Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	10
Day 24	10	9	10	10	10	10
Week 14	10	10	10	10	9	10
Globulin (g/dL)						
Day 4	1.3 ± 0.0	1.4 ± 0.0	1.3 ± 0.0	1.3 ± 0.1	1.3 ± 0.0	1.3 ± 0.1
Day 24	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.0 ± 0.0	1.9 ± 0.0	2.0 ± 0.1
Week 14	2.3 ± 0.1	2.4 ± 0.0	2.4 ± 0.0	2.4 ± 0.0	2.3 ± 0.1	2.4 ± 0.0
A/G ratio						
Day 4	3.0 ± 0.1	2.9 ± 0.1	3.2 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	3.2 ± 0.1
Day 24	2.2 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.2 ± 0.1
Week 14	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.1 ± 0.1	2.0 ± 0.0
Cholesterol (mg/dL)						
Day 4	116 ± 2	114 ± 2	110 ± 1	112 ± 3	109 ± 3	108 ± 1
Day 24	90 ± 2	92 ± 3	93 ± 3	89 ± 1	88 ± 2	88 ± 2
Week 14	88 ± 2	86 ± 2	88 ± 1	88 ± 2	90 ± 2	87 ± 2
Triglyceride (mg/dL)						
Day 4	74 ± 4	90 ± 8	97 ± 9	106 ± 8**	92 ± 6	88 ± 5
Day 24	117 ± 10	130 ± 16	122 ± 11	155 ± 16	125 ± 11	144 ± 13
Week 14	150 ± 27	179 ± 15	133 ± 8	181 ± 20	182 ± 14	163 ± 17
High-density lipoproteins (mg/dL)						
Day 4	73.200 ± 1.705	71.375 ± 1.772[b]	67.750 ± 1.709*[b]	72.111 ± 1.448[c]	68.375 ± 1.832[b]	66.556 ± 1.492**[c]
Day 24	65.550 ± 1.871	69.133 ± 1.684	69.270 ± 2.296	67.410 ± 1.938	65.260 ± 1.767	62.970 ± 1.541
Week 14	71.140 ± 3.611	66.480 ± 1.471	64.710 ± 0.648	66.270 ± 2.066	66.600 ± 2.541	65.370 ± 1.464
Low-density lipoproteins (mg/dL)						
Day 4	27.880 ± 1.686	24.225 ± 2.354[b]	24.050 ± 2.017[b]	16.978 ± 2.759**[c]	22.550 ± 2.186[b]	23.733 ± 1.380[c]
Day 24	3.090 ± 0.968	6.556 ± 2.186	7.470 ± 1.409	10.870 ± 3.085*	5.800 ± 1.545	7.070 ± 1.538
Week 14	16.080 ± 5.114	16.740 ± 2.275	6.330 ± 1.121	15.710 ± 3.802	15.022 ± 2.649	13.030 ± 2.678
Very-low-density lipoproteins (mg/dL)						
Day 4	14.820 ± 0.771	18.020 ± 1.573	19.480 ± 1.823	21.260 ± 1.528**	18.480 ± 1.167	17.680 ± 0.955
Day 24	23.320 ± 2.011	26.089 ± 3.145	24.440 ± 2.155	30.960 ± 3.159	24.920 ± 2.195	28.780 ± 2.542
Week 14	30.020 ± 5.303	35.700 ± 3.081	26.540 ± 1.515	36.140 ± 3.905	36.356 ± 2.867	32.560 ± 3.380
Alkaline aminotransferase (IU/L)						
Day 4	80 ± 2	84 ± 4	82 ± 2	84 ± 2	83 ± 3	75 ± 2
Day 24	58 ± 1	56 ± 2	59 ± 2	59 ± 2	57 ± 2	58 ± 2
Week 14	101 ± 8	98 ± 8	83 ± 5	99 ± 10	89 ± 6	80 ± 6
Alkaline phosphatase (IU/L)						
Day 4	827 ± 23	892 ± 16	855 ± 26	854 ± 19	860 ± 19	801 ± 14
Day 24	655 ± 10	638 ± 13	634 ± 16	638 ± 11	595 ± 15**	605 ± 14**
Week 14	285 ± 8	282 ± 5	274 ± 5	279 ± 4	302 ± 5	270 ± 7
Creatine kinase (IU/L)						
Day 4	618 ± 80	768 ± 188	751 ± 184	884 ± 222	710 ± 134[c]	639 ± 134[c]
Day 24	442 ± 65[c]	446 ± 64	363 ± 48	464 ± 69	607 ± 121	1041 ± 336
Week 14	301 ± 21	284 ± 25	264 ± 24	228 ± 20	205 ± 18*	291 ± 27
Sorbitol dehydrogenase (IU/L)						
Day 4	13 ± 1	13 ± 1	14 ± 1	12 ± 1	14 ± 1	14 ± 1
Day 24	21 ± 1	23 ± 1	21 ± 1	20 ± 1	19 ± 1	19 ± 1
Week 14	38 ± 2	40 ± 3	39 ± 1	42 ± 3	40 ± 2	37 ± 2

TABLE B1
Clinical Pathology Data for Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	10
Day 24	10	9	10	10	10	10
Week 14	10	10	10	10	9	10
Total bile acids (µmol/L)						
Day 4	28.4 ± 2.8	32.4 ± 3.4	34.7 ± 3.2	30.6 ± 3.1	30.9 ± 3.7	28.3 ± 2.3
Day 24	37.1 ± 1.8	27.6 ± 2.2	26.2 ± 2.3*	33.0 ± 1.9	27.2 ± 2.5*	31.6 ± 3.8
Week 14	25.3 ± 1.4	27.3 ± 2.4	28.5 ± 1.9	28.4 ± 2.4	33.1 ± 2.2*	37.1 ± 2.6**
Female						
Hematology						
n						
Day 4	8	8	8	10	9	9
Day 24	10	9	8	9	9	9
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 4	45.2 ± 1.3	47.0 ± 1.1	42.9 ± 0.6	44.6 ± 1.3	43.7 ± 0.7	46.4 ± 1.1
Day 24	48.7 ± 1.0	48.0 ± 0.7	48.4 ± 0.6	48.4 ± 0.4	49.2 ± 0.5	49.0 ± 0.5
Week 14	49.6 ± 0.6	48.7 ± 0.3	49.4 ± 0.4	48.1 ± 0.4*	48.3 ± 0.3	49.1 ± 0.2
Hemoglobin (g/dL)						
Day 4	15.2 ± 0.4	15.6 ± 0.4	14.4 ± 0.2	15.1 ± 0.4	14.7 ± 0.2	15.7 ± 0.4
Day 24	16.2 ± 0.3	16.0 ± 0.2	16.0 ± 0.2	15.8 ± 0.1	16.2 ± 0.1	16.2 ± 0.1
Week 14	16.5 ± 0.2	16.4 ± 0.1	16.5 ± 0.1	16.1 ± 0.1	16.2 ± 0.1	16.4 ± 0.1
Erythrocytes (10^6/µL)						
Day 4	7.17 ± 0.21	7.36 ± 0.19	6.73 ± 0.10	7.02 ± 0.22	6.87 ± 0.11	7.32 ± 0.18
Day 24	7.49 ± 0.16	7.37 ± 0.10	7.49 ± 0.10	7.47 ± 0.07	7.53 ± 0.09	7.55 ± 0.11
Week 14	8.12 ± 0.10	8.07 ± 0.07	8.12 ± 0.07	7.89 ± 0.06	7.93 ± 0.06	8.07 ± 0.04
Reticulocytes (10^6/µL)						
Day 4	0.43 ± 0.04	0.45 ± 0.04	0.44 ± 0.02	0.44 ± 0.03	0.46 ± 0.02	0.47 ± 0.04
Day 24	0.30 ± 0.02	0.26 ± 0.02	0.26 ± 0.02	0.25 ± 0.02	0.26 ± 0.01	0.25 ± 0.01
Week 14	0.19 ± 0.02	0.17 ± 0.03	0.20 ± 0.01	0.21 ± 0.02	0.21 ± 0.02	0.21 ± 0.01
Nucleated erythrocytes/100 leukocytes						
Day 4	0.63 ± 0.26	0.50 ± 0.27	0.38 ± 0.18	0.70 ± 0.26	0.78 ± 0.28	0.44 ± 0.18
Day 24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.20 ± 0.13	0.10 ± 0.10	0.20 ± 0.13	0.10 ± 0.10	0.30 ± 0.21	0.00 ± 0.00
Mean cell volume (fL)						
Day 4	63.0 ± 0.3	64.0 ± 0.3*	63.9 ± 0.3	63.5 ± 0.2	63.8 ± 0.2	63.4 ± 0.2
Day 24	65.1 ± 0.2	65.2 ± 0.4	64.6 ± 0.3	64.9 ± 0.5	65.3 ± 0.5	64.8 ± 0.4
Week 14	61.0 ± 0.1	60.3 ± 0.3	60.9 ± 0.1	60.9 ± 0.1	61.0 ± 0.2	61.0 ± 0.1
Mean cell hemoglobin (pg)						
Day 4	21.2 ± 0.2	21.2 ± 0.1	21.5 ± 0.1	21.5 ± 0.2	21.4 ± 0.1	21.4 ± 0.1
Day 24	21.7 ± 0.2	21.7 ± 0.1	21.4 ± 0.1	21.2 ± 0.2	21.5 ± 0.2	21.4 ± 0.2
Week 14	20.3 ± 0.1	20.3 ± 0.1	20.4 ± 0.1	20.4 ± 0.0	20.4 ± 0.1	20.3 ± 0.0

