NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicity Studies of Benzyltrimethylammonium Chloride (CAS No. 56-93-9) Administered by Gavage to F344/N Rats and B6C3F$_1$ Mice

NTP TOX 57

FEBRUARY 2000

National Toxicology Program
Toxicity Report Series
Number 57

NTP Technical Report on the Toxicity Studies of

Benzyltrimethylammonium Chloride

(CAS No. 56-93-9)

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

February 2000
NIH Publication No. 00-4405

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

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

The studies described in this Toxicity Study Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Toxicity Study Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). Other information about NTP studies is available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

National Toxicology Program
Toxicity Report Series
Number 57

NTP Technical Report
on the Toxicity Studies of

Benzyltrimethylammonium Chloride

(CAS No. 56-93-9)

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

Kamal M. Abdo, Ph.D., Study Scientist
National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and report findings

K.M. Abdo, Ph.D., Study Scientist
J.R. Bucher, Ph.D.
R.E. Chapin, Ph.D.
G.J. Harry, Ph.D.
J. Mahler, D.V.M.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
K.L. Witt, M.S., Integrated Laboratory Systems, Inc.

Microbiological Associates, Inc.
Conducted studies

M.L. Wenk, Ph.D., Principal Investigator
J.M. Pletcher, D.V.M.

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
S. Botts, D.V.M, M.S., Ph.D.

Pathology Associates International
Evaluated pathology findings

L.H. Brennecke, D.V.M.
R.M. Kovatch, D.V.M.

NTP Pathology Review
Evaluated slides and prepared pathology report (22 November 1996)

J.C. Seely, D.V.M., Chairperson
PATHCO, Inc.
J. Mahler, D.V.M.
National Toxicology Program

Analytical Sciences, Inc.
Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
K.P. McGowan, M.B.A.
M.A. Mauney, M.S.
N.G. Mintz, B.S.
J.T. Scott, M.S.

Biotechnical Services, Inc.
Prepared Toxicity Study Report

S.R. Gunnels, M.A., Principal Investigator
A.M. Macri-Hanson, M.A., M.F.A.
M.L. Rainer, B.S.
W.D. Sharp, B.A., B.S.
D.P. Shaw, B.A.

PEER REVIEW

The draft report on the toxicity studies of benzyltrimethylammonium chloride 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 the Toxicity Study Report presents the experimental results and conclusions fully and clearly.

Mohamed S. Abdel-Rahman, Ph.D.
Department of Pharmacology and Physiology
New Jersey Medical School
Newark, NJ

James S. Bus, Ph.D.
Health and Environmental Sciences
Dow Chemical Company
Midland, MI

Linda A. Chatman, D.V.M.
Pfizer, Inc.
Groton, CT

CONTENTS

ABSTRACT

$C_{10}H_{16}NCl$

BENZYLTRIMETHYLAMMONIUM CHLORIDE

CAS No. 56-93-9

Molecular Weight: 185.70

Synonyms: Ammonium, benzyltrimethyl-chloride (8Cl); BTM; BTMAC; N,N,N,-trimethyl-benzenemethanaminium chloride; trimethylbenzylammonium chloride (9Cl); TMBAC

Benzyltrimethylammonium chloride is widely used as a solvent for cellulose, a gelling inhibitor in polyester resins, a chemical intermediate, a paint dispersant, and an acrylic dyeing agent. It is also used in plant growth regulator compositions and synthetic processes. The National Institute of Environmental Health Sciences nominated benzyltrimethylammonium chloride for study due to its high production volume and the potential for occupational exposure, as well as the limited information on toxicity of this chemical. Male and female F344/N rats and B6C3F$_1$ mice received benzyltrimethylammonium chloride by gavage for 16 days or 13 weeks. Animals were evaluated for hematology, clinical chemistry, histopathology, neurotoxicity, and reproductive toxicity. Genetic toxicology studies were conducted in *Salmonella typhimurium* and in mouse peripheral blood erythrocytes.

In the 16-day studies, groups of five male and five female rats received 0, 16, 32, 63, 125, or 250 mg benzyltrimethylammonium chloride/kg body weight in deionized water by gavage, 5 days per week for 16 days. Groups of five male and five female mice received 0, 63, 125, 250, 500, or 1,000 mg/kg benzyltrimethylammonium chloride in deionized water by gavage, 5 days per week for 16 days. All rats in the 125 and 250 mg/kg groups, all mice in the 250, 500, and 1,000 mg/kg groups, and one 125 mg/kg female mouse died on day 1 of the studies. Clinical findings observed in 125 mg/kg male and female rats included abnormal breathing, ataxia, lethargy (males only), nasal and eye discharge, and tremors. Salivation was slightly increased in male and female rats in the 63 mg/kg groups. Female mice in the 125 mg/kg group had

a significantly greater absolute liver weight than that of the vehicle controls. No gross or microscopic changes observed in rats or mice were considered related to chemical administration.

In the 13-week studies, groups of 10 male and 10 female rats and mice received benzyltrimethylammonium chloride in deionized water by gavage at doses of 0, 12.5, 25, 50, or 100 mg/kg, 5 days per week for 13 weeks. Benzyltrimethylammonium chloride generally had little effect on the body weights of rats or mice. Final mean body weights of dosed animals were within 8% (rats) or 3% (mice) of the control group body weights. The deaths of two female rats and one male and one female mouse administered 100 mg/kg were the result of pharmacologic effects on the cardiovascular system. Some cholinergic effects including chromodacryorrhea, lacrimation, salivation, pupillary constriction, altered gait, and mild tremors were observed at nonlethal doses in rats; these effects were accompanied by alterations in body position. No significant target organ toxicity was observed in dosed rats or mice.

Benzyltrimethylammonium chloride was not mutagenic in *S. typhimurium* strain TA97, TA98, TA100, or TA1535, with or without S9 metabolic activation enzymes. However, significant increases in the frequency of micronucleated normochromatic erythrocytes were found in the peripheral blood of male and female mice administered benzyltrimethylammonium chloride by gavage for 13 weeks.

Based on the mortality observed in the 16-day and 13-week studies, rats and mice appeared to be equally sensitive to benzyltrimethylammonium chloride. The minimally toxic dose for rats and mice was estimated to be 50 mg/kg.

INTRODUCTION

CHEMICAL AND PHYSICAL PROPERTIES

Benzyltrimethylammonium chloride is a quaternary ammonium compound with a structural resemblance to acetylcholine and other chemicals having cholinergic activity. It is an off-white to yellow powder (*Sigma-Aldrich*, 1988) with a melting point of 236° to 239° C (Karsai *et al.*, 1986). It decomposes at 239° C, and the decomposition products include carbon monoxide, carbon dioxide, nitrogen oxides, hydrochloride gas, and ammonia (*Sigma-Aldrich*, 1988; Sax and Lewis, 1989). Benzyltrimethylammonium chloride is hygroscopic (*Sigma-Aldrich*, 1988) and soluble in water, ethanol, and butanol and slightly soluble in butyl phthalate and tributyl phosphate (Sax and Lewis, 1987; Weast, 1989).

PRODUCTION, USE, AND HUMAN EXPOSURE

Benzyltrimethylammonium chloride is prepared by boiling benzyl chloride and trimethylamine in absolute ethanol. In addition, a reaction involving benzyl dimethylamine and methyl chloride, with or without solvent, may be used to manufacture this compound (Karsai *et al.*, 1986). Benzyltrimethylammonium chloride is also prepared by dissolving phenylmethyl chloride in ether and adding 25% trimethylamine in methanol. The product is collected and recrystallized from alcohol and ether (Hume and Holland, 1965).

In 1983, 16 companies were listed as manufacturers of benzyltrimethylammonium chloride. Ten of the manufacturers reported a total production volume ranging from 1.5 to 15.5 million pounds (USEPA, 1990). In 1986 and 1988, respectively, 4,132,000 and 3,985,000 pounds of benzyltrimethylammonium chloride were produced (USITC, 1986, 1987, 1989).

Benzyltrimethylammonium chloride is used as a solvent for cellulose, a gelling inhibitor in polyester resins, a chemical intermediate (Sax and Lewis, 1987), and a paint dispersant for the rubber industry (*Chemical Marketing Reporter*, 1983). It is also used extensively as an acrylic dyeing agent in the textile industry (Moore *et al.*, 1987). Benzyltrimethylammonium chloride is patented for use in plant growth regulator compositions and synthetic processes (Karsai *et al.*, 1986).

Occupational exposure may result from the use of this compound in the chemical (Sax and Lewis, 1987), rubber (*Chemical Marketing Reporter*, 1983), and textile industries (Moore *et al.*, 1987). Data from the National Occupational Exposure Survey, conducted by the National Institute for Occupational Safety and Health during the years 1981 to 1983, estimated that 5,000 workers were potentially exposed to benzyltrimethylammonium chloride (NIOSH, 1990). No exposure or threshold limit values for benzyltrimethylammonium chloride have been established.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

Male Wistar rats intravenously administered benzyltrimethylammonium iodide eliminated the compound from plasma in two phases. One phase had a half-life of 3 minutes, and the other had a half-life of 70 minutes. Benzyltrimethylammonium chloride had an overall half-life of 30 minutes following intravenous administration. Greater than 90% of the dose was eliminated in the urine; 0.9% was eliminated in the bile. The highest tissue concentration following intravenous dosing was found in the liver and kidney 10 minutes after injection. No metabolites were found in the urine, bile, or plasma (Neef *et al.*, 1984).

Absorption from the gastrointestinal tract did not exceed 40% for F344/N rats or 15% for B6C3F$_1$ mice administered 0.63 mg benzyltrimethylammonium chloride (ring-U-^{14}C)/kg body weight by gavage in water and did not exceed 10% for rats or mice administered 63 mg/kg dermally for 24 hours. Greater than 90% of the gavage dose was excreted in the urine and feces within 24 hours of administration. There was no evidence of metabolism of the parent compound in rats or mice because greater than 90% of the excreted radiolabel was composed of the parent compound (Sanders *et al.*, 1995).

Humans

No absorption, distribution, metabolism, or excretion studies of benzyltrimethylammonium chloride in humans were found in a review of the literature.

TOXICITY

Experimental Animals

An acute median lethal oral dose of 250 mg/kg has been reported for rats of an unspecified strain (Dewitt *et al.*, 1953). The acute oral lethality of 1,600 mg benzyltrimethylammonium chloride/kg body weight in an unspecified number of male TAC:SWfBr mice was reported to be 100% (Ellis *et al.*, 1980). In that study, benzyltrimethylammonium chloride was administered orally as a 2% suspension in 0.5% methylcellulose. The results of those studies indicate that rats are more sensitive than mice to the lethality of benzyltrimethylammonium chloride; these results were used to select the doses for the 16-day NTP studies. A 48-hour median lethal concentration for benzyltrimethylammonium chloride in water fleas *(Daphnia pulex)* was determined to be 11.94 ppm in an aquatic static bioassay (Moore *et al.*, 1987). Sanders *et al.* (1995) studied the acute oral toxicity of 125, 175, 210, and 250 mg benzyltrimethylammonium chloride/kg body weight in groups of five male F344/N rats (300 to 350 g). Mortality was observed in the 175, 210, and 250 mg/kg groups. In addition, benzyltrimethylammonium chloride induced muscarinic cholinergic symptoms of salivation and chromodacryorrhea. These symptoms were relieved by atropine injection, but atropine administration did not reduce mortality.

Humans

No data were found on the acute toxicity of benzyltrimethylammonium chloride in humans. However, 10 human fatalities resulting from overexposure to aryl/alkyl quaternary ammonium compounds have been reported. Three of these fatalities involved benzalkonium chloride, and the subjects suffered complete cardiovascular collapse. A common symptom observed in these fatalities was curariform paralysis. Intramuscular injection reportedly resulted in liver and kidney necrosis (Gosselin *et al.*, 1984).

NEUROTOXICITY

The cholinergic activities of benzyltrimethylammonium chloride were studied in isolated muscle preparations *in vitro* and through quantitation of saliva collected from the submaxillary duct of cats following intravenous administration (Hamilton and Rubinstein, 1968). Researchers concluded that benzyltrimethylammonium chloride is capable of stimulating both nicotinic and muscarinic receptors and speculated that it acts at the same ganglionic site as acetylcholine. This was confirmed by studies in which 25 mg benzyltrimethylammonium chloride/kg body weight, dissolved in tetrahydrofurfuryl alcohol and administered intravenously, induced a gastrocnemius muscle twitch in an unspecified strain of rats (Ellis *et al.*, 1980). Benzyltrimethylammonium chloride (40 μg/kg), administered without atropine to mongrel dogs via a femoral vein canula, mimicked

muscarinic acetylcholinergic activity and caused a decrease in blood pressure. A 400 μg/kg dose administered to atropinized dogs induced a nicotinic increase in blood pressure (Hume and Holland, 1965).

Anticholinergic actions of benzyltrimethylammonium bromide have been demonstrated *in vitro* using superfused frog rectus abdominis muscle and rabbit sciatic nerve gastrocnemius muscle preparations (Dretchen *et al.*, 1971). At low concentrations, the salt inhibited cholinergic receptors. At high concentrations, the salt appeared to have acted by a nonspecific mechanism that may have involved increased potassium efflux.

CARCINOGENICITY

No carcinogenicity studies of benzyltrimethylammonium chloride in experimental animals or humans were found in a review of the literature.

GENETIC TOXICITY

Only one set of published mutagenicity data for benzyltrimethylammonium chloride was identified; therefore, there is insufficient information to fully characterize the genetic activity of this compound. Results of mutagenicity testing in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA1535, with and without induced rat or hamster liver S9 activation enzymes, were uniformly negative (Zeiger *et al.*, 1988). Concentrations of benzyltrimethylammonium chloride ranged from 100 to 10,000 μg/plate. Slight toxicity was noted at the higher doses in some trials, but no increases in the number of mutant colonies were seen.

STUDY RATIONALE AND DESIGN

Benzyltrimethylammonium chloride was nominated for toxicity testing by the National Institute of Environmental Health Sciences because of its high production volume, the potential for occupational exposure, and the limited information on toxicity. Sixteen-day and 13-week toxicity studies were conducted in F344/N rats and B6C3F$_1$ mice administered benzyltrimethylammonium chloride by gavage. This route was selected because benzyltrimethylammonium chloride was found to be poorly absorbed via the dermal route (Sanders *et al.*, 1995). Micronucleus tests were conducted on mouse peripheral blood erythrocytes following treatment with benzyltrimethylammonium chloride by gavage for 13 weeks.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF BENZYLTRIMETHYLAMMONIUM CHLORIDE

Benzyltrimethylammonium chloride was obtained from Fluka Chemical Corporation (Ronkonkoma, NY) in one lot (306793/1). Information on the identity, purity, and stability of the bulk chemical was provided by the manufacturer; identity was confirmed by the study laboratory. Reports on analyses performed in support of the benzyltrimethylammonium chloride studies are on file at the National Institute of Environmental Health Sciences.

The manufacturer identified the chemical, an off-white to yellow crystalline powder, as benzyltrimethylammonium chloride by nuclear magnetic resonance spectroscopy. The purity of lot 306793/1, determined by argentometric titration, was 100.4% or greater. The study laboratory confirmed the identity of the chemical with infrared spectroscopy. The spectrum was consistent with a literature reference for benzyltrimethylammonium bromide (*Aldrich*, 1990).