TABLE B1
Clinical Pathology Data for Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	8	8	8	10	9	9
Day 24	10	9	8	9	9	9
Week 14	10	10	10	10	10	10
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.6 ± 0.2	33.2 ± 0.2	33.6 ± 0.2	33.8 ± 0.3	33.6 ± 0.2	33.8 ± 0.2
Day 24	33.3 ± 0.3	33.3 ± 0.2	33.1 ± 0.2	32.7 ± 0.2	32.8 ± 0.2	33.0 ± 0.2
Week 14	33.2 ± 0.2	33.6 ± 0.1	33.5 ± 0.1	33.5 ± 0.1	33.5 ± 0.2	33.4 ± 0.1
Platelets (10^3/µL)						
Day 4	780.6 ± 38.3	654.1 ± 89.2	732.6 ± 24.3	683.3 ± 46.6	811.6 ± 21.3	753.9 ± 48.1
Day 24	614.9 ± 35.5	538.2 ± 23.2	592.6 ± 21.1	598.2 ± 33.0	539.8 ± 30.9	556.1 ± 33.0
Week 14	525.3 ± 12.5	500.6 ± 15.2	517.4 ± 11.9	519.7 ± 10.7	497.7 ± 15.1	513.1 ± 11.8
Leukocytes (10^3/µL)						
Day 4	8.83 ± 0.41	8.03 ± 0.46	8.10 ± 0.64	7.55 ± 0.45	7.97 ± 0.39	8.60 ± 0.80
Day 24	9.02 ± 0.62	9.08 ± 0.91	8.88 ± 0.83	8.78 ± 0.60	8.82 ± 0.69	9.02 ± 0.77
Week 14	8.52 ± 0.43	8.82 ± 0.30	7.39 ± 0.49	7.88 ± 0.42	7.49 ± 0.28	8.17 ± 0.59
Segmented neutrophils (10^3/µL)						
Day 4	0.99 ± 0.08	1.03 ± 0.09	0.76 ± 0.11	0.88 ± 0.12	0.88 ± 0.06	0.94 ± 0.12
Day 24	1.54 ± 0.19	1.39 ± 0.15	1.39 ± 0.21	1.55 ± 0.24	1.11 ± 0.14	1.66 ± 0.24
Week 14	1.03 ± 0.09	1.22 ± 0.14	1.02 ± 0.14	0.91 ± 0.11	0.82 ± 0.08	1.12 ± 0.13
Lymphocytes (10^3/µL)						
Day 4	7.80 ± 0.44	6.94 ± 0.43	7.31 ± 0.61	6.63 ± 0.46	7.03 ± 0.36	7.62 ± 0.73
Day 24	7.11 ± 0.54	7.23 ± 0.87	7.09 ± 0.75	6.75 ± 0.58	7.24 ± 0.57	6.77 ± 0.52
Week 14	7.26 ± 0.39	7.50 ± 0.32	6.30 ± 0.42	6.88 ± 0.33	6.61 ± 0.31	6.98 ± 0.51
Atypical lymphocytes (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 24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes (10^3/µL)						
Day 4	0.03 ± 0.02	0.04 ± 0.03	0.03 ± 0.02	0.00 ± 0.00	0.02 ± 0.01	0.02 ± 0.01
Day 24	0.30 ± 0.05	0.44 ± 0.08	0.38 ± 0.08	0.41 ± 0.06	0.38 ± 0.09	0.53 ± 0.08
Week 14	0.17 ± 0.06	0.06 ± 0.03	0.06 ± 0.03	0.08 ± 0.02	0.03 ± 0.02	0.03 ± 0.02
Basophils (10^3/µL)						
Day 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 24	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)						
Day 4	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.02
Day 24	0.07 ± 0.03	0.03 ± 0.01	0.02 ± 0.02	0.06 ± 0.02	0.10 ± 0.05	0.06 ± 0.02
Week 14	0.07 ± 0.02	0.04 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.04 ± 0.02

TABLE B1
Clinical Pathology Data for Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Female (continued)						
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	9
Day 24	10	9	9	9	8	10
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	12.4 ± 0.7	12.4 ± 0.7	14.2 ± 0.6	14.4 ± 0.8	14.7 ± 0.5	13.4 ± 0.8
Day 24	15.5 ± 0.3	15.0 ± 0.7	15.8 ± 0.7	14.8 ± 0.5	15.9 ± 0.5	15.5 ± 0.4
Week 14	15.8 ± 0.4	16.4 ± 0.5	16.5 ± 0.4	16.1 ± 0.6	14.8 ± 0.5	16.1 ± 0.7
Creatinine (mg/dL)						
Day 4	0.34 ± 0.02	0.39 ± 0.02	0.33 ± 0.02	0.33 ± 0.02	0.33 ± 0.02	0.33 ± 0.02
Day 24	0.40 ± 0.03	0.37 ± 0.02	0.40 ± 0.03	0.37 ± 0.02	0.39 ± 0.02	0.40 ± 0.02
Week 14	0.51 ± 0.01	0.48 ± 0.01	0.53 ± 0.02	0.51 ± 0.02	0.53 ± 0.02	0.49 ± 0.02
Total protein (g/dL)						
Day 4	6.1 ± 0.1	6.4 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1
Day 24	6.8 ± 0.1	6.5 ± 0.1**	6.3 ± 0.1**	6.4 ± 0.1**	6.4 ± 0.1**	6.3 ± 0.1**
Week 14	7.1 ± 0.1	6.8 ± 0.1	7.2 ± 0.1	6.8 ± 0.1	7.0 ± 0.1	6.8 ± 0.1
Albumin (g/dL)						
Day 4	4.6 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.7 ± 0.0	4.7 ± 0.1
Day 24	4.7 ± 0.0	4.6 ± 0.0	4.5 ± 0.1**	4.6 ± 0.0*	4.5 ± 0.1*	4.5 ± 0.1**
Week 14	5.1 ± 0.1	4.9 ± 0.1	5.2 ± 0.1	5.0 ± 0.1	5.1 ± 0.1	4.8 ± 0.0
Globulin (g/dL)						
Day 4	1.6 ± 0.1	1.7 ± 0.0	1.4 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.5 ± 0.1
Day 24	2.0 ± 0.0	1.9 ± 0.1	1.8 ± 0.1**	1.8 ± 0.0*	1.9 ± 0.0	1.8 ± 0.0*
Week 14	2.0 ± 0.0	1.9 ± 0.0	2.0 ± 0.1	1.9 ± 0.0	1.9 ± 0.0	2.0 ± 0.0
A/G ratio						
Day 4	3.0 ± 0.1	2.8 ± 0.1	3.3 ± 0.1	3.2 ± 0.1*	3.3 ± 0.1	3.2 ± 0.1
Day 24	2.3 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.4 ± 0.0	2.5 ± 0.1
Week 14	2.5 ± 0.0	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.0	2.7 ± 0.0	2.5 ± 0.0
Cholesterol (mg/dL)						
Day 4	111 ± 2	118 ± 4	109 ± 3	110 ± 4	111 ± 3	103 ± 3
Day 24	84 ± 2	82 ± 3	77 ± 2	82 ± 2	83 ± 2	85 ± 3
Week 14	91 ± 2	78 ± 4*	95 ± 3	84 ± 2	85 ± 3	84 ± 2
Triglyceride (mg/dL)						
Day 4	108 ± 10	103 ± 11	108 ± 7	116± 10	112 ± 12	104 ± 11
Day 24	76 ± 11	86 ± 19	75 ± 9	80 ± 7	86 ± 7	88 ± 8
Week 14	75 ± 11	137 ± 21*	96 ± 13	98 ± 8	71 ± 5	76 ± 7
High density lipoproteins (mg/dL)						
Day 4	82.537 ± 3.259[b]	82.540 ± 4.458[d]	80.200 ± 4.320[e]	79.950 ± 3.415[b]	74.111 ± 4.530[c]	74.200 ± 3.225[b]
Day 24	65.750 ± 1.333	60.889 ± 1.932	56.800 ± 1.556**	62.600 ± 1.846	60.238 ± 1.465	63.590 ± 2.190
Week 14	69.960 ± 2.221	59.020 ± 2.690**	71.980 ± 2.021	63.990 ± 2.152	64.050 ± 1.988	62.350 ± 1.649
Low density lipoproteins (mg/dL)						
Day 4	12.313 ± 2.349[b]	14.740 ± 3.482[d]	9.343 ± 2.059[e]	13.225 ± 2.387[b]	16.111 ± 4.296[c]	11.343 ± 1.876[e]
Day 24	6.370 ± 1.171	7.911 ± 1.466	6.711 ± 1.262	5.022 ± 0.879	5.738 ± 1.545	5.230 ± 1.214
Week 14	7.760 ± 1.549	13.980 ± 3.647	7.360 ± 1.949	4.150 ± 1.124	7.150 ± 1.444	6.770 ± 1.577
Very low density lipoproteins (mg/dL)						
Day 4	21.580 ± 2.067	20.560 ± 2.215	21.680 ± 1.498	23.200 ± 2.046	22.440 ± 2.424	20.800 ± 2.113
Day 24	15.100 ± 2.197	17.289 ± 3.732	14.978 ± 1.754	15.956 ± 1.338	17.225 ± 1.489	17.640 ± 1.672
Week 14	15.000 ± 2.139	27.420 ± 4.266*	19.120 ± 2.647	19.500 ± 1.670	14.140 ± 1.035	15.280 ± 1.434