Based on the manufacturer's stability information, the bulk chemical was stored at room temperature in sealed containers flushed with nitrogen to expel moisture.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations for the 16-day studies were prepared once within 7 days of the beginning of dosing. Dose formulations for the 13-week studies were prepared within 8 days of the first day of dosing, then every 2 to 3 weeks until the end of the study. To prepare the dose formulations, a weighed amount of benzyltrimethylammonium chloride was dissolved in deionized water and diluted to achieve the highest concentration required. Serial dilutions of the highest concentration were made to obtain each of the lower concentrations. Dose formulations were stored in sealed serum vials (16-day studies) or amber glass bottles (13-week studies) at room temperature.

Stability studies of 3.2 and 1.25 mg/mL dose formulations were performed by the study laboratory using ultraviolet spectroscopy. Stability was confirmed for at least 21 days (3.2 mg/mL) or 28 days (1.25 mg/mL) for formulations stored in sealed containers at room temperature.

The study laboratory analyzed the dose formulations used in the 16-day studies and the initial, mid-point, and final dose formulations used in the 13-week studies. The study laboratory also analyzed animal room samples of the same dose formulations after they had been in use for 2 to 3 weeks. All dose formulations administered to rats and mice and all animal room samples were within 10% of the target concentrations.

16-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 13 (rats) or 14 (mice) days and were 6 weeks old on the first day of the studies. Groups of five male and five female rats received 0, 16, 32, 63, 125, or 250 mg benzyltrimethylammonium chloride per kg body weight in deionized water by gavage, 5 days per week for 16 days. Groups of five male and five female mice received 0, 63, 125, 250, 500, or 1,000 mg/kg in deionized water by gavage, 5 days per week for 16 days. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage; male mice were housed individually. Animals were observed daily, and clinical findings and body weights were recorded initially, on day 8, and at the end of the studies. Prior to terminal sacrifice, a functional observation battery was performed on all surviving rats. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 16-day studies, the animals were anesthetized with a mixture of carbon dioxide and oxygen, and blood was collected from the retroorbital sinus of all rats and mice for hematology and clinical chemistry analyses. Blood samples for hematology analysis were collected in tubes containing EDTA as an anticoagulant. Blood samples for clinical chemistry analysis were collected in untreated clot tubes, and the sera were separated by centrifugation. Hematology parameters were analyzed on a Serono-Baker 9000 Automated Cell Counter (Serono-Baker Diagnostics, Allentown, PA). Manual hematocrit values were determined using an Adams Microhematocrit Centrifuge, CT2900 (Clay Adams, Sparks, MD). Leukocyte differentials and erythrocyte, leukocyte, lymphocyte, and platelet morphology were determined by light microscopy from blood smears stained with modified Wright's stain using an Ames Hematek Slide Stainer (Miles Laboratory, Ames Division, Elkhart, IN). Reticulocyte counts were determined by light microscopy using blood samples stained with new methylene blue (Sigma Chemical Company, St. Louis, MO). Clinical chemistry parameters were measured

using a Hitachi 717® chemistry analyzer (Boehringer Mannheim, Indianapolis, IN). All reagents were obtained from the manufacturer with the exception of the reagents for sorbitol dehydrogenase and total bile acid determinations, which were obtained from Sigma Chemical Company. The parameters measured are listed in Table 1.

A necropsy was performed on all rats and mice. The heart, right kidney, liver, lung, spleen, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 μm, and stained with hematoxylin and eosin. Histopathologic examinations were performed on vehicle control rats and mice, rats receiving 63, 125, or 250 mg/kg, and mice receiving 125, 250, 500, or 1,000 mg/kg. Table 1 lists the tissues and organs examined.

13-WEEK STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). Upon receipt, the rats and mice were 4 weeks old. Animals were quarantined for 12 to 15 days and were 6 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 untreated mice at the end of the 13-week studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). All results were negative.

Core study groups of 10 male and 10 female rats and mice received benzyltrimethylammonium chloride in deionized water by gavage at doses of 0, 12.5, 25, 50, or 100 mg/kg, 5 days per week for 13 weeks. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings were recorded and animals were weighed initially, on day 8, and weekly until the end of the studies. A functional observation battery was performed on core study rats on days 10 and 85. Details of the study design and animal maintenance are summarized in Table 1.

On days 3 and 21, blood was collected from the retroorbital sinus of groups of 10 special study rats administered the same doses as core study rats for hematology and clinical chemistry analyses. At the end of the 13-week studies, blood was collected from the retroorbital sinus of all core study rats for hematology and clinical chemistry analyses and from all core study mice for clinical chemistry analyses. Methods used for

hematology and clinical chemistry analyses were the same as those used in the 16-day studies. The parameters measured are listed in Table 1.

At the end of the 13-week studies, samples were collected for sperm motility and vaginal cytology evaluations on core study rats and mice receiving 0, 25, 50, or 100 mg/kg. The parameters evaluated are listed in Table 1. Methods used were those described in the NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1991). For 12 consecutive days prior to the 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 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.

A necropsy was performed on all core study rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 μm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on vehicle control groups and all 100 mg/kg rats and mice. 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. 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 Benzyltrimethylammonium Chloride

16-Day Studies	13-Week Studies
Study Laboratory Microbiological Associates, Inc. (Bethesda, MD)	Microbiological Associates, Inc. (Bethesda, MD)
Strain and Species Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$
Animal Source Taconic Farms (Germantown, NY)	Taconic Farms (Germantown, NY)
Time Held Before Studies Rats: 13 days Mice: 14 days	Rats: 12 days (males) or 13 days (females) Mice: 14 days (males) or 15 days (females)
Average Age When Studies Began 6 weeks	6 weeks
Date of First Dose Rats: 10 August 1992 Mice: 11 August 1992	Rats: 12 (males) or 13 (females) October 1993 Mice: 14 (males) or 15 (females) October 1993
Duration of Dosing 16 days (5 days/week)	13 weeks (5 days/week)
Date of Last Dose Rats: 25 August 1992 Mice: 26 August 1992	Rats: 10 (males) or 11 (females) January 1994 Mice: 12 (males) or 13 (females) January 1994
Necropsy Dates Rats: 26 August 1992 Mice: 27 August 1992	Rats: 11 (males) or 12 (females) January 1994 Mice: 13 (males) or 14 (females) January 1994
Average Age at Necropsy 8 weeks	19 weeks
Size of Study Groups 5 males and 5 females	10 males and 10 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 16-day studies
Animals per cage Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 5 Mice: 1 (males) or 5 (females)
Method of Animal Identification Tail tattoo	Tail tattoo
Diet NIH-07 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Benzyltrimethylammonium Chloride

16-Day Studies	13-Week Studies
Water Tap water (Washington Suburban Sanitary Commission Potomac Plant) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 16-day studies
Cages Polycarbonate (Lab Products, Inc., Rochelle Park, NJ), changed twice weekly for group-housed animals and once weekly for individually housed animals	Same as 16-day studies
Bedding Sani-Chips® (P.J. Murphy Forest Products, Montville, NJ) changed twice weekly for group-housed animals and once weekly for individually housed animals	Same as 16-day studies
Racks Stainless steel (Lab Products, Inc., Rochelle Park, NJ), changed and rotated every 2 weeks	Same as 16-day studies
Animal Room Environment Temperature: 20.6°-23.9° C Relative humidity: 35%-65% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 20.6°-23.9° C Relative humidity: 35%-65% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses Rats: 0, 16, 32, 63, 125, or 250 mg/kg in deionized water by gavage (dosing volume=5 mL/kg body weight) Mice: 0, 63, 125, 250, 500, or 1,000 mg/kg in deionized water by gavage (dosing volume=10 mL/kg body weight)	0, 12.5, 25, 50, or 100 mg/kg in deionized water by gavage (dosing volume=5 mL/kg body weight for rats and 10 mL/kg body weight for mice)
Type and Frequency of Observation Observed twice daily; clinical findings and body weights were recorded initially, on day 8, and at the end of the studies.	Observed twice daily; animals were weighed and clinical findings were recorded initially, on day 8, and weekly thereafter until the end of the studies.
Method of Sacrifice CO_2 asphyxiation	Same as 16-day studies
Necropsy Necropsy performed on all animals. Organs weighed were the heart, right kidney, liver, lung, spleen, right testis, and thymus.	Necropsy performed on all core study animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Benzyltrimethylammonium Chloride

16-Day Studies	13-Week Studies
Clinical Pathology Blood was collected from the retroorbital sinus of all rats and mice surviving to the end of the studies for hematology and clinical chemistry. ***Hematology*:** automated and manual hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and nucleated erythrocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet count; and total leukocyte count and differentials ***Clinical chemistry*:** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, serum cholinesterase, erythrocyte cholinesterase, and bile acids	Blood was collected from the retroorbital sinus of all special study rats on days 3 and 21 and from all core study rats surviving until the end of the study for hematology and clinical chemistry. Blood was collected from the retroorbital sinus of all mice surviving to the end of the study for clinical chemistry. ***Hematology*:** automated and manual hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and nucleated erythrocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet count; and total leukocyte count and differentials ***Clinical chemistry*:** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, serum cholinesterase, and bile acids
Histopathology Complete histopathology was performed on all vehicle control animals, 63, 125, and 250 mg/kg rats, and 125, 250, 500, and 1,000 mg/kg mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone and marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (except male mice), 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 Zymbal's gland (rats).	Complete histopathology was performed on all core study vehicle control and 100 mg/kg rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, muscle, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spinal cord, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, uterus, and Zymbal's gland (rats). The lung of all 50 mg/kg rats was also examined.
Sperm Motility and Vaginal Cytology None	At the end of the studies, sperm samples were collected from all core study male rats and mice in the 0, 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 counts, and epididymal spermatozoal motility and concentration. The left cauda epididymis, 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 all core study females administered 0, 25, 50, or 100 mg/kg for vaginal cytology evaluations. The parameters evaluated were estrous cycle length and the percentage of cycle spent in the estrous cycle stages.
Functional Observation Battery At the end of the study, all surviving rats were subjected to a functional observation battery. The parameters evaluated were body position, activity level, coordination, gait, general behavior, head-flick, head-searching, compulsive licking or biting, backward walking, self-mutilation, circling, convulsions, tremors, lacrimation or chromodacryorrhea, salivation, piloerection, pupillary dilation or constriction, unusual respiration, diarrhea, excessive or diminished urination, and vocalization.	Core study rats were subjected to a functional observation battery on days 10 and 85. The parameters evaluated were the same as those evaluated in the 16-day studies.

STATISTICAL METHODS

Calculation and Analysis of Lesion Incidences

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

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and vehicle control groups in the analysis of continuous variables. Organ and body weight data, which 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) 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 (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. 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 data to test for simultaneous equality of measurements across doses. The Fisher exact test was used to determine the significance of the functional observation battery data (Gart *et al.*, 1979).

QUALITY ASSURANCE METHODS

The 13-week studies of benzyltrimethylammonium chloride were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The quality assurance unit of Microbiological Associates, Inc., 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.* (1988). Benzyltrimethylammonium chloride 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 benzyltrimethylammonium chloride. In the absence of toxicity, 10,000 μg/plate was selected as the high dose.

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 13-week 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 1,000 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 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 dose 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 13-week studies 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 differing 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 Study Report represent a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

RATS

16-DAY STUDY

All male and female rats in the 125 and 250 mg/kg groups died on day 1 of the study (Table 2). All other rats survived to the end of the study. The final mean body weights and body weight gains of dosed males and females were similar to those of the vehicle controls (Table 2).

TABLE 2
Survival and Body Weights of Rats in the 16-Day Gavage Study of Benzyltrimethylammonium Chloride

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	151 ± 3	240 ± 4	89 ± 2	
16	5/5	150 ± 3	231 ± 5	81 ± 4	96
32	5/5	147 ± 7	233 ± 12	86 ± 6	97
63	5/5	146 ± 4	226 ± 8	80 ± 5	94
125	0/5[c]	150 ± 4	—	—	—
250	0/5[c]	149 ± 5	—	—	—
Female					
0	5/5	103 ± 4	137 ± 5	34 ± 1	
16	5/5	103 ± 3	144 ± 5	41 ± 2	105
32	5/5	106 ± 2	142 ± 4	36 ± 2	104
63	5/5	103 ± 2	137 ± 4	34 ± 2	100
125	0/5[c]	103 ± 2	—	—	—
250	0/5[c]	105 ± 4	—	—	—

[a] Number of animals surviving at 16 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the vehicle control groups were not significant by Dunnett's test. No data were calculated for groups with 100% mortality.
[c] Day of death: 1

Chemical-related clinical findings observed in three males and one female administered 125 mg/kg included abnormal breathing, ataxia, lethargy (males only), nasal and eye discharge, and tremors on day 1; one 63 mg/kg male was also lethargic and had nasal and eye discharge. A functional observation battery was performed on males and females surviving to the end of the study. All males in the 63 mg/kg group exhibited salivation (Table B1). Compared to the vehicle controls, incidences of pupillary dilation and mild tremors were slightly, but not significantly, increased in 63 mg/kg males. The only chemical-related effect observed in females was salivation in two of five rats in the 63 mg/kg group.

Because of 100% mortality in the 125 and 250 mg/kg groups, no hematology or clinical chemistry evaluations were performed and no organ weight data were collected for these groups. For the groups with survivors, there were no treatment-related changes in the hematology, clinical chemistry, or organ weight variables (Tables C1 and D1). No chemical-related gross or microscopic changes were observed. In some animals that died early, necrosis of thymic lymphocytes and pulmonary edema were observed; these were thought to be stress-related or agonal changes. Based on the 100% mortality observed at 125 and 250 mg/kg, doses of 0, 12.5, 25, 50, and 100 mg/kg were selected for the 13-week gavage study in rats.

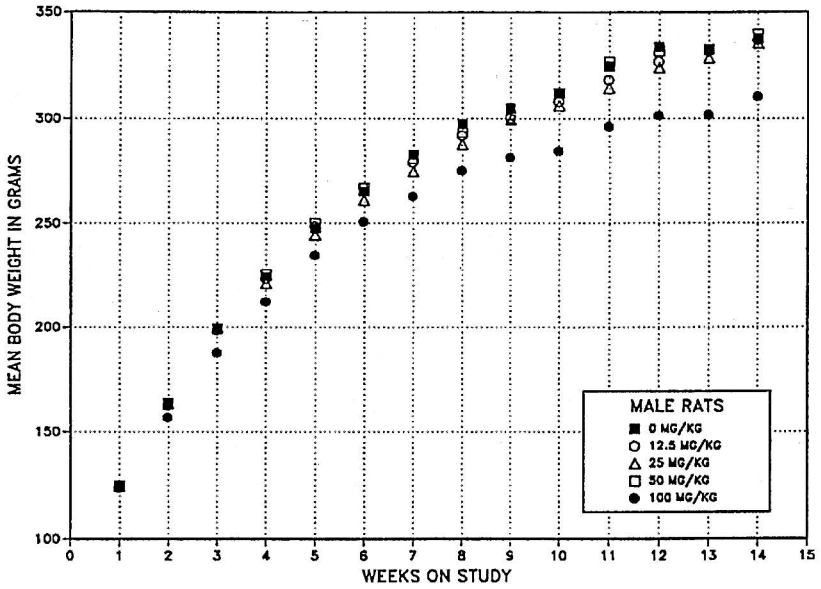
13-WEEK STUDY

One 25 mg/kg and two 100 mg/kg female rats died before the end of the study (Table 3); the deaths of the 100 mg/kg females were considered to be due to pharmacologic effects of benzyltrimethylammonium chloride on the cardiovascular system. All other rats survived to the end of the study. The mean body weight gain of 100 mg/kg males was significantly less than that of the vehicle controls (Table 3 and Figure 1). Chemical-related clinical findings included nasal and eye discharge in 12.5 (1/10), 25 (6/10), 50 (6/10), and 100 (10/10) mg/kg males and in 50 (6/10) and 100 (6/10) mg/kg females, oral discharge in 50 (2/10) and 100 (3/10) mg/kg males and in 100 mg/kg females (9/10), and tremors in 100 mg/kg males (4/10) and females (2/10).