TABLE B1
Clinical Pathology Data for Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	9
Day 24	10	9	9	9	8	10
Week 14	10	10	10	10	10	10
Alanine aminotransferase (IU/L)						
Day 4	70 ± 2	76 ± 3	72 ± 2	75 ± 2	77 ± 2	77 ± 3
Day 24	50 ± 5	47 ± 2	59 ± 7	46 ± 2	49 ± 1	50 ± 2
Week 14	57 ± 5	81 ± 12	83 ± 9	63 ± 5	49 ± 4	42 ± 2
Alkaline phosphatase (IU/L)						
Day 4	668 ± 16	731 ± 23	670 ± 11	680 ± 16	690 ± 11	680 ± 20
Day 24	456 ± 8	442 ± 11	441 ± 9	446 ± 12	437 ± 8	458 ± 8
Week 14	239 ± 7	283 ± 19	247 ± 5	226 ± 6	233 ± 6	212 ± 6*
Creatine kinase (IU/L)						
Day 4	379 ± 50	640 ± 229	633 ± 188	334 ± 52[c]	309 ± 50	511 ± 112
Day 24	743 ± 193	832 ± 201	743 ± 115	403 ± 58	747 ± 183	696 ± 95
Week 14	263 ± 34	288 ± 36	259 ± 31	300 ± 34	307 ± 31	416 ± 48
Sorbitol dehydrogenase (IU/L)						
Day 4	16 ± 1	19 ± 2	17 ± 2	20 ± 1	18 ± 2	16 ± 1
Day 24	26 ± 3	19 ± 2	32 ± 7	23 ± 2	20 ± 2	21 ± 3
Week 14	34 ± 2	34 ± 3	40 ± 2	34 ± 2	33 ± 1	26 ± 2*
Total bile acids (µmol/L)						
Day 4	19.180 ± 0.764	26.690 ± 2.111*	28.710 ± 1.557**	25.420 ± 1.901	21.810 ± 1.171	33.867 ± 4.091**
Day 24	33.770 ± 5.691	31.011 ± 2.823	38.222 ± 5.148	29.044 ± 1.375	29.550 ± 3.041	45.420 ± 4.424
Week 14	23.750 ± 3.027	22.450 ± 1.435	28.170 ± 3.450	28.000 ± 3.633	24.210 ± 3.159	38.790 ± 3.365**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
**$P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=8
[c] n=9
[d] n=5
[e] n=7

TABLE B2
Hematology Data for Mice in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male						
Hematology						
n	10	10	10	10	10	10
Hematocrit (%)	49.0 ± 0.6	49.0 ± 0.9	49.0 ± 0.7	47.6 ± 0.5	47.3 ± 0.4	48.1 ± 1.1
Hemoglobin (g/dL)	16.5 ± 0.2	16.5 ± 0.4	16.6 ± 0.2	16.2 ± 0.1	15.9 ± 0.1*	16.3 ± 0.4
Erythrocytes (10^6/µL)	9.82 ± 0.14	9.86 ± 0.18	9.86 ± 0.16	9.62 ± 0.12	9.53 ± 0.11	9.70 ± 0.24
Reticulocytes (10^6/µL)	0.25 ± 0.02	0.27 ± 0.02	0.27 ± 0.02	0.25 ± 0.02	0.26 ± 0.02	0.26 ± 0.02
Nucleated erythrocytes/ 100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	49.9 ± 0.2	49.7 ± 0.2	49.8 ± 0.2	49.6 ± 0.2	49.9 ± 0.2	49.8 ± 0.2
Mean cell hemoglobin (pg)	16.8 ± 0.1	16.7 ± 0.1	16.8 ± 0.1	16.9 ± 0.1	16.7 ± 0.2	16.8 ± 0.2
Mean cell hemoglobin concentration (g/dL)	33.7 ± 0.3	33.6 ± 0.2	33.8 ± 0.2	34.0 ± 0.2	33.6 ± 0.2	33.8 ± 0.3
Platelets (10^3/µL)	794.4 ± 18.6	798.0 ± 39.1	806.2 ± 22.7	821.6 ± 17.8	857.9 ± 31.4	881.1 ± 15.7*
Leukocytes (10^3/µL)	5.75 ± 0.42	5.71 ± 0.31	5.10 ± 0.21	5.64 ± 0.39	5.71 ± 0.19	5.01 ± 0.26
Segmented neutrophils (10^3/µL)	0.75 ± 0.07	0.73 ± 0.09	0.57 ± 0.05	0.70 ± 0.10	0.80 ± 0.06	0.67 ± 0.06
Lymphocytes (10^3/µL)	4.99 ± 0.39	4.97 ± 0.29	4.51 ± 0.20	4.92 ± 0.34	4.88 ± 0.19	4.31 ± 0.22
Atypical lymphocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Female						
Hematology						
n	10	10	10	10	10	7
Hematocrit (%)	48.4 ± 0.7	47.9 ± 1.0	46.8 ± 0.4	47.3 ± 0.5	47.0 ± 0.6	45.5 ± 0.3**
Hemoglobin (g/dL)	16.5 ± 0.2	16.4 ± 0.3	16.1 ± 0.1	16.2 ± 0.1	16.1 ± 0.2	15.7 ± 0.1*
Erythrocytes (10^6/µL)	9.64 ± 0.17	9.54 ± 0.19	9.28 ± 0.08	9.42 ± 0.12	9.39 ± 0.10	9.08 ± 0.12*
Reticulocytes (10^6/µL)	0.32 ± 0.02	0.30 ± 0.02	0.30 ± 0.02	0.29 ± 0.02	0.30 ± 0.02	0.29 ± 0.02
Nucleated erythrocytes /100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	50.3 ± 0.2	50.3 ± 0.2	50.6 ± 0.2	50.4 ± 0.2	50.1 ± 0.2	50.1 ± 0.4
Mean cell hemoglobin (pg)	17.1 ± 0.1	17.2 ± 0.1	17.3 ± 0.1	17.2 ± 0.1	17.1 ± 0.1	17.3 ± 0.2
Mean cell hemoglobin concentration (g/dL)	34.1 ± 0.1	34.2 ± 0.3	34.4 ± 0.2	34.3 ± 0.1	34.2 ± 0.3	34.4 ± 0.3
Platelets (10^3/µL)	670.4 ± 34.9	677.8 ± 54.7	713.9 ± 31.3	726.4 ± 28.0	717.7 ± 18.8	793.9 ± 6.9*
Leukocytes (10^3/µL)	4.25 ± 0.20	4.79 ± 0.32	4.57 ± 0.24	4.59 ± 0.39	4.74 ± 0.46	4.73 ± 0.58
Segmented neutrophils (10^3/µL)	0.34 ± 0.05	0.51 ± 0.11	0.48 ± 0.04	0.48 ± 0.09	0.54 ± 0.09	0.54 ± 0.08
Lymphocytes (10^3/µL)	3.90 ± 0.19	4.26 ± 0.24	4.07 ± 0.20	4.09 ± 0.34	4.19 ± 0.43	4.15 ± 0.49
Atypical lymphocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.10	0.01 ± 0.01	0.04 ± 0.02

* Significantly different ($P \le 0.05$) from the vehicle control group by Dunn's test
** $P \le 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

APPENDIX C
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	164 ± 4	166 ± 4	177 ± 9	169 ± 4	169 ± 5	165 ± 9
Heart						
Absolute	0.588 ± 0.007	0.591 ± 0.013	0.627 ± 0.032	0.595 ± 0.014	0.601 ± 0.014	0.647 ± 0.060
Relative	3.595 ± 0.079	3.566 ± 0.081	3.540 ± 0.040	3.530 ± 0.039	3.559 ± 0.071	3.989 ± 0.490
R. Kidney						
Absolute	0.683 ± 0.012	0.708 ± 0.014	0.750 ± 0.050	0.706 ± 0.013	0.735 ± 0.026	0.688 ± 0.047
Relative	4.170 ± 0.057	4.271 ± 0.072	4.222 ± 0.083	4.190 ± 0.064	4.344 ± 0.046	4.171 ± 0.121
Liver						
Absolute	7.956 ± 0.207	7.929 ± 0.230	8.710 ± 0.491	8.425 ± 0.172	8.270 ± 0.307	7.606 ± 0.535
Relative	48.585 ± 1.392	47.745 ± 0.428	49.151 ± 0.635	49.988 ± 0.410	48.920 ± 0.879	46.069 ± 0.933
Lung						
Absolute	1.100 ± 0.048	1.017 ± 0.057	1.136 ± 0.097	1.194 ± 0.065	1.037 ± 0.034	0.985 ± 0.127[b]
Relative	6.717 ± 0.300	6.149 ± 0.401	6.499 ± 0.728	7.082 ± 0.366	6.144 ± 0.160	5.909 ± 0.415[b]
R. Testis						
Absolute	1.009 ± 0.036	1.027 ± 0.019	1.052 ± 0.026	1.005 ± 0.029	0.965 ± 0.082	1.007 ± 0.059
Relative	6.150 ± 0.159	6.192 ± 0.090	5.976 ± 0.182	5.966 ± 0.175	5.691 ± 0.417	6.115 ± 0.125
Thymus						
Absolute	0.404 ± 0.013	0.392 ± 0.017	0.434 ± 0.018	0.393 ± 0.014	0.404 ± 0.025	0.377 ± 0.031
Relative	2.465 ± 0.087	2.376 ± 0.157	2.469 ± 0.121	2.335 ± 0.108	2.400 ± 0.174	2.281 ± 0.108
Female						
Necropsy body wt	134 ± 2	132 ± 3	132 ± 3	135 ± 2	134 ± 4	131 ± 3
Heart						
Absolute	0.481 ± 0.010	0.485 ± 0.010	0.496 ± 0.014	0.505 ± 0.010	0.479 ± 0.009	0.457 ± 0.013
Relative	3.595 ± 0.087	3.665 ± 0.057	3.767 ± 0.036	3.750 ± 0.111	3.582 ± 0.057	3.490 ± 0.084
R. Kidney						
Absolute	0.573 ± 0.012	0.564 ± 0.019	0.571 ± 0.023	0.590 ± 0.011	0.572 ± 0.018	0.574 ± 0.018
Relative	4.279 ± 0.057	4.266 ± 0.121	4.341 ± 0.118	4.380 ± 0.074	4.273 ± 0.087	4.373 ± 0.073
Liver						
Absolute	5.662 ± 0.071	5.592 ± 0.174	5.530 ± 0.116	5.960 ± 0.153	5.817 ± 0.248	5.632 ± 0.180
Relative	42.303 ± 0.975	42.277 ± 1.129	42.075 ± 0.687	44.259 ± 1.230	43.410 ± 1.281	42.932 ± 0.639
Lung						
Absolute	0.907 ± 0.011	0.944 ± 0.047	0.943 ± 0.051	0.958 ± 0.019	0.877 ± 0.049	0.837 ± 0.027
Relative	6.769 ± 0.094	7.125 ± 0.252	7.158 ± 0.273	7.116 ± 0.189	6.559 ± 0.388	6.405 ± 0.293
Thymus						
Absolute	0.332 ± 0.017	0.324 ± 0.009	0.344 ± 0.014	0.340 ± 0.011	0.330 ± 0.011	0.322 ± 0.017
Relative	2.478 ± 0.133	2.461 ± 0.117	2.623 ± 0.113	2.532 ± 0.113	2.466 ± 0.066	2.452 ± 0.103

[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=4

TABLE C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male						
n	10	10	10	10	9	10
Body wt	331 ± 6	329 ± 5	335 ± 7	333 ± 5	306 ± 6*	313 ± 6
Heart						
Absolute	0.926 ± 0.016	0.885 ± 0.017	0.920 ± 0.029	0.907 ± 0.019	0.828 ± 0.023**	0.892 ± 0.020
Relative	2.805 ± 0.066	2.692 ± 0.034	2.747 ± 0.079	2.724 ± 0.030	2.707 ± 0.032	2.856 ± 0.047
R. Kidney						
Absolute	1.000 ± 0.020	0.964 ± 0.024	1.005 ± 0.024	0.978 ± 0.025	0.888 ± 0.027**	0.978 ± 0.019
Relative	3.028 ± 0.069	2.928 ± 0.050	3.000 ± 0.040	2.937 ± 0.060	2.903 ± 0.055	3.132 ± 0.051
Liver						
Absolute	10.590 ± 0.183	10.987 ± 0.337[b]	10.781 ± 0.248	10.706 ± 0.273	9.904 ± 0.223	10.207 ± 0.311
Relative	32.057 ± 0.530	33.368 ± 0.548[b]	32.180 ± 0.428	32.120 ± 0.499	32.402 ± 0.313	32.611 ± 0.617
Lung						
Absolute	1.393 ± 0.056	1.379 ± 0.032	1.361 ± 0.027	1.407 ± 0.029	1.362 ± 0.059	1.404 ± 0.035
Relative	4.207 ± 0.143	4.194 ± 0.077	4.069 ± 0.081	4.227 ± 0.069	4.450 ± 0.146	4.503 ± 0.141
R. Testis						
Absolute	1.422 ± 0.031	1.438 ± 0.031	1.428 ± 0.029	1.488 ± 0.030	1.389 ± 0.022	1.422 ± 0.016
Relative	4.305 ± 0.099	4.373 ± 0.074	4.264 ± 0.048	4.472 ± 0.090	4.553 ± 0.080*	4.558 ± 0.082*
Thymus						
Absolute	0.269 ± 0.008	0.280 ± 0.009	0.273 ± 0.016	0.280 ± 0.013	0.237 ± 0.010	0.234 ± 0.013
Relative	0.813 ± 0.023	0.852 ± 0.030	0.812 ± 0.041	0.840 ± 0.031	0.776 ± 0.034	0.748 ± 0.37
Female						
n	10	10	10	10	10	10
Body wt	189 ± 4	183 ± 3	188 ± 4	191 ± 4	192 ± 2	190 ± 4
Heart						
Absolute	0.607 ± 0.012	0.580 ± 0.006	0.580 ± 0.013	0.604 ± 0.014	0.604 ± 0.009	0.605 ± 0.018
Relative	3.214 ± 0.055	3.172 ± 0.045	3.091 ± 0.049	3.166 ± 0.056	3.151 ± 0.036	3.186 ± 0.085
R. Kidney						
Absolute	0.622 ± 0.018	0.593 ± 0.010	0.611 ± 0.017	0.632 ± 0.010	0.628 ± 0.008	0.648 ± 0.015
Relative	3.286 ± 0.042	3.249 ± 0.068	3.250 ± 0.053	3.318 ± 0.046	3.278 ± 0.023	3.406 ± 0.030
Liver						
Absolute	5.682 ± 0.155	5.960 ± 0.133	5.728 ± 0.209	5.723 ± 0.168	5.900 ± 0.133	5.916 ± 0.236
Relative	30.043 ± 0.484	32.603 ± 0.680*	30.431 ± 0.661	29.967 ± 0.612	30.788 ± 0.542	31.074 ± 0.963
Lung						
Absolute	0.921 ± 0.029	0.972 ± 0.040	0.890 ± 0.024	0.942 ± 0.029	0.926 ± 0.018	0.938 ± 0.021
Relative	4.876 ± 0.142	5.327 ± 0.236	4.736 ± 0.057	4.932 ± 0.118	4.836 ± 0.079	4.943 ± 0.133
Thymus						
Absolute	0.215 ± 0.007	0.187 ± 0.008*	0.209 ± 0.007	0.216 ± 0.006	0.213 ± 0.004	0.200 ± 0.006
Relative	1.137 ± 0.023	1.019 ± 0.041*	1.114 ± 0.033	1.132 ± 0.027	1.115 ± 0.025	1.050 ± 0.033

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Body weights from day 92; organ weights from day 93. Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