TABLE 3
Survival and Body Weights of Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	125 ± 4	338 ± 8	213 ± 7	
12.5	10/10	124 ± 4	337 ± 8	213 ± 6	100
25	10/10	125 ± 3	336 ± 8	211 ± 5	99
50	10/10	125 ± 4	340 ± 5	215 ± 4	101
100	10/10	125 ± 3	311 ± 9	186 ± 7**	92
Female					
0	10/10	106 ± 3	190 ± 3	85 ± 2	
12.5	10/10	106 ± 3	198 ± 4	93 ± 2	104
25	9/10[c]	104 ± 2	193 ± 3	88 ± 2	101
50	10/10	107 ± 3	192 ± 4	85 ± 3	101
100	8/10[d]	107 ± 3	187 ± 4	81 ± 2	98

** Significantly different ($P \leq 0.01$) from the vehicle control group by Dunnett's test
[a] Number of animals surviving at 13 weeks/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 (gavage accident)
[d] Week of death: 10, 12



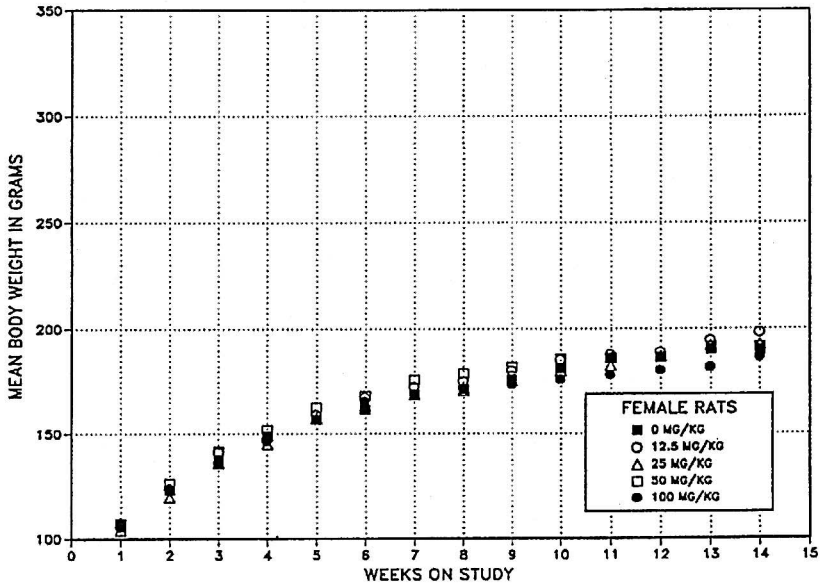


FIGURE 1
Body Weights of Rats Administered Benzyltrimethylammonium Chloride by Gavage for 13 Weeks

A functional observation battery was conducted on days 10 and 85. Clinical evaluation demonstrated chromodacryorrhea and increased salivation in male and female rats in the 100 mg/kg group on day 85 (Tables 4 and B2). In female rats, slight lacrimation was observed in all dosed groups (30% to 75%) on day 85. Chemical-related effects on the motor system were evident on day 85 in male and female rats in the 100 mg/kg groups. These effects were characterized by an altered gait (males: 40%; females: 25%) and mild to severe tremors (males: 50%; females: 63%) and were accompanied by alterations in motor coordination and, in some cases, altered body position (males: 40%; females: 38%). Pupillary constriction was observed in 3 of 10 females in the 50 mg/kg group and 5 of 10 females in the 100 mg/kg group.

TABLE 4
Summary of Functional Observation Battery for Rats on Day 85 in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male					
n	10	10	10	10	10
Body position					
Crouched over	0	0	0	0	2
Head bobbing	0	0	0	0	2
Coordination of movement					
Moderately impaired	0	0	0	0	1
Severely impaired	0	0	0	0	1
Gait					
Hunched or crouched	0	0	0	0	2
Body drags/is flattened	0	0	0	0	2
Lacrimation or chromodacryorrhea					
Slight	0	0	0	0	5*
Severe	0	0	0	0	1
Pupillary constriction or dilation					
Constricted	0	0	0	0	1
Salivation					
Slight	0	1	2	4*	2
Severe	0	0	0	2	8**
Tremors					
Mild	0	0	0	0	2
Severe whole body	0	0	0	0	3

TABLE 4
Summary of Functional Observation Battery for Rats on Day 85 in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Female					
n	10	10	9	10	8
Body position					
Flattened	0	0	0	0	1
Crouched over	0	0	0	0	2
Coordination of movement					
Slightly impaired	0	0	0	0	3
Gait					
Ataxia	0	0	0	0	2
Lacrimation or chromodacryorrhea					
Slight	0	3	3	4*	6**
Pupillary constriction or dilation					
Constricted	0	0	0	3	4*
Salivation					
Slight	1	1	1	0	3
Severe	0	0	0	1	1
Tremors					
Mild	0	0	0	0	4*
Mild whole body	0	0	0	0	1

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$

Significant differences were observed in the hematology and clinical chemistry variables (Table C2). The majority of these differences were sporadic or minimal, did not demonstrate a treatment relationship, or were inconsistent between genders and consequently were not considered to be toxicologically relevant. However, at week 13, there were very minimal, treatment-related increases in the mean cell volumes of rats. These increases in mean cell volume, which is an estimate of the average size (expressed as a volume) of a population of erythrocytes, suggest that the erythrocytes were minimally larger in the dosed animals than in the vehicle controls. Additionally, females administered 25 mg/kg or greater appeared to have minimally decreased total protein and albumin concentrations. The biologic significance of the differences in mean cell volumes and protein concentrations is unknown; because these changes were minimal and no other hematologic, clinical chemistry, and pathologic alterations occurred, the differences were not considered to be clinically significant.

Benzyltrimethylammonium chloride administration had no effect on the absolute or relative organ weights of males or females (Table D2). No chemical-related gross or microscopic lesions were observed (Tables A1 and A2). There were no differences in reproductive tissue parameters in males (Table E1). A minimal shortening of diestrus and prolongation of proestrus occurred in 25 mg/kg females; there was no alteration in the length of the estrous cycle (Table E2).

MICE

16-DAY STUDY

All male and female mice in the 250, 500, and 1,000 mg/kg groups and one 125 mg/kg female died on day 1 of the study; all other mice survived to the end of the study (Table 5). The mean body weight gains of females in the 63 and 125 mg/kg groups were significantly greater than that of the vehicle controls (Table 5). The final mean body weights of dosed males and females and mean body weight gains of dosed males were similar to those of the vehicle controls. Clinical findings occurred sporadically and were not considered to be related to chemical administration.

TABLE 5
Survival and Body Weights of Mice in the 16-Day Gavage Study of Benzyltrimethylammonium Chloride

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	23.8 ± 0.5	26.4 ± 0.7	2.6 ± 0.3	
63	5/5	23.8 ± 0.6	26.0 ± 0.5	2.2 ± 0.2	98
125	5/5	23.6 ± 0.4	26.1 ± 0.6	2.6 ± 0.4	99
250	0/5[c]	23.7 ± 0.4	—	—	—
500	0/5[c]	23.5 ± 0.5	—	—	—
1,000	0/5[c]	23.7 ± 0.6	—	—	—
Female					
0	5/5	20.0 ± 0.4	21.2 ± 0.4	1.2 ± 0.1	
63	5/5	19.2 ± 0.5	21.9 ± 0.5	2.7 ± 0.2**	103
125	4/5[c]	19.3 ± 0.5	22.4 ± 0.1	2.6 ± 0.3**	105
250	0/5[c]	19.6 ± 0.3	—	—	—
500	0/5[c]	19.7 ± 0.3	—	—	—
1,000	0/5[c]	19.4 ± 0.5	—	—	—

** Significantly different ($P \leq 0.01$) from the vehicle control group by Dunnett's test
[a] Number of animals surviving at 16 days/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. No data were calculated for groups with 100% mortality.
[c] Day of death: 1

Because of 100% mortality in the groups receiving 250 mg/kg or greater, no hematology or clinical chemistry evaluations were performed and no organ weight data were collected for these groups. For the groups with survivors, there were no treatment-related changes in hematology or clinical chemistry variables (Table C3). For 125 mg/kg females, the absolute liver weight was significantly greater and the relative lung weight was significantly less than those of the vehicle controls (Table D3). No chemical-related gross or microscopic changes were observed. Pulmonary congestion and edema were observed in some animals that died early and were interpreted to be an agonal change. Based on the 100% mortality observed at 250 mg/kg and greater, doses of 0, 12.5, 25, 50, and 100 mg/kg were selected for the 13-week gavage study in mice.

13-WEEK STUDY

One male and one female in the 100 mg/kg groups died before the end of the study; the deaths were the result of pharmacologic effects of benzyltrimethylammonium chloride on the cardiovascular system (Table 6). All other mice survived until the end of the study. Final mean body weights and body weight gains of dosed males and females were similar to those of the vehicle controls (Table 6 and Figure 2). Beginning at week 10, hyperactivity was observed in 100 mg/kg females immediately following administration of benzyltrimethylammonium chloride. However, the hyperactivity diminished within an hour after dosing. No other clinical findings were observed.

TABLE 6
Survival and Body Weights of Mice in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	24.4 ± 0.5	34.9 ± 0.7	10.6 ± 0.5	
12.5	10/10	24.3 ± 0.5	34.9 ± 1.0	10.6 ± 0.5	100
25	10/10	24.3 ± 0.5	34.5 ± 0.6	10.1 ± 0.5	99
50	10/10	24.6 ± 0.5	34.8 ± 0.9	10.2 ± 0.5	100
100	9/10[c]	24.4 ± 0.5	33.9 ± 0.9	9.4 ± 0.4	97
Female					
0	10/10	19.3 ± 0.4	29.1 ± 1.0	9.8 ± 0.8	
12.5	10/10	19.2 ± 0.4	29.9 ± 0.9	10.6 ± 0.7	103
25	10/10	18.4 ± 0.4	28.7 ± 0.9	10.2 ± 0.7	98
50	10/10	19.2 ± 0.6	29.2 ± 1.3	10.0 ± 0.8	100
100	9/10[d]	18.7 ± 0.4	28.2 ± 0.9	9.3 ± 0.7	97

[a] Number of animals surviving at 13 weeks/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 groups were not significant by Dunnett's test.
[c] Week of death: 9
[d] Week of death: 6

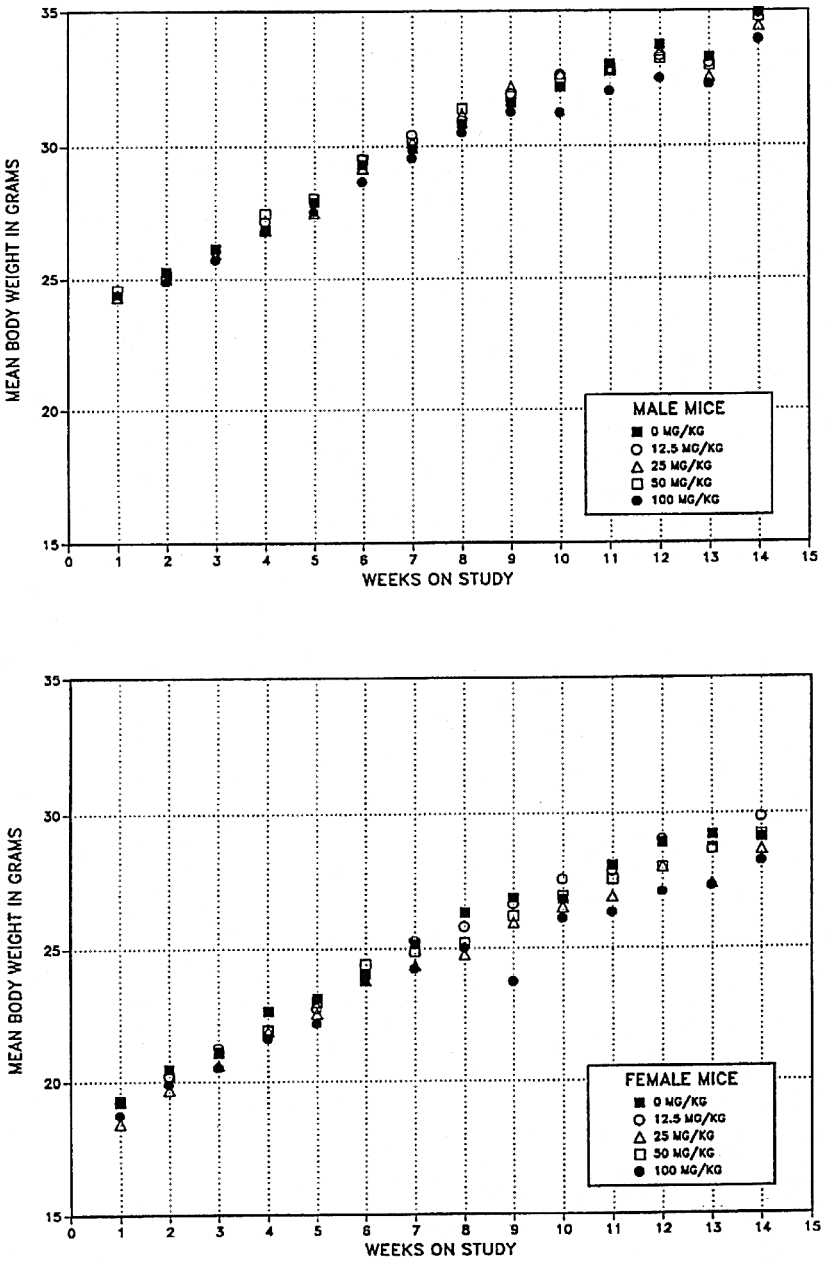


FIGURE 2
Body Weights of Mice Administered Benzyltrimethylammonium Chloride by Gavage for 13 Weeks

In male mice, kidney weights were increased in the 50 mg/kg group, and the relative kidney weight was also increased in the 100 mg/kg group (Table D4). Relative heart weights were increased in the 25 mg/kg or greater males. However, no chemical-related gross or microscopic lesions were observed (Tables A3 and A4). Males administered 25 mg/kg or greater had minimally decreased total protein concentrations (Table C4). The biologic significance of the protein concentration difference was unknown; because the change was minimal and no other clinical chemistry and pathologic alterations occurred, this difference was not considered to be clinically significant. No treatment-related differences were detected in reproductive tissue evaluations or estrous cycle characterizations (Tables E3 and E4).

GENETIC TOXICOLOGY

Benzyltrimethylammonium chloride (100 to 10,000 μg/plate) was not mutagenic in *Salmonella typhimurium* strain TA97, TA98, TA100, or TA1535 with or without induced rat or hamster liver S9 metabolic activation enzymes (Zeiger *et al.*, 1988; Table F1). Slight toxicity was noted at the two highest concentrations tested in all four strains. *In vivo*, benzyltrimethylammonium chloride induced a significant dose-related increase in the frequency of micronucleated normochromatic erythrocytes in the peripheral blood of male and female mice administered 12.5 to 100 mg/kg by gavage for 13 weeks (Table F2). Micronucleus analyses yielded positive trends ($P \leq 0.025$) for both the male and female data, but only the highest dose tested in males and females produced an increase in micronuclei that was significantly different from the control frequency ($P \leq 0.006$).