TABLE C3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male						
n	5	5	5	5	5	5
Necropsy body wt	25.6 ± 0.6	26.1 ± 0.5	25.7 ± 0.7	25.0 ± 0.9	25.0 ± 1.0	24.9 ± 0.6
Heart						
Absolute	0.123 ± 0.005	0.128 ± 0.002	0.123 ± 0.004	0.120 ± 0.004	0.124 ± 0.004	0.122 ± 0.003
Relative	4.788 ± 0.119	4.894 ± 0.057	4.794 ± 0.040	4.793 ± 0.043	4.968 ± 0.180	4.892 ± 0.141
R. Kidney						
Absolute	0.242 ± 0.007	0.260 ± 0.006	0.255 ± 0.009	0.252 ± 0.011	0.259 ± 0.012	0.254 ± 0.009
Relative	9.465 ± 0.148	9.986 ± 0.325	9.921 ± 0.170	10.109 ± 0.188	10.365 ± 0.350	10.206 ± 0.304
Liver						
Absolute	1.415 ± 0.059	1.443 ± 0.020	1.423 ± 0.038	1.334 ± 0.048	1.383 ± 0.061	1.295 ± 0.079
Relative	55.249 ± 1.452	55.376 ± 1.022	55.427 ± 0.530	53.482 ± 0.910	55.344 ± 0.758	52.123 ± 3.297
Lung						
Absolute	0.155 ± 0.008	0.186 ± 0.030	0.178 ± 0.005	0.171 ± 0.005	0.190 ± 0.013	0.159 ± 0.003
Relative	6.069 ± 0.287	7.095 ± 1.045	6.927 ± 0.103	6.877 ± 0.209	7.588 ± 0.403	6.381 ± 0.157
R. Testis						
Absolute	0.098 ± 0.005	0.099 ± 0.004	0.102 ± 0.002	0.102 ± 0.002	0.098 ± 0.006	0.097 ± 0.004
Relative	3.825 ± 0.144	3.785 ± 0.167	3.966 ± 0.104	4.104 ± 0.070	3.897 ± 0.114	3.892 ± 0.083
Thymus						
Absolute	0.045 ± 0.003	0.054 ± 0.004	0.052 ± 0.002	0.055 ± 0.005	0.057 ± 0.004	0.049 ± 0.006
Relative	1.785 ± 0.136	2.067 ± 0.183	2.014 ± 0.101	2.210 ± 0.182	2.313 ± 0.178	1.993 ± 0.261
Female						
n	4	5	5	5	5	5
Necropsy body wt	20.9 ± 0.6	20.8 ± 0.2	21.5 ± 0.2	21.4 ± 0.6	21.7 ± 0.7	19.8 ± 0.7
Heart						
Absolute	0.105 ± 0.004	0.108 ± 0.005	0.106 ± 0.002	0.112 ± 0.003	0.101 ± 0.001	0.104 ± 0.003
Relative	5.008 ± 0.146	5.176 ± 0.190	4.901 ± 0.066	5.235 ± 0.057	4.672 ± 0.101	5.277 ± 0.140
R. Kidney						
Absolute	0.158 ± 0.008	0.168 ± 0.005	0.166 ± 0.002	0.166 ± 0.004	0.163 ± 0.003	0.163 ± 0.006
Relative	7.535 ± 0.277	8.063 ± 0.166	7.699 ± 0.092	7.749 ± 0.112	7.519 ± 0.120	8.210 ± 0.132*
Liver						
Absolute	1.113 ± 0.021	1.052 ± 0.024	1.094 ± 0.013	1.084 ± 0.026	1.102 ± 0.061	1.075 ± 0.035
Relative	53.269 ± 1.631	50.551 ± 0.847	50.807 ± 0.216	50.583 ± 0.406	50.593 ± 1.326	54.199 ± 0.588
Lung						
Absolute	0.179 ± 0.021	0.185 ± 0.017	0.170 ± 0.008	0.194 ± 0.026	0.157 ± 0.003	0.140 ± 0.006
Relative	8.580 ± 1.124	8.870 ± 0.733	7.908 ± 0.388	8.976 ± 1.006	7.265 ± 0.311	7.082 ± 0.178
Thymus						
Absolute	0.070 ± 0.003	0.060 ± 0.006	0.066 ± 0.002	0.070 ± 0.004	0.063 ± 0.004	0.066 ± 0.004
Relative	3.330 ± 0.186	2.870 ± 0.306	3.055 ± 0.085	3.277 ± 0.170	2.938 ± 0.227	3.385 ± 0.300

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test

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

TABLE C4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	39.1 ± 1.6	37.1 ± 1.7	38.6 ± 1.1	35.3 ± 1.2	38.8 ± 1.4	39.2 ± 1.6
Heart						
Absolute	0.161 ± 0.005	0.158 ± 0.005	0.164 ± 0.005	0.154 ± 0.003	0.161 ± 0.004	0.163 ± 0.005
Relative	4.160 ± 0.136	4.311 ± 0.138	4.260 ± 0.111	4.397 ± 0.102	4.180 ± 0.124	4.171 ± 0.074
R. Kidney						
Absolute	0.325 ± 0.011	0.327 ± 0.011	0.317 ± 0.009	0.302 ± 0.008	0.313 ± 0.012	0.327 ± 0.008
Relative	8.392 ± 0.284	8.906 ± 0.306	8.229 ± 0.137	8.592 ± 0.200	8.086 ± 0.213	8.420 ± 0.235
Liver						
Absolute	1.678 ± 0.069	1.610 ± 0.073	1.684 ± 0.060	1.509 ± 0.047	1.666 ± 0.089	1.641 ± 0.096
Relative	43.014 ± 0.485	43.549 ± 0.997	43.560 ± 0.614	42.776 ± 0.404	42.819 ± 0.939	41.702 ± 0.934
Lung						
Absolute	0.238 ± 0.014	0.224 ± 0.017	0.240 ± 0.012	0.207 ± 0.011	0.201 ± 0.008	0.209 ± 0.013
Relative	6.140 ± 0.325	6.175 ± 0.640	6.261 ± 0.402	5.827 ± 0.157	5.216 ± 0.214	5.372 ± 0.296
R. Testis						
Absolute	0.126 ± 0.004	0.125 ± 0.003	0.124 ± 0.002	0.126 ± 0.002	0.122 ± 0.003	0.122 ± 0.002
Relative	3.243 ± 0.105	3.439 ± 0.182	3.230 ± 0.107	3.594 ± 0.103	3.175 ± 0.116	3.158 ± 0.122
Thymus						
Absolute	0.045 ± 0.005	0.043 ± 0.003	0.042 ± 0.003	0.042 ± 0.003	0.039 ± 0.003	0.042 ± 0.003
Relative	1.137 ± 0.105	1.159 ± 0.065	1.092 ± 0.086	1.175 ± 0.065	0.998 ± 0.075	1.080 ± 0.082
Female						
n	10	10	10	10	10	7
Necropsy body wt	30.9 ± 0.8	30.1 ± 1.0	29.0 ± 1.0	28.3 ± 0.7	28.0 ± 0.9*	28.4 ± 1.2[b]
Heart						
Absolute	0.123 ± 0.002	0.122 ± 0.002	0.123 ± 0.002	0.119 ± 0.002	0.121 ± 0.002	0.117 ± 0.003
Relative	4.014 ± 0.096	4.064 ± 0.107	4.281 ± 0.127	4.237 ± 0.095	4.333 ± 0.140	4.147 ± 0.144
R. Kidney						
Absolute	0.180 ± 0.008	0.173 ± 0.004	0.176 ± 0.004	0.164 ± 0.003	0.173 ± 0.003	0.167 ± 0.005
Relative	5.869 ± 0.276	5.797 ± 0.148	6.091 ± 0.136	5.826 ± 0.157	6.191 ± 0.173	5.873 ± 0.164
Liver						
Absolute	1.195 ± 0.024	1.202 ± 0.043	1.239 ± 0.050	1.141 ± 0.030	1.151 ± 0.042	1.157 ± 0.041
Relative	38.737 ± 0.457	39.974 ± 0.749	42.686 ± 0.897**	40.393 ± 0.740	41.118 ± 1.080	40.641 ± 0.669
Lung						
Absolute	0.231 ± 0.013	0.225 ± 0.014	0.208 ± 0.011	0.186 ± 0.006*	0.193 ± 0.009*	0.178 ± 0.010**
Relative	7.536 ± 0.525	7.529 ± 0.493	7.207 ± 0.376	6.601 ± 0.191	6.907 ± 0.355	6.238 ± 0.169
Thymus						
Absolute	0.049 ± 0.002	0.046 ± 0.002	0.048 ± 0.002	0.046 ± 0.002	0.049 ± 0.002	0.043 ± 0.002
Relative	1.581 ± 0.075	1.539 ± 0.074	1.670 ± 0.067	1.634 ± 0.033	1.753 ± 0.058	1.507 ± 0.043

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=8

APPENDIX D
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE D1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
n	10	10	9	10
Weights (g)				
Necropsy body wt	331 ± 6	333 ± 6	307 ± 5*	309 ± 6**
L. Cauda epididymis	0.1609 ± 0.0042	0.1593 ± 0.0049	0.1461 ± 0.0044	0.1511 ± 0.0039
L. Epididymis	0.3051 ± 0.0100	0.3168 ± 0.0114	0.3017 ± 0.0126	0.2935 ± 0.0061
L. Testis	1.5234 ± 0.0211	1.5116 ± 0.0227	1.4611 ± 0.0275	1.4790 ± 0.0168
Spermatid measurements				
Spermatid heads (10^7/g testis)	115.822 ± 7.331	136.343 ± 5.181*	123.000 ± 3.969	114.492 ± 2.960
Spermatid heads (10^7/testis)	165.250 ± 9.628	193.250 ± 6.501*	167.778 ± 5.597	157.000 ± 4.938
Spermatid heads (10^7/g cauda)	160.100 ± 37.899	375.800 ± 106.646	191.000 ± 54.185	230.900 ± 56.581
Spermatid heads (10^7/cauda)	39.800 ± 5.095	66.150 ± 14.304	41.000 ± 6.920	47.100 ± 5.620
Epididymal spermatozoal measurements				
Motility (%)	81.900 ± 0.862	79.100 ± 1.329	80.333 ± 0.866	79.300 ± 1.116

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test (body weights) or Dunn's test (spermatid measurements)
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Differences from the vehicle control group for tissue weights are not significant by Dunnett's test. Differences from the vehicle control group for spermatozoal measurements are not significant by Dunn's test.

TABLE D2
Estrous Cycle Characterization for Female Rats in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
n	10	10	10	10
Necropsy body wt	188 ± 4	193 ± 3	192 ± 2	192 ± 3
Estrous cycle length (days)	4.650 ± 0.198	4.300 ± 0.153	4.700 ± 0.133	4.700 ± 0.082
Estrous stages (% of cycle)				
Diestrus	35.8	35.0	45.0	43.3
Proestrus	7.5	9.2	8.3	10.0
Estrus	35.8	33.3	29.2	27.5
Metestrus	20.8	22.5	17.5	19.2

[a] Necropsy body weights and estrous cycle lengths are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weights) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females did not differ significantly from vehicle control females in the relative length of time spent in the estrous stages.

TABLE D3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	39.1 ± 1.6	35.3 ± 1.2	38.8 ± 1.4	39.2 ± 1.6
L. Cauda epididymis	0.0196 ± 0.0010	0.0190 ± 0.0009	0.0197 ± 0.0011	0.0203 ± 0.0012
L. Epididymis	0.0323 ± 0.0015	0.0304 ± 0.0017	0.0302 ± 0.0010	0.0306 ± 0.0017
L. Testis	0.1189 ± 0.0039	0.1202 ± 0.0027	0.1183 ± 0.0031	0.1169 ± 0.0009
Spermatid measurements				
Spermatid heads (10^7/g testis)	185.587 ± 8.921	192.867 ±10.239	185.045 ± 7.926	176.979 ± 7.577
Spermatid heads (10^7/testis)	20.529 ± 1.045	21.464 ± 0.881	20.384 ± 1.247	19.076 ± 0.795
Spermatid heads (10^7/g cauda)	8.140 ± 1.900	9.800 ± 1.726	9.700 ± 1.388	7.970 ± 1.058
Spermatid heads (10^7/cauda)	311.700 ± 122.901	407.800 ± 105.990	413.100 ± 117.055	264.600 ± 65.967
Epididymal spermatozoal measurements				
Motility (%)	70.100 ± 7.827	78.800 ± 0.867	80.000 ± 1.022	77.100 ± 1.602

[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and spermatozoal measurements).

TABLE D4
Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of Dimethylaminopropyl Chloride, Hydrochloride[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
n	10	10	10	9
Necropsy body wt	30.9 ± 0.8	28.3 ± 0.7	28.0 ± 0.9	27.5 ± 1.4*
Estrous cycle length (days)	3.922 ± 0.097[b]	4.189 ± 0.177[b]	4.260 ± 0.148	4.611 ± 0.439
Estrous stages (% of cycle)				
Diestrus	35.0	32.5	27.5	25.0
Proestrus	0.0	0.0	0.0	0.0
Estrus	43.3	48.3	50.0	51.9
Metestrus	21.7	19.2	22.5	23.1

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
[a] Necropsy body weights and estrous cycle lengths are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females did not differ significantly from vehicle control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

APPENDIX E
GENETIC TOXICOLOGY

TABLE E1
Mutagenicity of Dimethylaminopropyl Chloride, Hydrochloride in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+30% hamster S9 Trial 1	+30% hamster S9 Trial 2	+30% rat S9 Trial 1	+30% rat S9 Trial 2
TA100	0	133 ± 5.8	107 ± 5.0	135 ± 9.8	136 ± 6.2	123 ± 5.4	126 ± 16.3
	100	105 ± 4.5	100 ± 7.4	132 ± 7.0	166 ± 11.3	107 ± 3.2	179 ± 2.5
	333	122 ± 6.2	176 ± 4.7	133 ± 9.8	176 ± 9.5	128 ± 9.3	192 ± 8.5
	1,000	156 ± 12.2	183 ± 9.0	165 ± 5.4	191 ± 12.5	167 ± 12.3	196 ± 4.6
	3,333	220 ± 24.2	237 ± 15.0	278 ± 8.6	369 ± 12.6	205 ± 4.7	263 ± 12.2
	10,000	441 ± 24.5	457 ± 15.2	455 ± 17.1	696 ± 50.7	453 ± 21.3	626 ± 26.1
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control[c]		837 ± 14.4	958 ± 67.4	559 ± 21.4	905 ± 85.6	437 ± 10.6	712 ± 36.2
TA1535	0	9 ± 1.9	12 ± 1.5	10 ± 2.8	10 ± 1.8	13 ± 2.1	16 ± 1.5
	100	20 ± 0.3	34 ± 6.1	11 ± 3.8	30 ± 2.6	21 ± 2.0	19 ± 0.6
	333	23 ± 3.4	41 ± 5.0	20 ± 5.2	65 ± 5.5	28 ± 4.7	48 ± 2.4
	1,000	56 ± 3.2	136 ± 16.6	76 ± 6.2	151 ± 8.6	63 ± 7.1	155 ± 26.8
	3,333	148 ± 11.0	194 ± 14.8	183 ± 20.9	445 ± 21.4	166 ± 8.9	280 ± 7.5
	10,000	365 ± 10.4	470 ± 14.2	509 ± 11.7	846 ± 45.2	502 ± 9.6	654 ± 30.7
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control		955 ± 8.3	1,133 ± 65.9	333 ± 15.3	591 ± 67.2	231 ± 32.4	198 ± 11.6
TA97	0	158 ± 10.4		172 ± 10.5		175 ± 9.8	
	100	172 ± 7.5		171 ± 16.6		184 ± 0.9	
	333	159 ± 14.8		186 ± 4.6		182 ± 7.1	
	1,000	171 ± 9.7		181 ± 6.4		185 ± 18.6	
	3,333	189 ± 2.4		145 ± 12.0		176 ± 14.3	
	10,000	169 ± 15.8		147 ± 9.2		163 ± 7.8	
Trial summary		Negative		Negative		Negative	
Positive control		623 ± 23.5		626 ± 24.9		586 ± 38.7	
TA98	0	22 ± 1.8		19 ± 4.1		21 ± 3.2	
	100	15 ± 3.0		19 ± 1.9		17 ± 4.2	
	333	17 ± 1.3		13 ± 2.0		21 ± 1.5	
	1,000	17 ± 1.5		18 ± 5.5		13 ± 1.3	
	3,333	13 ± 2.3		17 ± 4.7		17 ± 2.6	
	10,000	13 ± 2.0		19 ± 2.3		21 ± 5.3	
Trial summary		Negative		Negative		Negative	
Positive control		340 ± 9.2		534 ± 13.2		514 ± 6.7	

[a] Studies were performed at SRI International. The detailed protocol is presented by Zeiger *et al.* (1992). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Frequency of Micronucleated Normochromatic Erythrocytes in Peripheral Blood of Mice Administered Dimethylaminopropyl Chloride, Hydrochloride by Gavage for 3 Months[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	Pairwise P Value[c]
Male				
Water[d]	0	10	1.10 ± 0.28	
Dimethylaminopropyl chloride, hydrochloride				
	6.25	10	1.55 ± 0.25	0.1080
	12.5	10	1.80 ± 0.26	0.0329
	25	10	1.10 ± 0.15	0.5000
	50	10	1.35 ± 0.26	0.2374
	100	10	1.50 ± 0.29	0.1335
			P = 0.395[e]	
Female				
Water	0	10	0.70 ± 0.13	
Dimethylaminopropyl chloride, hydrochloride				
	6.25	10	0.75 ± 0.17	0.4263
	12.5	10	1.25 ± 0.25	0.0390
	25	10	1.15 ± 0.26	0.0694
	50	10	1.25 ± 0.24	0.0390
	100	7	1.00 ± 0.27	0.1713
			P = 0.188	

[a] Study was performed at SITEK Research Laboratories, Inc. The detailed protocol is presented by MacGregor *et al.* (1990).
[b] NCE = normochromatic erythrocyte
Mean ± standard error
[c] Pairwise comparison with the vehicle control. Dosed group values are significant at $P \leq 0.005$ (ILS, 1990).
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the Cochran Armitage one-tailed trend test, significant at $P \leq 0.025$ (ILS, 1990).