DISCUSSION

Benzyltrimethylammonium chloride is widely used in the chemical, textile, and rubber industries (USEPA, 1990). It was nominated for toxicity testing by the National Institute of Environmental Health Sciences because of its high production volume, potential for occupational exposure, and the paucity of toxicity information concerning the chemical.

Based on the doses at which mortality occurred in the 16-day studies, rats and mice appear to be equally sensitive to benzyltrimethylammonium chloride. On day 1 of the studies, 100% mortality occurred in 125 and 250 mg/kg male and female rats and in 250, 500, and 1,000 mg/kg male and female mice; one of five 125 mg/kg female mice died. The high rate of mortality in rats and mice in the 16-day studies combined with the absence of an identifiable target organ for benzyltrimethylammonium chloride toxicity suggests that the cause of death was the result of a pharmacologic effect. The differences in lung and liver weights in 125 mg/kg female mice in the 16-day study were not associated with gross or histologic changes and, accordingly, were not considered to be related to chemical administration. Benzyltrimethylammonium chloride mimics the action of acetylcholine by activating the muscarinic and nicotinic receptors and was shown to be a vasodepressor that could lead to total cardiovascular collapse (Hume and Holland, 1965; Hamilton and Rubinstein, 1968; Gosselin *et al.*, 1984). The cholinergic activity of benzyltrimethylammonium chloride in rats in the 16-day study was evidenced by salivation. Benzyltrimethylammonium chloride was four times more active than acetylcholine in its ability to induce salivation in dogs (Long *et al.*, 1965). However, pupillary dilation was observed in 63 mg/kg male rats but not in females and therefore was not considered to be related to chemical administration. Neither salivation nor pupillary constriction occurred in dosed mice.

The NTP also conducted 14-day dermal studies (unpublished) of benzyltrimethylammonium chloride. Male and female F344/N rats were administered 0, 11.9, 39.6, or 118.8 mg per day (equivalent to 0, 170, 340, or 680 mg/kg per day for males and 0, 260, 520, or 860 mg/kg per day for females), and B6C3F$_1$ mice were administered 0, 3.96, 11.9, or 39.6 mg per day (equivalent to 0, 385, 790, or 1,580 mg/kg per day for males and 0, 450, 900, or 1,800 mg/kg per day for females). Results of these dermal studies were similar to those of the 16-day gavage studies except that the animals were more sensitive to toxic effects following gavage administration. Deaths, ataxia, and tremors occurred in rats administered 118.8 mg and mice administered 39.6 mg; these doses were 1.5 to 3.5 orders of magnitude greater than the doses used in the gavage studies.

This difference in sensitivity is supported by the findings of Sanders *et al.* (1995), which showed that benzyltrimethylammonium chloride was poorly absorbed from the skin of rats and mice.

Based on the mortality in the 16-day studies, doses of 0, 12.5, 25, 50, and 100 mg/kg were administered in deionized water by gavage to rats and mice in the 13-week studies. Three female rats and one male and one female mouse died before the end of the studies. There were no significant differences in final mean body weights of dosed male or female rats or mice compared to the vehicle controls. Because the changes in kidney and heart weights observed in 50 and 100 mg/kg male mice were not associated with gross or histopathologic changes, these effects were not considered to be related to chemical administration. Clinical findings and functional observations in 100 mg/kg rats included eye, nasal, and oral discharges, lacrimation or chromodacryorrhea, salivation, tremors, pupillary constriction, and impaired coordination. Between 10 and 13 weeks of dosing, female mice displayed increased activity levels immediately following dosing at 100 mg/kg; the activity level returned to normal levels within 1 hour. The cholinergic nature of these effects suggest an acetylcholine-mimicking activity of benzyltrimethylammonium chloride (Long *et al.*, 1965; Strycker and Long, 1969).

Significant decreases in total serum protein concentrations were observed in 25 and 50 mg/kg female rats on day 3, in 25, 50, and 100 mg/kg female rats at week 13, and in 25, 50, and 100 mg/kg male mice at week 13. The biological significance of this decrease is unknown because the effect was minimal in magnitude and was not accompanied by other clinical or pathologic alterations. In addition, total serum protein concentrations of all dosed groups fell within control values reported for rats (7.52 ± 0.20 g/dL) and mice (2.73 ± 0.30 g/dL); it is therefore unlikely that the decrease was due to chemical administration (Kaneko, 1989). Similarly, the increase in the mean cell volumes was considered biologically insignificant because the effect was minimal in magnitude and was not accompanied by hematologic or pathologic alterations. In the 16-day study, edema was observed in the lung of 250 mg/kg female rats and was considered secondary to lethality induced through cholinergic stimulation. Lung edema is likely to be the result of a decreased heart rate leading to reduced blood pressure and the force of contraction prior to death. No histopathologic changes that could be attributed to benzyltrimethylammonium chloride administration were observed in rats or mice.

Benzyltrimethylammonium chloride was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA1535, with or without S9 metabolic activation enzymes (Zeiger *et al.*, 1988). However, it did induce significant increases in the frequency of micronucleated normochromatic erythrocytes in peripheral blood of male and female mice in the 13-week study. Elevated micronucleus frequencies were observed in male and female mice administered 50 mg/kg or greater, although statistically significant increases were seen

only at 100 mg/kg. The observation of micronucleus induction suggests that benzyltrimethylammonium chloride induced chromosomal damage in maturing erythrocytes in the form of breakage and/or mitotic disruption leading to numerical aberrations (chromosome loss). No alteration in the percentage of normochromatic erythrocytes in the blood was observed in male or female mice, indicating no overt toxicity to the bone marrow and no stimulation of erythropoiesis.

Based on the mortality observed in the 16-day and 13-week studies, rats and mice appeared to be equally sensitive to benzyltrimethylammonium chloride. The minimally toxic dose for rats and mice was estimated to be 50 mg/kg.

REFERENCES

Aldrich Catalog/Handbook of Fine Chemicals 1990-1991 (1990), p. 146. Aldrich Chemical Company, Inc., Milwaukee, WI.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Boorman, G.A., Hickman, R.L., Davis, G.W., Rhodes, L.S., White, N.W., Griffen, T.A., Mayo, J., and Hamm, T.E., Jr. (1986). Serological titers to murine viruses in 90-day and 2-year studies. In *Complications of Viral and Mycoplasmal Infections in Rodents to Toxicology Research and Testing* (T.E. Hamm, Jr., Ed.), pp. 11-23. Hemisphere Publishing Corporation, Washington, DC.

Chemical Marketing Reporter (April 11, 1983), p. 13.

Code of Federal Regulations (CFR) **21**, Part 58.

Dewitt, J.B., Bellack, E., Klingensmith, C.W., Ward, J.C., and Treichler, R. (1953). Relationship Between Chemical Structure and Toxic Action on Rats. Chemical and Biological Research Center, Review No. 5, p. 39. National Research Council, Washington, DC.

Dixon, W.J., and Massey, F.J., Jr. (1951). *Introduction to Statistical Analysis*, 1st ed., pp. 145-147. McGraw-Hill Book Company, Inc., New York.

Dretchen, K., Diecke, F.P.J., and Long, J.P. (1971). Studies on the nonspecific blocking action of benzyltrimethylammonium bromide (BTM). *J. Pharmacol. Exp. Ther.* **177**, 369-376.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Ellis, K.O., White, R.L., Jr., Wright, G.C., and Wessels, F.L. (1980). Synthesis and skeletal muscle relaxant activity of quarternary ammonium salts of dantrolene and clodanolene. *J. Pharm. Sci.* **69**, 327-331.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *JNCI* **62**, 957-974.

Gosselin, R.E., Smith, R.P., Hodge, H.C., and Braddock, J.E. (1984). *Clinical Toxicity of Commercial Products: Acute Poisoning*, 5th ed., pp. II-278, III-63 to III-66. Williams and Wilkins, Baltimore, MD.

Hamilton, J.T., and Rubinstein, H.M. (1968). Nicotinic and muscarinic activity of benzyltrimethyl-ammonium and its alpha-, beta-, and gamma-substituted pyridylmethylammonium analogs. *J. Pharmacol. Exp. Ther.* **160**, 112-123.

Hume, A.S., and Holland, W.C. (1965). Vasopressor and depressor activity of phenylalkyltrimethylammonium compounds. *Arch. Int. Pharmacodyn.* **154**, 155-160.

Integrated Laboratory Systems (ILS) (1990). Micronucleus Data Management and Statistical Analysis Software, Version 1.4. ILS, P.O. Box 13501, Research Triangle Park, NC 72207.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kaneko, J.J. (1989). *Clinical Biochemistry of Domestic Animals*, 4th ed., p. 892. Academic Press, Inc., New York.

Karsai, J., Sebestyén, E., Gaál, S., Gárdi, I., Siki, K., and Kíss, G. (1986). Plant growth regulating compositions and process for regulating plant growth. International Patent Application, Patent No. WO 86/07237.

Long, J.P., Wong, K.C., and Witt, D.L. (1965). Cholinergic and anticholinergic activity of benzyltrimethylamine and fluorobenzyl isomers. *Arch. Int. Parmacodyn.* **155**, 282-288.

MacGregor, J.T., Wehr, C.M., Henika, P.R., and Shelby, M.D. (1990). The *in vivo* erythrocyte micronucleus test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.* **14**, 513-522.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

Moore, S.B., Diehl, R.A., Barnhardt, J.M., and Avery, G.B. (1987). Aquatic toxicities of textile surfactants. *Text. Chemist Colorist* **19**, 29-32.

Morrison, D.F. (1976). *Multivariate Statistical Methods*, 2nd ed., pp. 170-179. McGraw-Hill Book Company, New York.

National Institute for Occupational Safety and Health (NIOSH) (1990). National Occupational Exposure Survey (1981-1983), unpublished provisional data as of July 1, 1990. NIOSH, Cincinnati, OH.

National Toxicology Program (NTP) (1991). Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluations in Toxicity Testing for Rats and Mice, 10/31/82 version (updated May 1991). Research Triangle Park, NC.

Neef, C., Oosting, R., and Meijer, D.K.F. (1984). Structure-pharmacokinetics relationship of quaternary ammonium compounds. Elimination and distribution characteristics. *Naunyn Schmiedeberg's Arch. Pharmacol.* **328**, 103-110.

Rao, G.N., Haseman, J.K., and Edmondson, J. (1989a). Influence of viral infections on body weight, survival, and tumor prevalence in Fischer 344/NCr rats on two-year studies. *Lab. Anim. Sci.* **39**, 389-393.

Rao, G.N., Piegorsch, W.W., Crawford, D.D., Edmondson, J., and Haseman, J.K. (1989b). Influence of viral infections on body weight, survival, and tumor prevalence of B6C3F_1 (C57BL/6N $\times$ C3H/HeN) mice in carcinogenicity studies. *Fundam. Appl. Toxicol.* **13**, 156-164.

Sanders, M., Griffin, R.J., Burka, L.T., and Matthews, H.B. (1995). Toxicokinetics of the cholinomimetic compound benzyltrimethylammonium chloride in the male rat and mouse. *Xenobiotica* **25**, 303-313.

Sax, N.I., and Lewis, R.J., Sr. (1987). *Hawley's Condensed Chemical Dictionary*, 11th ed., p. 138. Van Nostrand Reinhold, New York.

Sax, N.I., and Lewis, R.J., Sr. (1989). *Dangerous Properties of Industrial Materials*, 7th ed., p. 426. Van Nostrand Reinhold, New York.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

The Sigma-Aldrich Library of Chemical Safety Data (1988). 2nd ed. (R.E. Lenga, Ed.). Sigma-Aldrich Corp., Milwaukee, WI.

Strycker, S.J., and Long, J.P. (1969). Studies on the muscarinic and antimuscarinic activity of benzyltrimethylammonium bromide (BTM). *J. Pharm. Sci*. **58**, 671-675.

United States Environmental Protection Agency (USEPA) (1990). U.S. Environmental Protection Agency, Computer Printout (TSCAPP): 1983 Production Statistics for Chemicals in the Non-confidential TSCA Chemical Substances Inventory. Office of Pesticides and Toxic Substances, Washington, DC.

United States International Trade Commission (USITC) (1986). Synthetic Organic Chemicals, United States Production and Sales, 1986, pp. 145, 167, 177-178. U.S. Publication No. 2009. Government Printing Office, Washington, DC.

United States International Trade Commission (USITC) (1987). Synthetic Organic Chemicals, United States Production and Sales, 1987, pp. 3-4, 12-6. Publication No. 2118. U.S. Government Printing Office, Washington, DC.

United States International Trade Commission (USITC) (1989). Synthetic Organic Chemicals, United States Production and Sales, 1988, pp. 12-6, 12-27, A-5, A-14 to A-18. Publication No. 2219. U.S. Government Printing Office, Washington, DC.

Weast, R.C., Ed. (1989). *CRC Handbook of Chemistry and Physics* (1988-1989), 69th ed., p. C-150. CRC Press, Inc., Boca Raton, FL.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1988). *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. Mol. Mutagen*. **11** (Suppl. 12), 1-158.

APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS IN RATS AND MICE

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary					
Animals initially in study	10	10	10	10	10
Survivors					
Terminal sacrifice	10	10	10	10	10
Animals examined microscopically	10	2	1	10	10
Alimentary System					
Liver	(10)	(2)			(10)
Inflammation, focal	4 (40%)				5 (50%)
Hepatocyte, centrilobular, vacuolization cytoplasmic					1 (10%)
Cardiovascular System					
Heart	(10)				(10)
Inflammation, focal	8 (80%)				5 (50%)
Endocrine System					
Pituitary gland	(10)				(10)
Cyst	1 (10%)				1 (10%)
Thyroid gland	(10)				(10)
Follicle, cyst	2 (20%)				1 (10%)
General Body System					
None					
Genital System					
Epididymis	(10)		(1)		(10)
Inflammation, focal, granulomatous			1 (100%)		
Prostate	(10)				(10)
Infiltration cellular, focal, lymphocyte	1 (10%)				
Inflammation					1 (10%)
Testes	(10)		(1)		(10)
Atrophy			1 (100%)		
Hematopoietic System					
Spleen	(10)				(10)
Hematopoietic cell proliferation	3 (30%)				4 (40%)
Integumentary System					
None					

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Musculoskeletal System					
None					
Nervous System					
None					
Respiratory System					
Lung	(10)			(10)	(10)
Inflammation, focal	6 (60%)			1 (10%)	5 (50%)
Nose	(10)				(10)
Inflammation	2 (20%)				
Special Senses System					
None					
Urinary System					
Kidney	(10)		(1)		(10)
Renal tubule, hemorrhage, focal	1 (10%)				

[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 13-Week Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary					
Animals initially in study	10	10	10	10	10
Early death					
Accidental death			1		
Natural deaths					2
Survivors					
Died last week of study				1	
Terminal sacrifice	10	10	9	9	8
Animals examined microscopically	10	3	2	10	10
Alimentary System					
Liver	(10)	(1)	(1)		(10)
Inflammation, focal	5 (50%)				4 (40%)
Hepatocyte, centrilobular, vacuolization cytoplasmic			1 (100%)		
Mesentery	(1)		(1)		
Fat, necrosis	1 (100%)		1 (100%)		
Pancreas	(10)		(1)		(10)
Acinus, atrophy					1 (10%)
Acinus, degeneration, focal	1 (10%)				
Cardiovascular System					
Heart	(10)		(1)		(10)
Inflammation, focal	5 (50%)		1 (100%)		4 (40%)
Endocrine System					
Pituitary gland	(10)				(10)
Pars distalis, cyst					1 (10%)
Thyroid gland	(10)		(1)		(10)
Follicle, cyst	1 (10%)				
General Body System					
None					
Genital System					
Uterus	(10)	(2)	(1)	(1)	(10)
Bilateral, cyst		2 (100%)		1 (100%)	
Bilateral, dilatation	2 (20%)				2 (20%)
Hematopoietic System					
Spleen	(10)		(1)		(10)
Hematopoietic cell proliferation	1 (10%)		1 (100%)		

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Integumentary System					
None					
Musculoskeletal System					
None					
Nervous System					
None					
Respiratory System					
Lung	(10)		(1)	(10)	(10)
Congestion					1 (10%)
Inflammation, focal	3 (30%)		1 (100%)	3 (30%)	6 (60%)
Nose	(10)				(10)
Inflammation, chronic active	1 (10%)				
Special Senses System					
None					
Urinary 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 13-Week Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary					
Animals initially in study	10	10	10	10	10
Early death					
Natural death					1
Survivors					
Died last week of study				1	
Terminal sacrifice	10	10	10	9	9
Animals examined microscopically	10				10
Alimentary System					
Liver	(10)				(10)
Inflammation, chronic, focal					1 (10%)
Hepatocyte, centrilobular, hypertrophy	3 (30%)				3 (30%)
Salivary glands	(10)				(10)
Infiltration cellular, focal, lymphocyte	1 (10%)				
Cardiovascular System					
None					
Endocrine System					
Adrenal cortex	(10)				(10)
Capsule, hyperplasia, focal					1 (10%)
General Body System					
None					
Genital System					
Prostate	(10)				(10)
Infiltration cellular, lymphocyte	1 (10%)				
Hematopoietic System					
Spleen	(10)				(10)
Hematopoietic cell proliferation	1 (10%)				1 (10%)
Lymphoid follicle, hyperplasia					2 (20%)
Integumentary System					
None					
Musculoskeletal System					
None					

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	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					
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 13-Week Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary					
Animals initially in study	10	10	10	10	10
Early death					
Natural death					1
Survivors					
Terminal sacrifice	10	10	10	10	9
Animals examined microscopically	10				10
Alimentary System					
Liver	(10)				(10)
Inflammation, chronic, focal	1 (10%)				
Necrosis, focal	2 (20%)				1 (10%)
Hepatocyte, centrilobular, hypertrophy	3 (30%)				3 (30%)
Cardiovascular System					
None					
Endocrine System					
Adrenal cortex	(10)				(10)
Bilateral, capsule, hyperplasia, focal	5 (50%)				5 (50%)
Capsule, hyperplasia, focal	1 (10%)				
Thyroid gland	(10)				(10)
Ultimobranchial cyst					1 (10%)
General Body System					
None					
Genital System					
Ovary	(10)				(10)
Hemorrhage					1 (10%)
Uterus	(10)				(10)
Endometrium, hyperplasia, cystic	3 (30%)				
Hematopoietic System					
Lymph node, mandibular	(10)				(10)
Hemorrhage					1 (10%)
Hyperplasia, lymphoid	1 (10%)				
Lymph node, mesenteric	(10)				(10)
Hyperplasia, lymphoid	1 (10%)				
Spleen	(10)				(10)
Hematopoietic cell proliferation	1 (10%)				3 (30%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Integumentary System					
None					
Musculoskeletal System					
None					
Nervous System					
None					
Respiratory System					
Lung	(10)				(10)
Interstitium, inflammation, chronic	1 (10%)				2 (20%)
Nose	(10)				(10)
Olfactory epithelium, cytoplasmic alteration					1 (10%)
Special Senses System					
None					
Urinary System					
Urinary bladder	(10)				(10)
Infiltration cellular, lymphocyte					1 (10%)

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

APPENDIX B
FUNCTIONAL OBSERVATION BATTERY RESULTS

TABLE B1
Selected Functional Observation Battery Data for Rats in the 16-Day Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	16 mg/kg	32 mg/kg	63 mg/kg
Male				
Activity				
Minimum exploratory movements	1/5	1/5	0/5	1/5
Somewhat high	0/5	0/5	1/5	0/5
Pupillary constriction or dilation				
Dilated	0/5	0/5	1/5	3/5
Salivation				
Slight	0/5	0/5	0/5	4/5*
Severe	0/5	0/5	0/5	1/5
Tremors				
Mild	0/5	0/5	0/5	2/5
Female				
Salivation				
Slight	0/5	1/5	0/5	2/5

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
[a] Number of animals with behavior/number of animals observed

TABLE B2
Functional Observation Battery Data for Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male					
Activity					
Day 10					
Minimum exploratory movements	2/10	1/10	1/10	3/10	1/10
Normal	8/10	9/10	9/10	7/10	9/10
Day 85					
Low	0/10	0/10	0/10	0/10	2/10
Minimum exploratory movements	4/10	0/10*	1/10	0/10*	1/10
Normal	6/10	10/10*	9/10	10/10*	7/10
Backward walking					
Day 10					
Not present	10/10	10/10	10/10	10/10	10/10
Day 85					
Not present	10/10	10/10	10/10	10/10	10/10
Body position					
Day 10					
Sitting/standing (normal)	10/10	10/10	10/10	10/10	10/10
Rearing (normal)	8/10	10/10	9/10	8/10	8/10
Day 85					
Sitting/standing (normal)	10/10	10/10	10/10	10/10	8/10
Rearing (normal)	10/10	10/10	8/10	10/10	5/10*
Crouched over	0/10	0/10	0/10	0/10	2/10
Head bobbing	0/10	0/10	0/10	0/10	2/10
Circling					
Day 10					
Not present	10/10	10/10	10/10	10/10	10/10
Day 85					
Not present	10/10	10/10	10/10	10/10	10/10
Compulsive biting or licking					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	10/10	10/10	10/10
Convulsions					
Day 10					
None	10/10	10/10	10/10	10/10	10/10
Day 85					
None	10/10	10/10	10/10	10/10	8/10
Mild	0/10	0/10	0/10	0/10	1/10
Severe	0/10	0/10	0/10	0/10	1/10
Coordination of movement					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	10/10	10/10	8/10
Moderately impaired	0/10	0/10	0/10	0/10	1/10
Severely impaired	0/10	0/10	0/10	0/10	1/10

TABLE B2
Functional Observation Battery Data for Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male (continued)					
Diarrhea					
Day 10					
Not present	10/10	10/10	10/10	10/10	10/10
Day 85					
Not present	10/10	10/10	10/10	10/10	10/10
Gait					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	10/10	10/10	7/10
Ataxia	0/10	0/10	0/10	0/10	1/10
Hind limbs impaired	0/10	0/10	0/10	0/10	1/10
Hunched or crouched	0/10	0/10	0/10	0/10	2/10
Body drags/is flattened	0/10	0/10	0/10	0/10	2/10
General behavior					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	10/10	10/10	8/10
Abnormal	0/10	0/10	0/10	0/10	2/10
Head flick					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	10/10	10/10	9/10
Mild	0/10	0/10	0/10	0/10	1/10
Head search					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	10/10	10/10	10/10
Lacrimation or chromodacryorrhea					
Day 10					
None	10/10	10/10	10/10	10/10	9/10
Slight	0/10	0/10	0/10	0/10	1/10
Day 85					
None	10/10	10/10	10/10	10/10	4/10**
Slight	0/10	0/10	0/10	0/10	5/10*
Severe	0/10	0/10	0/10	0/10	1/10
Piloerection					
Day 10					
Not present	10/10	10/10	10/10	10/10	10/10
Day 85					
Present	0/10	0/10	0/10	0/10	1/10
Not present	10/10	10/10	10/10	10/10	9/10
Pupillary constriction or dilation					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	10/10	10/10	9/10
Constricted	0/10	0/10	0/10	0/10	1/10

TABLE B2
Functional Observation Battery Data for Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male (continued)					
Respiration					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	10/10	10/10	9/10
Labored	0/10	0/10	0/10	0/10	1/10
Salivation					
Day 10					
None	9/10	10/10	10/10	9/10	7/10
Slight	1/10	0/10	0/10	1/10	2/10
Severe	0/10	0/10	0/10	0/10	1/10
Day 85					
None	10/10	9/10	8/10	4/10**	0/10**
Slight	0/10	1/10	2/10	4/10*	2/10
Severe	0/10	0/10	0/10	2/10	8/10**
Self mutilation					
Day 10					
Not present	10/10	10/10	10/10	10/10	10/10
Day 85					
Not present	10/10	10/10	10/10	10/10	10/10
Tremors					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	10/10	10/10	5/10*
Mild	0/10	0/10	0/10	0/10	2/10
Severe whole body	0/10	0/10	0/10	0/10	3/10
Urination					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	10/10	10/10	10/10
Vocalization					
Day 10					
Not present	10/10	10/10	10/10	10/10	10/10
Day 85					
Not present	10/10	10/10	10/10	10/10	10/10
Female					
Activity					
Day 10					
Minimum exploratory movements	0/10	4/10*	2/10	3/10	1/10
Normal	10/10	6/10*	8/10	7/10	9/10
Day 85					
Minimum exploratory movements	1/10	5/10	2/9	1/10	3/8
Normal	9/10	5/10	7/9	9/10	5/8

TABLE B2
Functional Observation Battery Data for Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Female (continued)					
Backward walking					
Day 10					
Not present	10/10	10/10	10/10	10/10	10/10
Day 85					
Not present	10/10	10/10	9/9	10/10	8/8
Body position					
Day 10					
Sitting/standing (normal)	10/10	10/10	10/10	10/10	10/10
Rearing (normal)	10/10	8/10	8/10	4/10**	8/10
Day 85					
Sitting/standing (normal)	10/10	10/10	9/9	10/10	7/8
Rearing (normal)	10/10	8/10	7/9	9/10	5/8
Flattened	0/10	0/10	0/9	0/10	1/8
Crouched over	0/10	0/10	0/9	0/10	2/8
Circling					
Day 10					
Not present	10/10	10/10	10/10	10/10	10/10
Day 85					
Not present	10/10	10/10	9/9	10/10	8/8
Compulsive biting or licking					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	9/9	10/10	8/8
Convulsions					
Day 10					
None	10/10	10/10	10/10	10/10	10/10
Day 85					
None	10/10	10/10	9/9	10/10	8/8
Coordination of movement					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	9/9	10/10	5/8
Slightly impaired	0/10	0/10	0/9	0/10	3/8
Diarrhea					
Day 10					
Not present	10/10	10/10	10/10	10/10	10/10
Day 85					
Not present	10/10	10/10	9/9	10/10	8/8
Gait					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	9/9	10/10	4/8*
Ataxia	0/10	0/10	0/9	0/10	2/8
Hind limbs impaired	0/10	0/10	0/9	0/10	2/8
Forelimbs drag	0/10	0/10	0/9	0/10	1/8
Walks on tiptoes	0/10	0/10	0/9	0/10	1/8
Hunched or crouched	0/10	0/10	0/9	0/10	1/8
Body drags/is flattened	0/10	0/10	0/9	0/10	1/8

TABLE B2
Functional Observation Battery Data for Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Female (continued)					
General behavior					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	9/9	9/10	8/8
Abnormal	0/10	0/10	0/9	1/10	0/8
Head flick					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	9/9	10/10	7/8
Mild	0/10	0/10	0/9	0/10	1/8
Head search					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	9/9	10/10	8/8
Lacrimation or chromodacryorrhea					
Day 10					
None	9/10	10/10	9/10	10/10	10/10
Slight	1/10	0/10	1/10	0/10	0/10
Day 85					
None	10/10	7/10	6/9	6/10*	2/8**
Slight	0/10	3/10	3/9	4/10*	6/8**
Piloerection					
Day 10					
Not present	10/10	10/10	10/10	10/10	10/10
Day 85					
Not present	10/10	10/10	9/9	10/10	8/8
Pupillary constriction or dilation					
Day 10					
Normal	10/10	10/10	9/10	10/10	10/10
Dilated	0/10	0/10	1/10	0/10	0/10
Day 85					
Normal	10/10	10/10	9/9	7/10	4/8*
Constricted	0/10	0/10	0/9	3/10	4/8*
Respiration					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	9/9	10/10	8/8
Salivation					
Day 10					
None	10/10	10/10	10/10	10/10	9/10
Slight	0/10	0/10	0/10	0/10	1/10
Day 85					
None	9/10	9/10	8/9	9/10	4/8
Slight	1/10	1/10	1/9	0/10	3/8
Severe	0/10	0/10	0/9	1/10	1/8

TABLE B2
Functional Observation Battery Data for Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Female (continued)					
Self mutilation					
Day 10					
Not present	10/10	10/10	10/10	10/10	10/10
Day 85					
Not present	10/10	10/10	9/9	10/10	8/8
Tremors					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	9/9	10/10	3/8**
Mild	0/10	0/10	0/9	0/10	4/8*
Mild whole body	0/10	0/10	0/9	0/10	1/8
Urination					
Day 10					
Normal	10/10	10/10	10/10	10/10	10/10
Day 85					
Normal	10/10	10/10	9/9	10/10	8/8
Vocalization					
Day 10					
Not present	10/10	10/10	10/10	10/10	10/10
Day 85					
Not present	10/10	10/10	9/9	10/10	8/8

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with behavior/number of animals observed

APPENDIX C
HEMATOLOGY AND CLINICAL CHEMISTRY RESULTS

TABLE C1
Hematology and Clinical Chemistry Data for Rats in the 16-Day Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	16 mg/kg	32 mg/kg	63 mg/kg
Male				
n	5	5	5	5
Hematology				
Automated hematocrit (%)	40.4 ± 0.6	40.3 ± 0.6	40.7 ± 1.0	40.3 ± 0.4
Manual hematocrit (%)	45.4 ± 0.7	45.8 ± 0.7	45.8 ± 1.2	45.4 ± 0.5
Hemoglobin (g/dL)	15.0 ± 0.3	15.1 ± 0.1	15.1 ± 0.3	14.8 ± 0.1
Erythrocytes ($10^6/\mu L$)	7.06 ± 0.10	7.08 ± 0.12	7.13 ± 0.20	6.99 ± 0.11
Reticulocytes ($10^6/\mu L$)	0.29 ± 0.02	0.24 ± 0.06	0.25 ± 0.04	0.46 ± 0.13
Nucleated erythrocytes/100 leukocytes	0.40 ± 0.25	0.40 ± 0.25	0.20 ± 0.20	0.40 ± 0.25
Mean cell volume (fL)	57.1 ± 0.2	56.9 ± 0.3	57.1 ± 0.3	57.6 ± 0.5
Mean cell hemoglobin (pg)	21.2 ± 0.1	21.3 ± 0.3	21.3 ± 0.2	21.3 ± 0.2
Mean cell hemoglobin concentration (g/dL)	37.1 ± 0.2	37.4 ± 0.5	37.3 ± 0.3	36.8 ± 0.2
Platelets ($10^3/\mu L$)	838.4 ± 15.1	820.0 ± 34.2	857.4 ± 21.1	774.0 ± 9.4
Leukocytes ($10^3/\mu L$)	9.10 ± 0.32	8.42 ± 0.18	8.88 ± 0.55	9.06 ± 0.40
Segmented neutrophils ($10^3/\mu L$)	0.86 ± 0.16	0.82 ± 0.14	0.82 ± 0.14	1.00 ± 0.14
Lymphocytes ($10^3/\mu L$)	8.06 ± 0.31	7.38 ± 0.26	7.91 ± 0.35	7.63 ± 0.26
Monocytes ($10^3/\mu L$)	0.07 ± 0.05	0.20 ± 0.09	0.10 ± 0.06	0.37 ± 0.12
Eosinophils ($10^3/\mu L$)	0.12 ± 0.06	0.02 ± 0.02	0.06 ± 0.04	0.06 ± 0.04
Clinical Chemistry				
Urea nitrogen (mg/dL)	24.0 ± 0.5	25.8 ± 0.4	23.0 ± 0.7	25.0 ± 0.5
Creatinine (mg/dL)	0.70 ± 0.00	0.70 ± 0.00	0.70 ± 0.00	0.70 ± 0.00
Total protein (g/dL)	6.6 ± 0.2	6.3 ± 0.1	6.6 ± 0.1	6.2 ± 0.1
Albumin (g/dL)	4.8 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.6 ± 0.1
Alanine aminotransferase (IU/L)	48 ± 1	56 ± 4	43 ± 1	43 ± 4
Alkaline phosphatase (IU/L)	545 ± 15	553 ± 13	566 ± 16	559 ± 13
Creatine kinase (IU/L)	440 ± 81	542 ± 147	505 ± 81	444 ± 79
Sorbitol dehydrogenase (IU/L)	33 ± 2	32 ± 3	31 ± 2	32 ± 2
Serum cholinesterase (IU/L)	676.8 ± 28.8	753.2 ± 13.5	738.4 ± 23.4	789.0 ± 39.7*
Erythrocyte cholinesterase (IU/L)	530.2 ± 143.6	726.4 ± 113.2	694.4 ± 121.9	726.6 ± 71.4
Bile acids (μmol/L)	28.3 ± 4.5	28.6 ± 4.0	39.9 ± 7.2	34.5 ± 5.1

TABLE C1
Hematology and Clinical Chemistry Data for Rats in the 16-Day Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	16 mg/kg	32 mg/kg	63 mg/kg
Female				
n	5	5	5	5
Hematology				
Automated hematocrit (%)	44.5 ± 0.4	42.6 ± 0.8	42.5 ± 0.4	44.2 ± 1.1
Manual hematocrit (%)	46.4 ± 0.4	45.0 ± 0.5	44.8 ± 0.4	46.2 ± 0.9
Hemoglobin (g/dL)	15.6 ± 0.1	15.2 ± 0.2	15.0 ± 0.1	15.7 ± 0.3
Erythrocytes ($10^6/\mu L$)	7.59 ± 0.08	7.24 ± 0.13	7.20 ± 0.06	7.58 ± 0.20
Reticulocytes ($10^6/\mu L$)	0.20 ± 0.03	0.22 ± 0.03	0.18 ± 0.02	0.23 ± 0.03
Nucleated erythrocytes/100 leukocytes	0.00 ± 0.00	0.80 ± 0.37	0.20 ± 0.20	0.00 ± 0.00
Mean cell volume (fL)	58.6 ± 0.2	58.9 ± 0.1	59.1 ± 0.2	58.3 ± 0.3
Mean cell hemoglobin (pg)	20.6 ± 0.2	21.0 ± 0.2	20.8 ± 0.1	20.7 ± 0.2
Mean cell hemoglobin concentration (g/dL)	35.2 ± 0.3	35.7 ± 0.4	35.2 ± 0.3	35.5 ± 0.2
Platelets ($10^3/\mu L$)	714.8 ± 13.5	755.2 ± 23.9	763.2 ± 14.2	710.0 ± 23.9
Leukocytes ($10^3/\mu L$)	9.28 ± 0.50	9.46 ± 0.19	9.38 ± 0.93	8.24 ± 0.30
Segmented neutrophils ($10^3/\mu L$)	1.30 ± 0.26	1.21 ± 0.22	1.17 ± 0.23	0.77 ± 0.18
Lymphocytes ($10^3/\mu L$)	7.72 ± 0.37	7.89 ± 0.27	8.05 ± 0.84	7.32 ± 0.44
Monocytes ($10^3/\mu L$)	0.19 ± 0.05	0.27 ± 0.05	0.13 ± 0.04	0.09 ± 0.05
Eosinophils ($10^3/\mu L$)	0.07 ± 0.03	0.10 ± 0.03	0.04 ± 0.02	0.06 ± 0.02
Clinical Chemistry				
Urea nitrogen (mg/dL)	23.8 ± 0.4	23.6 ± 0.2	22.6 ± 1.3	22.4 ± 0.8
Creatinine (mg/dL)	0.70 ± 0.00	0.68 ± 0.02	0.66 ± 0.02	0.66 ± 0.04
Total protein (g/dL)	6.0 ± 0.1	6.0 ± 0.1	5.8 ± 0.1	6.0 ± 0.1
Albumin (g/dL)	4.5 ± 0.1	4.5 ± 0.1	4.3 ± 0.1	4.4 ± 0.1
Alanine aminotransferase (IU/L)	40 ± 2	37 ± 1	37 ± 2	37 ± 1
Alkaline phosphatase (IU/L)	505 ± 8	490 ± 18	501 ± 31	518 ± 9
Creatine kinase (IU/L)	432 ± 61	272 ± 32	421 ± 70	378 ± 86
Sorbitol dehydrogenase (IU/L)	30 ± 1	28 ± 0	27 ± 2	26 ± 1
Serum cholinesterase (IU/L)	2,364.0 ± 120.6	2,577.8 ± 189.9	2,061.0 ± 123.8	2,138.4 ± 223.7
Erythrocyte cholinesterase (IU/L)	673.8 ± 175.9	400.6 ± 164.4	1,185.8 ± 193.3	819.2 ± 204.7
Bile acids (μmol/L)	29.2 ± 2.9	31.4 ± 5.2	28.5 ± 5.0	28.9 ± 6.2

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data. No data are available for the 125 and 250 mg/kg groups due to 100% mortality.

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male					
n					
Day 3	10	10	10	10	10
Day 21	10	10	10	10	10
Week 13	10	9	10	10	10
Hematology					
Automated hematocrit (%)					
Day 3	38.4 ± 0.5	39.0 ± 0.6	37.9 ± 0.4	38.7 ± 0.4	39.1 ± 0.7
Day 21	44.3 ± 0.4	44.3 ± 0.4	44.1 ± 0.3	44.8 ± 0.5	43.5 ± 0.7
Week 13	46.8 ± 0.4	48.5 ± 0.3*	47.9 ± 0.5	46.5 ± 0.5	46.5 ± 0.4
Manual hematocrit (%)					
Day 3	42.2 ± 0.7	41.5 ± 0.7	41.2 ± 0.4	43.1 ± 0.4	42.5 ± 0.8
Day 21	47.7 ± 0.5	47.5 ± 0.7	47.0 ± 0.5	48.0 ± 0.6	47.6 ± 0.6
Week 13	48.9 ± 0.4	50.0 ± 0.4	49.5 ± 0.4	48.7 ± 0.5	48.9 ± 0.4
Hemoglobin (g/dL)					
Day 3	13.7 ± 0.2	13.8 ± 0.2	13.5 ± 0.1	13.8 ± 0.1	13.8 ± 0.2
Day 21	15.4 ± 0.1	15.6 ± 0.1	15.5 ± 0.1	15.6 ± 0.1	15.4 ± 0.2
Week 13	15.8 ± 0.1	15.9 ± 0.1	16.0 ± 0.1	15.8 ± 0.2	15.8 ± 0.1
Erythrocytes ($10^6/\mu L$)					
Day 3	6.27 ± 0.07	6.33 ± 0.09	6.18 ± 0.06	6.28 ± 0.05	6.36 ± 0.12
Day 21	7.25 ± 0.08	7.31 ± 0.06	7.34 ± 0.05	7.28 ± 0.08	7.09 ± 0.10
Week 13	8.96 ± 0.07	9.08 ± 0.06	9.03 ± 0.09	8.71 ± 0.09	8.67 ± 0.06*
Reticulocytes ($10^6/\mu L$)					
Day 3	0.53 ± 0.03	0.58 ± 0.02	0.54 ± 0.04	0.50 ± 0.02	0.55 ± 0.03
Day 21	0.24 ± 0.01	0.23 ± 0.01	0.20 ± 0.02*	0.20 ± 0.01**	0.21 ± 0.01**
Week 13	0.21 ± 0.01	0.24 ± 0.02	0.24 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)					
Day 3	0.17 ± 0.05	0.13 ± 0.06	0.12 ± 0.05	0.16 ± 0.02	0.13 ± 0.03
Day 21	0.05 ± 0.03	0.04 ± 0.03	0.01 ± 0.01	0.06 ± 0.03	0.07 ± 0.03
Week 13	0.02 ± 0.02	0.04 ± 0.03	0.00 ± 0.00[b]	0.02 ± 0.02[b]	0.00 ± 0.00
Mean cell volume (fL)					
Day 3	61.2 ± 0.3	61.5 ± 0.3	61.3 ± 0.3	61.6 ± 0.2	61.4 ± 0.2
Day 21	61.0 ± 0.4	60.7 ± 0.4	60.0 ± 0.3	61.6 ± 0.4	61.3 ± 0.3
Week 13	52.2 ± 0.2	53.4 ± 0.1**	53.0 ± 0.2**	53.4 ± 0.2**	53.6 ± 0.1**
Mean cell hemoglobin (pg)					
Day 3	21.8 ± 0.1	21.8 ± 0.2	21.9 ± 0.1	22.0 ± 0.1	21.7 ± 0.1
Day 21	21.2 ± 0.2	21.3 ± 0.1	21.1 ± 0.1	21.4 ± 0.1	21.7 ± 0.1
Week 13	17.6 ± 0.1	17.5 ± 0.1	17.7 ± 0.1	18.1 ± 0.1**	18.2 ± 0.1**
Mean cell hemoglobin concentration (g/dL)					
Day 3	35.6 ± 0.1	35.5 ± 0.2	35.7 ± 0.2	35.7 ± 0.2	35.4 ± 0.2
Day 21	34.8 ± 0.3	35.1 ± 0.2	35.2 ± 0.2	34.8 ± 0.3	35.4 ± 0.2
Week 13	33.7 ± 0.2	32.8 ± 0.2*	33.4 ± 0.3	33.9 ± 0.2	34.0 ± 0.2
Platelets ($10^3/\mu L$)					
Day 3	1,091.1 ± 30.5	1,086.9 ± 25.3	1,080.6 ± 14.8	1,025.7 ± 11.2	1,069.5 ± 26.3
Day 21	793.1 ± 19.9	807.4 ± 8.6	820.8 ± 10.4	824.7 ± 20.0	838.7 ± 28.7
Week 13	649.8 ± 10.3	640.6 ± 9.7	646.3 ± 10.8	641.4 ± 6.6	653.7 ± 13.7
Leukocytes ($10^3/\mu L$)					
Day 3	8.09 ± 0.28	8.49 ± 0.24	7.70 ± 0.24	7.89 ± 0.22	8.91 ± 0.54
Day 21	11.03 ± 0.41	12.07 ± 0.30	10.78 ± 0.45	10.78 ± 0.25	10.90 ± 0.59
Week 13	11.96 ± 0.53	12.60 ± 0.61	11.90 ± 0.38	12.86 ± 0.60	11.67 ± 0.36

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male (continued)					
n					
Day 3	10	10	10	10	10
Day 21	10	10	10	10	10
Week 13	10	9	10	10	10
Hematology (continued)					
Segmented neutrophils ($10^3/\mu L$)					
Day 3	1.28 ± 0.21	1.14 ± 0.07	0.96 ± 0.11	0.10 ± 0.11	1.14 ± 0.08
Day 21	1.17 ± 0.07	1.37 ± 0.19	1.18 ± 0.14	1.35 ± 0.12	1.15 ± 0.14
Week 13	2.13 ± 0.17	1.59 ± 0.11*	1.56 ± 0.16*[b]	1.54 ± 0.20*[b]	1.25 ± 0.18**
Lymphocytes ($10^3/\mu L$)					
Day 3	6.69 ± 0.32	7.18 ± 0.19	6.64 ± 0.27	6.72 ± 0.18	7.57 ± 0.49
Day 21	9.55 ± 0.43	10.16 ± 0.25	9.17 ± 0.41	9.01 ± 0.27	9.49 ± 0.47
Week 13	9.06 ± 0.43	10.35 ± 0.65	9.82 ± 0.42[b]	10.29 ± 0.61[b]	9.97 ± 0.50
Atypical lymphocytes ($10^3/\mu L$)					
Day 21	0.04 ± 0.03	0.06 ± 0.05	0.00 ± 0.00	0.03 ± 0.02	0.00 ± 0.00
Week 13	0.22 ± 0.09	0.12 ± 0.05	0.18 ± 0.06[b]	0.19 ± 0.09[b]	0.16 ± 0.07
Monocytes ($10^3/\mu L$)					
Day 3	0.09 ± 0.04	0.14 ± 0.06	0.08 ± 0.02	0.14 ± 0.05	0.19 ± 0.04
Day 21	0.22 ± 0.07	0.44 ± 0.09	0.37 ± 0.09	0.36 ± 0.07	0.24 ± 0.10
Week 13	0.46 ± 0.12	0.35 ± 0.07	0.18 ± 0.05[b]	0.53 ± 0.10[b]	0.21 ± 0.04
Eosinophils ($10^3/\mu L$)					
Day 3	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.02	0.01 ± 0.01
Day 21	0.05 ± 0.04	0.02 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.01 ± 0.01
Week 13	0.08 ± 0.03	0.16 ± 0.04	0.12 ± 0.04[b]	0.11 ± 0.04[b]	0.07 ± 0.03
Clinical Chemistry					
Urea nitrogen (mg/dL)					
Day 3	20.1 ± 0.6	19.3 ± 0.4	19.4 ± 0.3	19.8 ± 0.3	19.2 ± 0.3
Day 21	22.4 ± 1.5	21.4 ± 0.5	20.9 ± 0.3	23.4 ± 1.8	21.8 ± 0.5
Week 13	23.5 ± 0.5	24.2 ± 1.3	23.6 ± 0.6	22.6 ± 0.3	22.3 ± 0.6
Creatinine (mg/dL)					
Day 3	0.58 ± 0.01	0.59 ± 0.01	0.58 ± 0.01	0.56 ± 0.02	0.57 ± 0.02
Day 21	0.68 ± 0.01	0.67 ± 0.02	0.68 ± 0.01	0.67 ± 0.02	0.67 ± 0.02
Week 13	0.66 ± 0.02	0.69 ± 0.01	0.70 ± 0.02	0.68 ± 0.01	0.67 ± 0.02
Total protein (g/dL)					
Day 3	5.7 ± 0.1	5.8 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.7 ± 0.1
Day 21	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.0
Week 13	6.8 ± 0.1	6.8 ± 0.1	7.0 ± 0.1	6.7 ± 0.1	6.7 ± 0.1
Albumin (g/dL)					
Day 3	4.3 ± 0.0	4.4 ± 0.0	4.3 ± 0.1	4.2 ± 0.1	4.3 ± 0.1
Day 21	4.6 ± 0.1	4.5 ± 0.0	4.5 ± 0.0	4.6 ± 0.1	4.6 ± 0.1
Week 13	5.0 ± 0.0	5.0 ± 0.1	5.0 ± 0.0	4.9 ± 0.0	4.9 ± 0.0
Alanine aminotransferase (IU/L)					
Day 3	42 ± 1	41 ± 2	41 ± 2	43 ± 2	42 ± 1
Day 21	47 ± 1	50 ± 1	46 ± 1	47 ± 1	46 ± 1
Week 13	67 ± 4	60 ± 4	69 ± 5	67 ± 5	56 ± 2