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF DIMETHYLAMINOPROPYL CHLORIDE, HYDROCHLORIDE

Dimethylaminopropyl chloride, hydrochloride was obtained from Aldrich Chemical Company (Milwaukee, WI) in one lot (07530KG) that was used in the 2-week and 3-month studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and the study laboratory, BioReliance Corporation (Rockville, MD). Reports on analyses performed in support of the study of dimethylaminopropyl chloride, hydrochloride are on file at the National Institute of Environmental Health Sciences.

The chemical, a hygroscopic, white powder, was identified as dimethylaminopropyl chloride, hydrochloride by the analytical chemistry laboratory using Fourier transform infrared (FT-IR) and proton nuclear magnetic resonance (NMR) spectroscopy and by the study laboratory using IR spectroscopy and comparison against a reference standard from the same lot. All spectra were consistent with the structure of dimethylaminopropyl chloride, hydrochloride and matched reference spectra (*Aldrich* 1983, 1985). The FT-IR and proton NMR spectra are presented in Figures F1 and F2.

The purity of lot 07530KG was determined by the analytical chemistry laboratory and the study laboratory using gas chromatography (GC). The analytical system utilized a gas chromatograph manufactured by Varian, Inc., or Hewlett-Packard Co. (Palo Alto, CA) with a flame ionization detector, a DB-5 column (30 m × 0.53 mm, 1.5-µm film thickness; J&W Scientific, Palo Alto, CA), helium or nitrogen as the carrier gas at 10 mL/minute, and an oven temperature held at 50° C for 5 minutes and then increased to 200° C at 10° C/minute.

Using this system, the analytical chemistry laboratory detected one major peak and one impurity peak in lot 07530KG; the impurity had an area of 0.53% relative to the total integrated peak area. Purity was determined by the study laboratory on two shipments of lot 07530KG using the same GC system. For the first shipment, purity was determined to be 99.25% (at receipt) and 98.76% (within 30 days prior to the start of the 2-week study). A bulk purity reanalysis was performed (within 30 days prior to the start of the 3-month study) on a second shipment of lot 07530KG, and the relative purity of this bulk dimethylaminopropyl chloride, hydrochloride to its bulk reference standard sample (taken from the first shipment and stored at less than or equal to –20° C) was 100%. Two significant impurities were detected, and they constituted 0.46% and 0.57% of the bulk dimethylaminopropyl chloride, hydrochloride. The overall purity of lot 07530KG was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored at ambient temperature under a headspace of inert gas and protected from light and moisture. Stability of the bulk chemical was monitored during the 3-month study by the study laboratory using the GC system described above. No degradation of the bulk chemical was observed.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Dose formulations were prepared once for the 2-week studies and four times for the 3-month studies. The dose formulations were prepared by adding the appropriate amount of chemical, diluting to the specified volume with deionized water, and sonicating and/or mixing on a magnetic stirrer until dissolved. Aliquots of the dose formulations sufficient for daily dosing were stored at ambient temperature under a headspace of inert gas in amber glass vials sealed with Teflon®-lined butyl rubber stoppers and crimped aluminum caps for up to 35 days.

Homogeneity of the dose formulations was not confirmed as they were in the form of solutions. Stability studies of dose formulations of approximately 0.3 mg/mL were performed by the analytical chemistry laboratory using the analysis method described above. Stability was confirmed for up to 35 days for dose formulations stored in amber glass vials sealed with Teflon®-lined septa and aluminum caps, at refrigerated (5° C) and ambient (approximately 25° C) temperatures, and for up to 7 days under simulated animal room conditions, exposed to air and light at room temperature.

Analyses of the dose formulations of dimethylaminopropyl chloride, hydrochloride were conducted by the study laboratory using the GC system described above. The aqueous solution of the test chemical was first neutralized with 1 N sodium hydroxide solution followed by extraction of the chemical with methylene chloride and quantification using the analysis method described above. During the 2-week studies, the dose formulations were analyzed once; all six of the dose formulations for rats and mice were within 10% of the target concentrations (Table F2). All five of the animal room samples of these dose formulations for rats and mice were within 10% of the target. During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; animal room samples of these dose formulations were also analyzed (Table F3). Of the dose formulations analyzed, all 18 were within 10% of the target concentrations; 14 of 15 animal room samples for rats and all 15 animal room samples for mice were within 10% of target.

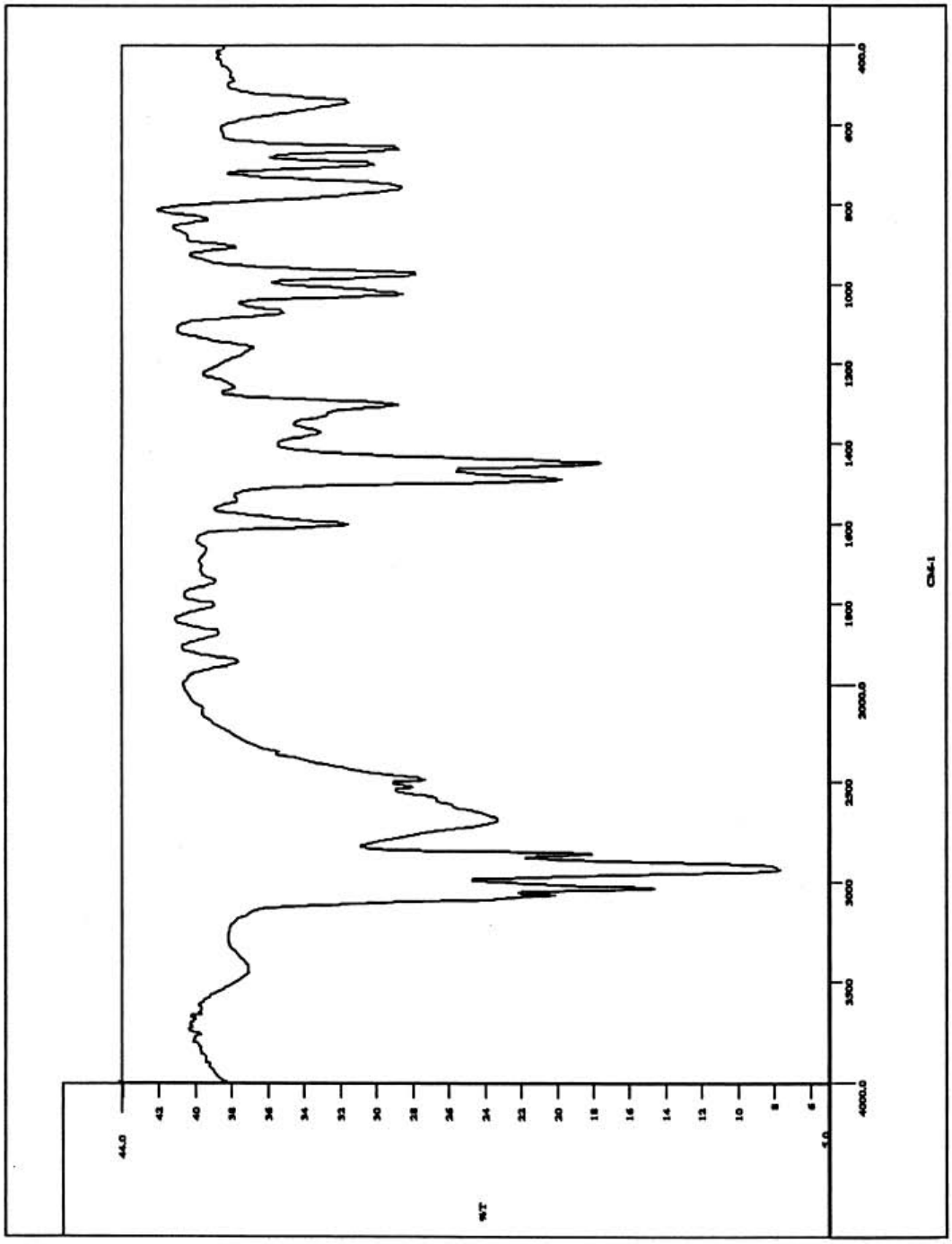

FIGURE F1
Fourier Transform Infrared Absorption Spectrum of Dimethylaminopropyl Chloride, Hydrochloride