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male (continued)					
n					
Day 3	10	10	10	10	10
Day 21	10	10	10	10	10
Week 13	10	9	10	10	10
Clinical Chemistry (continued)					
Alkaline phosphatase (IU/L)					
Day 3	708 ± 12	737 ± 17	730 ± 12	722 ± 15	700 ± 17
Day 21	533 ± 9	562 ± 17	543 ± 8	532 ± 10	544 ± 10
Week 13	270 ± 6	271± 8	276 ± 5	245 ± 5*	227 ± 7**
Creatine kinase (IU/L)					
Day 3	443 ± 47	577 ± 64	529 ± 38	429 ± 35	463 ± 31
Day 21	432 ± 31	457 ± 34	399 ± 20	467 ± 47	443 ± 47
Week 13	226 ± 23	255 ± 20	244 ± 14	256 ± 21	256 ± 30
Sorbitol dehydrogenase (IU/L)					
Day 3	16 ± 1	14 ± 2	15 ± 1	16 ± 1	16 ± 1
Day 21	19 ± 1	19 ± 1	20 ± 1	17 ± 1	20 ± 1
Week 13	25 ± 2	22 ± 2	24 ± 1	22 ± 1	19 ± 1**
Serum cholinesterase (IU/L)					
Day 3	859.6 ± 15.2	851.2 ± 17.9	853.5 ± 15.0	846.4 ± 14.4	904.1 ± 19.4
Day 21	692.9 ± 13.4	719.5 ± 11.1	712.4 ± 11.8	745.0 ± 32.0	777.3 ± 18.7**
Week 13	741.9 ± 20.7	760.0 ± 13.4	792.0 ± 25.2	792.5 ± 23.7	753.6 ± 16.3
Bile acids (μmol/L)					
Day 3	28.1 ± 4.2	18.0 ± 1.3	17.6 ± 1.3	23.3 ± 2.3	20.9 ± 1.6
Day 21	28.4 ± 2.9	15.6 ± 1.1*	18.6 ± 2.3	22.0 ± 2.5	26.8 ± 3.6
Week 13	19.4 ± 5.6	16.3 ± 2.7	17.3 ± 3.7	13.7 ± 0.8	17.6 ± 1.7
Female					
n					
Day 3	10	10	10	10	10
Day 21	10	10	10	10	10
Week 13	10	10	9	10	8
Hematology					
Automated hematocrit (%)					
Day 3	42.9 ± 0.4	41.7 ± 0.5	41.9 ± 0.5	40.9 ± 0.5*	42.7 ± 0.4
Day 21	45.0 ± 0.4	45.2 ± 0.6	46.1 ± 0.3	45.0 ± 0.3	45.7 ± 0.4
Week 13	45.2 ± 0.4	46.8 ± 0.3*	46.2 ± 0.4	46.4 ± 0.3	44.8 ± 0.3
Manual hematocrit (%)					
Day 3	43.9 ± 0.4	42.5 ± 0.4	43.0 ± 0.4	42.9 ± 0.4	43.9 ± 0.5
Day 21	48.6 ± 0.4	48.1 ± 0.9	49.4 ± 0.5	48.1 ± 0.6	49.7 ± 0.4
Week 13	46.1 ± 0.3	47.2 ± 0.4	46.7 ± 0.3	47.0 ± 0.4	45.8 ± 0.4
Hemoglobin (g/dL)					
Day 3	14.7 ± 0.1	14.2 ± 0.1	14.3 ± 0.2	14.1 ± 0.2*	14.7 ± 0.1
Day 21	15.7 ± 0.1	15.7 ± 0.2	16.0 ± 0.1	15.7 ± 0.1	16.0 ± 0.1
Week 13	15.3 ± 0.1	15.7 ± 0.1*	15.5 ± 0.1	15.5 ± 0.1	15.1 ± 0.1

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Female (continued)					
n					
Day 3	10	10	10	10	10
Day 21	10	10	10	10	10
Week 13	10	10	9	10	8
Hematology (continued)					
Erythrocytes ($10^6/\mu L$)					
Day 3	6.97 ± 0.08	6.74 ± 0.06	6.78 ± 0.10	6.61 ± 0.10*	6.91 ± 0.07
Day 21	7.15 ± 0.07	7.19 ± 0.12	7.31 ± 0.05	7.10 ± 0.07	7.31 ± 0.06
Week 13	7.88 ± 0.07	8.11 ± 0.05	7.97 ± 0.07	8.00 ± 0.06	7.70 ± 0.05
Reticulocytes ($10^6/\mu L$)					
Day 3	0.28 ± 0.02	0.27 ± 0.02	0.25 ± 0.01	0.28 ± 0.01	0.30 ± 0.02
Day 21	0.14 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.15 ± 0.01	0.14 ± 0.01
Week 13	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)					
Day 3	0.13 ± 0.04	0.05 ± 0.03	0.08 ± 0.04	0.12 ± 0.04	0.06 ± 0.02
Day 21	0.06 ± 0.02	0.00 ± 0.00*	0.01 ± 0.01	0.04 ± 0.02	0.01 ± 0.01
Week 13	0.04 ± 0.02	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Mean cell volume (fL)					
Day 3	61.6 ± 0.1	61.8 ± 0.2	61.8 ± 0.3	61.9 ± 0.3	61.8 ± 0.3
Day 21	63.0 ± 0.2	62.9 ± 0.3	63.0 ± 0.3	63.4 ± 0.3	62.6 ± 0.3
Week 13	57.4 ± 0.1	57.7 ± 0.1	57.9 ± 0.1**	57.9 ± 0.1**	58.2 ± 0.2**
Mean cell hemoglobin (pg)					
Day 3	21.1 ± 0.1	21.1 ± 0.1	21.1 ± 0.1	21.4 ± 0.2	21.3 ± 0.1
Day 21	21.9 ± 0.1	21.9 ± 0.2	21.9 ± 0.1	22.1 ± 0.2	21.9 ± 0.1
Week 13	19.4 ± 0.1	19.4 ± 0.1	19.4 ± 0.1	19.4 ± 0.1	19.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)					
Day 3	34.2 ± 0.1	34.2 ± 0.3	34.1 ± 0.2	34.5 ± 0.2	34.4 ± 0.2
Day 21	34.9 ± 0.1	34.8 ± 0.2	34.8 ± 0.1	34.9 ± 0.3	35.1 ± 0.2
Week 13	33.9 ± 0.1	33.7 ± 0.2	33.5 ± 0.2	33.4 ± 0.2	33.6 ± 0.2
Platelets ($10^3/\mu L$)					
Day 3	1,010.4 ± 33.0	1,056.0 ± 14.6	1,045.4 ± 20.1	1,016.0 ± 20.9	980.5 ± 24.8
Day 21	693.6 ± 15.7	696.2 ± 17.9	709.2 ± 16.2	723.9 ± 20.5	665.5 ± 12.8
Week 13	649.3 ± 13.8	653.2 ± 11.5	648.7 ± 20.6	708.8 ± 35.8	641.0 ± 16.4
Leukocytes ($10^3/\mu L$)					
Day 3	9.52 ± 0.28	9.39 ± 0.28	9.18 ± 0.26	9.24 ± 0.41	9.77 ± 0.33
Day 21	10.73 ± 0.48	10.20 ± 0.71	10.72 ± 0.58	9.71 ± 0.48	10.51 ± 0.36
Week 13	10.38 ± 0.32	9.85 ± 0.60	9.84 ± 0.42	10.71 ± 0.52	10.51 ± 0.63
Segmented neutrophils ($10^3/\mu L$)					
Day 3	1.08 ± 0.04	1.21 ± 0.11	1.23 ± 0.12	1.21 ± 0.08	1.10 ± 0.15
Day 21	1.06 ± 0.09	1.19 ± 0.15	1.02 ± 0.07	0.98 ± 0.11	1.06 ± 0.09
Week 13	1.30 ± 0.17	0.85 ± 0.11	1.72 ± 0.23	1.13 ± 0.18	1.05 ± 0.20
Lymphocytes ($10^3/\mu L$)					
Day 3	8.11 ± 0.29	7.71 ± 0.24	7.51 ± 0.29	7.53 ± 0.41	8.26 ± 0.23
Day 21	9.42 ± 0.45	8.79 ± 0.58	9.46 ± 0.61	8.57 ± 0.48	9.33 ± 0.41
Week 13	8.53 ± 0.34	8.45 ± 0.50	7.48 ± 0.41	8.84 ± 0.38	8.93 ± 0.61
Atypical lymphocytes ($10^3/\mu L$)					
Day 3	0.01 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Week 13	0.02 ± 0.02	0.24 ± 0.20	0.08 ± 0.04	0.13 ± 0.05	0.11 ± 0.04

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Female (continued)					
n					
Day 3	10	10	10	10	10
Day 21	10	10	10	10	10
Week 13	10	10	9	10	8
Hematology (continued)					
Monocytes ($10^3/\mu L$)					
Day 3	0.29 ± 0.09	0.42 ± 0.09	0.36 ± 0.09	0.38 ± 0.10	0.35 ± 0.09
Day 21	0.09 ± 0.05	0.09 ± 0.05	0.11 ± 0.05	0.12 ± 0.06	0.05 ± 0.03
Week 13	0.35 ± 0.07	0.37 ± 0.08	0.40 ± 0.07	0.49 ± 0.05	0.39 ± 0.11
Eosinophils ($10^3/\mu L$)					
Day 3	0.02 ± 0.01	0.01 ± 0.01	0.05 ± 0.02	0.03 ± 0.01	0.04 ± 0.02
Day 21	0.07 ± 0.02	0.06 ± 0.03	0.06 ± 0.02	0.01 ± 0.01*	0.00 ± 0.00**
Week 13	0.17 ± 0.02	0.15 ± 0.05	0.14 ± 0.04	0.10 ± 0.02	0.01 ± 0.01**
Clinical Chemistry					
Urea nitrogen (mg/dL)					
Day 3	21.7 ± 0.6	20.8 ± 0.7	19.6 ± 0.5	21.5 ± 0.5	22.6 ± 0.9
Day 21	22.8 ± 0.7	22.7 ± 0.6	21.7 ± 0.7	24.8 ± 1.4	23.4 ± 0.7
Week 13	21.2 ± 0.6	24.2 ± 0.8*	21.4 ± 0.8	21.8 ± 1.3	22.8 ± 0.7
Creatinine (mg/dL)					
Day 3	0.57 ± 0.02	0.57 ± 0.02	0.57 ± 0.02	0.58 ± 0.01	0.58 ± 0.01
Day 21	0.60 ± 0.00	0.65 ± 0.02	0.65 ± 0.02	0.64 ± 0.02	0.62 ± 0.01
Week 13	0.69 ± 0.02	0.69 ± 0.01	0.68 ± 0.02	0.69 ± 0.02	0.65 ± 0.03
Total protein (g/dL)					
Day 3	5.9 ± 0.1	5.8 ± 0.1	5.6 ± 0.1*	5.6 ± 0.0*	5.7 ± 0.1
Day 21	5.8 ± 0.0	5.8 ± 0.0	5.8 ± 0.1	5.8 ± 0.1	5.7 ± 0.1
Week 13	6.8 ± 0.1	6.7 ± 0.1	6.5 ± 0.1*	6.4 ± 0.1**	6.4 ± 0.1**
Albumin (g/dL)					
Day 3	4.5 ± 0.0	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.1*	4.3 ± 0.0**
Day 21	4.5 ± 0.0	4.4 ± 0.0	4.5 ± 0.1	4.4 ± 0.0	4.4 ± 0.1
Week 13	5.0 ± 0.1	5.0 ± 0.0	4.9 ± 0.1	4.7 ± 0.1**	4.6 ± 0.1**
Alanine aminotransferase (IU/L)					
Day 3	37 ± 1	36 ± 1	36 ± 1	36 ± 2	36 ± 1
Day 21	36 ± 1	34 ± 1	34 ± 1	39 ± 1	39 ± 1
Week 13	42 ± 3	51 ± 4	41 ± 2	43 ± 2	48 ± 3
Alkaline phosphatase (IU/L)					
Day 3	541 ± 13	540 ± 13	537 ± 9	544 ± 13	544 ± 15
Day 21	398 ± 9	382 ± 8	400 ± 8	405 ± 13	383 ± 12
Week 13	212 ± 7	237 ± 5	235 ± 9	208 ± 6	233 ± 8
Creatine kinase (IU/L)					
Day 3	517 ± 44	574 ± 60	488 ± 44	484 ± 41	474 ± 33
Day 21	493 ± 60	505 ± 48	502 ± 29	387 ± 42	405 ± 46
Week 13	366 ± 29	311 ± 33	395 ± 25	309 ± 47	392 ± 44
Sorbitol dehydrogenase (IU/L)					
Day 3	13 ± 1	14 ± 2	13 ± 1	14 ± 1	14 ± 1
Day 21	18 ± 1	18 ± 2	19 ± 1	21 ± 1	18 ± 1
Week 13	17 ± 1	20 ± 1	15 ± 1	16 ± 2	13 ± 1

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Female (continued)					
n					
Day 3	10	10	10	10	10
Day 21	10	10	10	10	10
Week 13	10	10	9	10	8
Clinical Chemistry (continued)					
Serum cholinesterase (IU/L)					
Day 3	1,748.3 ± 49.9	1,774.0 ± 72.4	1,701.2 ± 94.9	1,764.3 ± 95.6	1,895.4 ± 76.1
Day 21	2,525.4 ± 68.3	2,872.1 ± 127.7	2,865.9 ± 143.1	2,819.0 ± 167.2	2,781.5 ± 181.6
Week 13	4,297.5 ± 144.8	4,412.9 ± 154.0	4,034.1 ± 206.4	3,284.1 ± 134.2**	2,797.9 ± 246.9**
Bile acids (μmol/L)					
Day 3	24.1 ± 2.3	20.8 ± 2.8	17.1 ± 1.5	21.8 ± 3.2	18.1 ± 2.6
Day 21	25.9 ± 1.5	20.9 ± 1.6	18.1 ± 2.6*	28.9 ± 4.7	20.4 ± 3.4
Week 13	14.9 ± 0.9	21.8 ± 4.0	20.1 ± 2.8	35.0 ± 8.6**	27.8 ± 3.9*