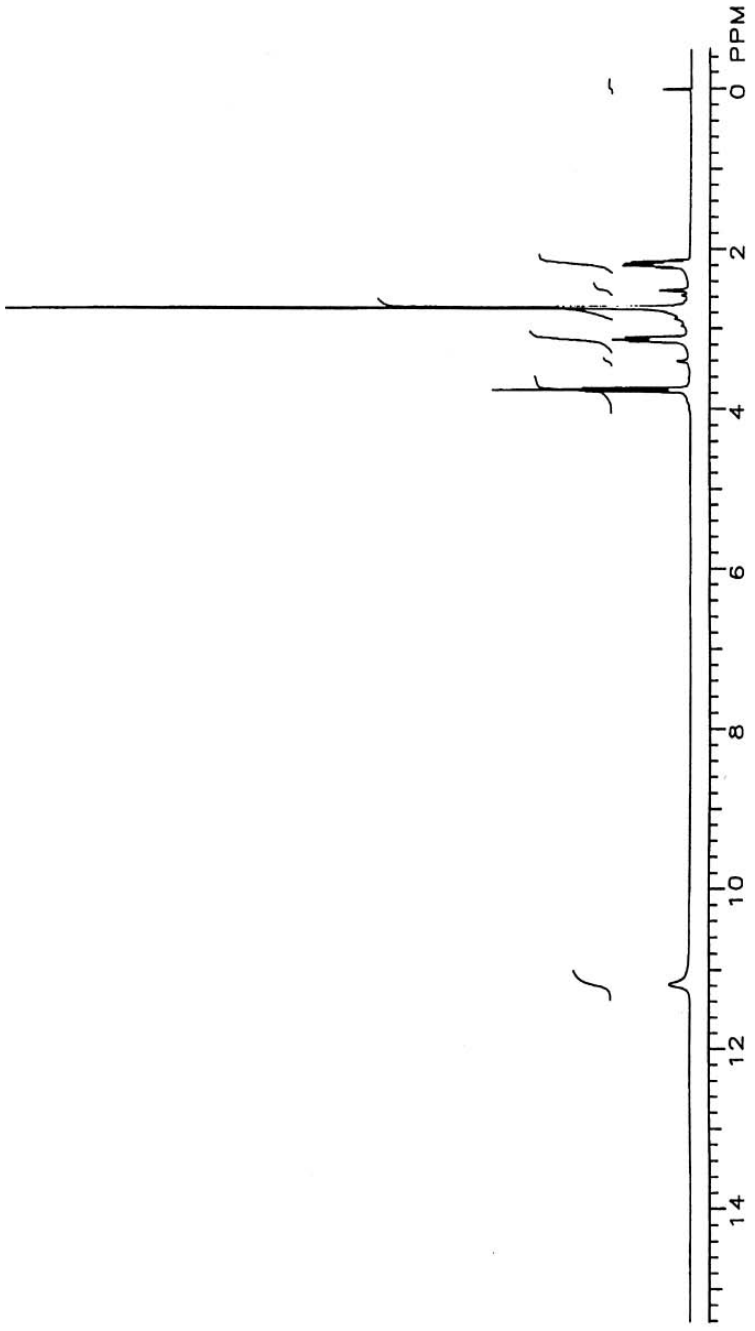

FIGURE F2
Proton Nuclear Magnetic Resonance Spectrum
of Dimethylaminopropyl Chloride, Hydrochloride

TABLE F1
Preparation and Storage of Dose Formulations in the Gavage Studies of Dimethylaminopropyl Chloride, Hydrochloride

Preparation
Dose formulations were prepared once for the 2-week studies and four times for the 3-month studies. The dose formulations were prepared by dissolving the required amount of dimethylaminopropyl chloride, hydrochloride in the specified volume of deionized water in a volumetric flask by sonicating and/or mixing on a magnetic stirrer.

Chemical Lot Number
07530KG

Maximum Storage Time
35 days

Storage Conditions
Aliquots of the dose formulations sufficient for daily dosing were stored at ambient temperature under a headspace of inert gas in amber glass vials sealed with Teflon®-lined rubber stoppers and crimped aluminum caps.

Study Laboratory
BioReliance (Rockville, MD)

TABLE F2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Week Gavage Studies of Dimethylaminopropyl Chloride, Hydrochloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
August 25, 1999	August 25-26, 1999	0.625	0.586	–6
		1.25	1.21	–3
		2.50	2.38	–5
		5.00	4.69	–6
		10.0	9.79	–2
		20.0	20.5	+3
	September 22-24, 1999[b]	1.25	1.31	+5
		2.50	2.56	+2
		5.00	5.32	+6
		10.0	10.4	+4
		20.0	21.4	+7
	September 21-23, 1999[c]	0.625	0.636	+2
		1.25	1.36	+9
		2.50	2.67	+7
		5.00	5.17	+3
		10.0	10.6	+6

[a] Results of duplicate analyses. Dosing volume for rats=5 mL/kg; 1.25 mg/mL=6.25 mg/kg, 2.50 mg/mL=12.5 mg/kg, 5.0 mg/mL=25.0 mg/kg, 10.0 mg/mL=50 mg/kg, and 20.0 mg/mL=100.0 mg/kg. Dosing volume for mice=10 mL/kg; 0.625 mg/mL=6.25 mg/kg, 1.25 mg/mL=12.5 mg/kg, 2.50 mg/mL=25.0 mg/kg, 5.0 mg/mL=50.0 mg/kg, and 10.0 mg/mL=100 mg/kg.
[b] Rat animal room samples
[c] Mouse animal room samples

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of Dimethylaminopropyl Chloride, Hydrochloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
June 6, 2000	June 6-7, 2000	0.625	0.621 ± 0.0019	–1
		1.25	1.24 ± 0.0068	–1
		2.50	2.43 ± 0.0042	–3
		5.00	4.94 ± 0.0064	–1
		10.0	10.2 ± 0.011	+2
		20.0	20.3 ± 0.0056	+2
	July 18-19, 2000[b]	1.25	1.36 ± 0.0044	+9
		2.50	2.57 ± 0.0051	+3
		5.00	5.09 ± 0.0079	+2
		10.0	11.3 ± 0.021	+13
		20.0	21.9 ± 0.0096	+10
	July 18-19, 2000[c]	0.625	0.611 ± 0.0040	–2
		1.25	1.36 ± 0.012	+9
		2.50	2.66 ± 0.011	+6
		5.00	5.30 ± 0.013	+6
		10.0	10.7 ± 0.023	+7
August 1, 2000	August 1-2, 2000	0.625	0.624 ± 0.0029	0
		1.25	1.27 ± 0.016	+2
		2.50	2.53 ± 0.0079	+1
		5.00	4.91 ± 0.030	–2
		10.0	9.86 ± 0.020	–1
		20.0	20.0 ± 0.042	0
	September 14-15, 2000[b]	1.25	1.20 ± 0.051	–4
		2.50	2.58 ± 0.012	+3
		5.00	5.28 ± 0.013	+6
		10.0	10.2 ± 0.013	+2
		20.0	20.8 ± 0.045	+4
	September 14-15, 2000[c]	0.625	0.616 ± 0.029	–1
		1.25	1.26 ± 0.028	+1
		2.50	2.72 ± 0.011	+9
		5.00	4.97 ± 0.022	–1
		10.0	10.4 ± 0.021	+4

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of Dimethylaminopropyl Chloride, Hydrochloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
August 29, 2000	August 29-30, 2000	0.625	0.619 ± 0.011	–1
		1.25	1.27 ± 0.010	+2
		2.50	2.46 ± 0.022	–2
		5.00	4.86 ± 0.010	–3
		10.0	9.70 ± 0.0041	–3
		20.0	20.0 ± 0.0088	0
	September 20-21, 2000[b]	1.25	1.31 ± 0.021	+5
		2.50	2.74 ± 0.017	+10
		5.00	5.42 ± 0.016	+8
		10.0	10.8 ± 0.0060	+8
		20.0	21.5 ± 0.011	+8
	September 20-21, 2000[c]	0.625	0.647 ± 0.013	+4
		1.25	1.30 ± 0.018	+4
		2.50	2.71 ± 0.0076	+8
		5.00	5.47 ± 0.012	+9
		10.0	10.8 ± 0.0092	+8

[a] Results (mean ± standard deviation) of four analyses. Dosing volume for rats=5 mL/kg; 1.25 mg/mL=6.25 mg/kg, 2.50 mg/mL=12.5 mg/kg, 5.0 mg/mL=25.0 mg/kg, 10.0 mg/mL=50 mg/kg, and 20.0 mg/mL=100.0 mg/kg. Dosing volume for mice=10 mL/kg; 0.625 mg/mL=6.25 mg/kg, 1.25 mg/mL=12.5 mg/kg, 2.50 mg/mL=25.0 mg/kg, 5.0 mg/mL=50.0 mg/kg, and 10.0 mg/mL=100 mg/kg.
[b] Rat animal room samples
[c] Mouse animal room samples

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