* 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=9

TABLE C3
Hematology and Clinical Chemistry Data for Mice in the 16-Day Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	63 mg/kg	125 mg/kg
Male			
n	5	5	5
Hematology			
Automated hematocrit (%)	45.0 ± 0.3	45.5 ± 0.5	45.5 ± 0.7
Manual hematocrit (%)	50.2 ± 0.4	51.4 ± 0.4	50.4 ± 0.5
Hemoglobin (g/dL)	15.8 ± 0.2	15.7 ± 0.2	15.9 ± 0.2
Erythrocytes ($10^6/\mu L$)	9.00 ± 0.08	9.09 ± 0.11	9.10 ± 0.12
Reticulocytes ($10^6/\mu L$)	0.19 ± 0.03	0.15 ± 0.02	0.18 ± 0.02
Nucleated erythrocytes/100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	50.0 ± 0.2	50.0 ± 0.2	50.0 ± 0.1
Mean cell hemoglobin (pg)	17.5 ± 0.0	17.3 ± 0.1	17.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)	35.0 ± 0.1	34.6 ± 0.4	34.9 ± 0.3
Platelets ($10^3/\mu L$)	760.0 ± 24.3	718.6 ± 36.0	763.2 ± 38.4
Leukocytes ($10^3/\mu L$)	4.84 ± 0.39	4.98 ± 0.56	4.98 ± 0.54
Segmented neutrophils ($10^3/\mu L$)	0.53 ± 0.13	0.42 ± 0.06	0.59 ± 0.09
Lymphocytes ($10^3/\mu L$)	4.20 ± 0.27	4.44 ± 0.53	4.26 ± 0.44
Monocytes ($10^3/\mu L$)	0.08 ± 0.04	0.09 ± 0.02	0.05 ± 0.02
Eosinophils ($10^3/\mu L$)	0.03 ± 0.02	0.04 ± 0.02	0.08 ± 0.03
Clinical Chemistry			
Serum cholinesterase (IU/L)	5,620.2 ± 192.7	5,900.0 ± 160.0	5,941.8 ± 199.0
Erythrocyte cholinesterase (IU/L)	2,016.0 ± 185.2	1,891.4 ± 247.4	1,812.5 ± 82.1[b]

TABLE C3
Hematology and Clinical Chemistry Data for Mice in the 16-Day Gavage Study of Benzyltrimethylammonium Chloride

	Vehicle Control	63 mg/kg	125 mg/kg
Female			
n		5	4
Hematology			
Automated hematocrit (%)	44.1 ± 0.5	43.4 ± 0.5	43.9 ± 0.5
Manual hematocrit (%)	50.0 ± 0.4	49.4 ± 0.6	50.0 ± 0.9
Hemoglobin (g/dL)	15.6 ± 0.3	15.5 ± 0.2	15.4 ± 0.2
Erythrocytes ($10^6/\mu L$)	8.92 ± 0.09	8.75 ± 0.09	8.81 ± 0.14
Reticulocytes ($10^6/\mu L$)	0.22 ± 0.04	0.20 ± 0.06	0.16 ± 0.03
Nucleated erythrocytes/100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[c]
Mean cell volume (fL)	49.4 ± 0.1	49.6 ± 0.2	49.9 ± 0.3
Mean cell hemoglobin (pg)	17.5 ± 0.1	17.7 ± 0.1	17.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)	35.3 ± 0.3	35.6 ± 0.2	35.0 ± 0.1
Platelets ($10^3/\mu L$)	708.2 ± 21.8	738.6 ± 12.6	722.3 ± 22.9
Leukocytes ($10^3/\mu L$)	4.58 ± 0.48	5.26 ± 0.38	5.93 ± 0.49
Segmented neutrophils ($10^3/\mu L$)	0.64 ± 0.16	0.58 ± 0.09	0.51 ± 0.12
Lymphocytes ($10^3/\mu L$)	3.86 ± 0.46	4.50 ± 0.34	5.36 ± 0.37
Monocytes ($10^3/\mu L$)	0.05 ± 0.02	0.10 ± 0.06	0.07 ± 0.03
Eosinophils ($10^3/\mu L$)	0.03 ± 0.02	0.07 ± 0.02	0.00 ± 0.00
Clinical Chemistry			
Serum cholinesterase (IU/L)	8,155.8 ± 24.2	8,328.6 ± 186.0	8,038.5 ± 247.4
Erythrocyte cholinesterase (IU/L)	2,044.8 ± 443.0	2,888.2 ± 300.2	2,384.8 ± 560.3

[a] Mean ± standard error. Statistical tests were performed on unrounded data. No data are available for the 250, 500, and 1,000 mg/kg groups due to 100% mortality.
[b] n=4
[c] n=5

TABLE C4
Clinical Chemistry Data for Mice in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male					
n	8	10	10	10	9
Urea nitrogen (mg/dL)	27.0 ± 0.6[b]	31.6 ± 1.6	30.3 ± 1.8	30.8 ± 1.1	30.1 ± 1.7[c]
Creatinine (mg/dL)	0.43 ± 0.02	0.48 ± 0.02	0.46 ± 0.02	0.47 ± 0.02	0.44 ± 0.02[c]
Total protein (g/dL)	6.1 ± 0.1[d]	6.0 ± 0.1[b]	5.8 ± 0.1**	5.7 ± 0.1**[b]	5.7 ± 0.1**[e]
Albumin (g/dL)	4.3 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.1 ± 0.1[c]
Alanine aminotransferase (IU/L)	40 ± 8	53 ± 20	84 ± 31	71 ± 22	37 ± 8
Alkaline phosphatase ((IU/L)	91 ± 2[b]	86 ± 4	86 ± 2	86 ± 3	84 ± 3
Creatine kinase (IU/L)	276 ± 87[b]	255 ± 57	461 ± 113[b]	485 ± 135	310 ± 77
Sorbitol dehydrogenase (IU/L)	47 ± 2	46 ± 1	45 ± 2	45 ± 4	45 ± 1[c]
Serum cholinesterase (IU/L)	8,963 ± 210	8,923 ± 376	8,404 ± 107	8,380 ± 353*[b]	8,893 ± 369[c]
Bile acids (μmol/L)	14.0 ± 1.1	13.8 ± 0.6[b]	14.9 ± 0.8	15.2 ± 0.8[b]	13.3 ± 0.6[e]
Female					
n	10	10	10	10	9
Urea nitrogen (mg/dL)	26.0 ± 2.0	26.9 ± 1.2	28.9 ± 1.3	25.0 ± 1.8	26.3 ± 1.3
Creatinine (mg/dL)	0.59 ± 0.02	0.58 ± 0.02	0.60 ± 0.02	0.54 ± 0.02	0.58 ± 0.02
Total protein (g/dL)	6.0 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.8 ± 0.1	5.8 ± 0.1
Albumin (g/dL)	4.6 ± 0.1	4.6 ± 0.1	4.5 ± 0.1	4.4 ± 0.0	4.4 ± 0.1
Alanine aminotransferase (IU/L)	34 ± 6	40 ± 9	29 ± 2	26 ± 1	36 ± 9
Alkaline phosphatase ((IU/L)	139 ± 3	126 ± 6	124 ± 4	128 ± 6	125 ± 6
Creatine kinase (IU/L)	297 ± 69	615 ± 212	428 ± 55	474 ± 91	318 ± 76
Sorbitol dehydrogenase (IU/L)	47 ± 1	48 ± 3	48 ± 1	48 ± 1	46 ± 1
Serum cholinesterase (IU/L)	10,020 ± 235	10,147 ± 114	9,964 ± 179	10,080 ± 230	9,726 ± 146
Bile acids (μmol/L)	15.1 ± 0.6	15.5 ± 0.8	15.5 ± 0.5	15.2 ± 0.5	15.6 ± 0.2

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

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

TABLE D1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 16-Day Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	16 mg/kg	32 mg/kg	63 mg/kg
5n	5	5		5
Male				
Necropsy body wt	240 ± 4	231 ± 5	233 ± 12	226 ± 8
Heart				
Absolute	0.833 ± 0.013	0.810 ± 0.016	0.828 ± 0.046	0.829 ± 0.038
Relative	3.46 ± 0.05	3.51 ± 0.06	3.55 ± 0.04	3.67 ± 0.11
R. Kidney				
Absolute	1.078 ± 0.024	1.061 ± 0.029	1.058 ± 0.081	1.011 ± 0.020
Relative	4.49 ± 0.10	4.59 ± 0.09	4.51 ± 0.14	4.48 ± 0.08
Liver				
Absolute	12.166 ± 0.302	11.288 ± 0.400	11.509 ± 0.970	10.997 ± 0.626
Relative	50.58 ± 0.70	48.82 ± 0.78	48.98 ± 1.68	48.47 ± 1.20
Lung				
Absolute	1.383 ± 0.058	1.355 ± 0.087	1.491 ± 0.104	1.314 ± 0.053
Relative	5.75 ± 0.20	5.85 ± 0.27	6.39 ± 0.30	5.82 ± 0.21
Spleen				
Absolute	0.626 ± 0.017	0.585 ± 0.020	0.605 ± 0.029	0.579 ± 0.024
Relative	2.60 ± 0.05	2.53 ± 0.05	2.60 ± 0.04	2.56 ± 0.04
R. Testis				
Absolute	1.266 ± 0.013	1.247 ± 0.026	1.231 ± 0.055	1.231 ± 0.019
Relative	5.27 ± 0.09	5.41 ± 0.14	5.28 ± 0.06	5.46 ± 0.11
Thymus				
Absolute	0.521 ± 0.010	0.501 ± 0.010	0.523 ± 0.029	0.502 ± 0.035
Relative	2.17 ± 0.06	2.17 ± 0.05	2.26 ± 0.13	2.21 ± 0.09
Female				
Necropsy body wt	137 ± 5	144 ± 5	142 ± 4	137 ± 4
Heart				
Absolute	0.548 ± 0.020	0.577 ± 0.024	0.596 ± 0.009	0.560 ± 0.021
Relative	4.01 ± 0.05	4.02 ± 0.05	4.21 ± 0.08	4.10 ± 0.12
R. Kidney				
Absolute	0.636 ± 0.031	0.655 ± 0.023	0.663 ± 0.020	0.619 ± 0.025
Relative	4.65 ± 0.12	4.57 ± 0.05	4.67 ± 0.11	4.53 ± 0.11
Liver				
Absolute	5.966 ± 0.333	6.233 ± 0.278	6.147 ± 0.219	5.771 ± 0.324
Relative	43.58 ± 1.29	43.37 ± 0.80	43.30 ± 1.00	42.09 ± 1.13
Lung				
Absolute	0.897 ± 0.041	0.985 ± 0.043	0.944 ± 0.034	0.884 ± 0.063
Relative	6.56 ± 0.13	6.88 ± 0.29	6.65 ± 0.18	6.44 ± 0.27
Spleen				
Absolute	0.401 ± 0.013	0.416 ± 0.017	0.426 ± 0.016	0.366 ± 0.021
Relative	2.93 ± 0.02	2.90 ± 0.08	3.00 ± 0.11	2.67 ± 0.07
Thymus				
Absolute	0.357 ± 0.022	0.367 ± 0.012	0.366 ± 0.008	0.353 ± 0.023
Relative	2.61 ± 0.13	2.56 ± 0.08	2.58 ± 0.09	2.58 ± 0.11

[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). Differences from the vehicle control group were not significant by Dunnett's test. No data are available for the 125 and 250 mg/kg groups due to 100% mortality.

TABLE D2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male					
n	10	10	10	10	10
Necropsy body wt	338 ± 8	337 ± 8	336 ± 8	340 ± 5	311 ± 9
Heart					
Absolute	1.018 ± 0.030	1.010 ± 0.024	1.000 ± 0.024	1.016 ± 0.025	0.953 ± 0.028
Relative	3.02 ± 0.05	3.00 ± 0.04	2.98 ± 0.05	2.99 ± 0.06	3.07 ± 0.03
R. Kidney					
Absolute	1.266 ± 0.049	1.274 ± 0.044	1.307 ± 0.092	1.238 ± 0.036	1.208 ± 0.041
Relative	3.74 ± 0.07	3.78 ± 0.07	3.89 ± 0.24	3.64 ± 0.08	3.89 ± 0.05
Liver					
Absolute	12.136 ± 0.423	12.671 ± 0.412	11.893 ± 0.277	12.146 ± 0.262	11.409 ± 0.459
Relative	35.88 ± 0.50	37.58 ± 0.69	35.46 ± 0.34	35.78 ± 0.60	36.66 ± 0.73
Lung					
Absolute	1.540 ± 0.040	1.473 ± 0.045	1.503 ± 0.045	1.515 ± 0.042	1.388 ± 0.054
Relative	4.57 ± 0.08	4.37 ± 0.07	4.48 ± 0.09	4.46 ± 0.09	4.47 ± 0.10
R. Testis					
Absolute	1.459 ± 0.043	1.380 ± 0.036	1.385 ± 0.063	1.443 ± 0.029	1.423 ± 0.043
Relative	4.32 ± 0.07	4.11 ± 0.12	4.15 ± 0.21	4.25 ± 0.05	4.58 ± 0.02
Thymus					
Absolute	0.328 ± 0.016	0.343 ± 0.023	0.341 ± 0.022	0.360 ± 0.017	0.295 ± 0.012
Relative	0.97 ± 0.04	1.01 ± 0.05	1.02 ± 0.06	1.06 ± 0.05	0.96 ± 0.04
Female					
n	10	10	9	9	8
Necropsy body wt	190 ± 3	198 ± 4	193 ± 3	193 ± 4	187 ± 4
Heart					
Absolute	0.704 ± 0.011	0.679 ± 0.012	0.674 ± 0.016	0.693 ± 0.013	0.670 ± 0.014
Relative	3.71 ± 0.04	3.43 ± 0.05**	3.49 ± 0.05*	3.60 ± 0.07	3.59 ± 0.06
R. Kidney					
Absolute	0.716 ± 0.015	0.730 ± 0.020	0.710 ± 0.012	0.750 ± 0.014	0.730 ± 0.025
Relative	3.77 ± 0.07	3.68 ± 0.06	3.68 ± 0.05	3.90 ± 0.06	3.91 ± 0.10
Liver					
Absolute	6.435 ± 0.144	6.532 ± 0.221	6.449 ± 0.129	6.715 ± 0.204	6.731 ± 0.221
Relative	33.82 ± 0.32	32.92 ± 0.81	33.47 ± 0.54	34.79 ± 0.54	36.00 ± 0.68*
Lung					
Absolute	1.156 ± 0.048	1.184 ± 0.033	1.147 ± 0.037	1.195 ± 0.050	1.121 ± 0.045
Relative	6.06 ± 0.17	5.97 ± 0.13	5.97 ± 0.24	6.20 ± 0.24	6.00 ± 0.17
Thymus					
Absolute	0.263 ± 0.010	0.276 ± 0.014	0.263 ± 0.010	0.251 ± 0.013	0.257 ± 0.012
Relative	1.38 ± 0.04	1.39 ± 0.06	1.36 ± 0.04	1.30 ± 0.05	1.37 ± 0.06

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** Significantly different ($P \leq 0.01$) 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 D3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 16-Day Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	63 mg/kg	125 mg/kg
Male			
n	5	5	5
Necropsy body wt	26.4 ± 0.7	26.0 ± 0.5	26.1 ± 0.6
Heart			
Absolute	0.132 ± 0.005	0.130 ± 0.003	0.131 ± 0.003
Relative	5.00 ± 0.11	5.00 ± 0.06	5.01 ± 0.05
R. Kidney			
Absolute	0.249 ± 0.012	0.246 ± 0.007	0.255 ± 0.008
Relative	9.41 ± 0.22	9.43 ± 0.13	9.76 ± 0.11
Liver			
Absolute	1.456 ± 0.049	1.416 ± 0.044	1.437 ± 0.042
Relative	55.03 ± 0.66	54.35 ± 1.03	54.94 ± 0.64
Lung			
Absolute	0.180 ± 0.007	0.184 ± 0.007	0.194 ± 0.006
Relative	6.80 ± 0.20	7.08 ± 0.35	7.42 ± 0.18
Spleen			
Absolute	0.069 ± 0.003	0.067 ± 0.003	0.067 ± 0.003
Relative	2.61 ± 0.06	2.57 ± 0.08	2.57 ± 0.07
R. Testis			
Absolute	0.106 ± 0.002	0.103 ± 0.001	0.103 ± 0.004
Relative	4.03 ± 0.16	3.96 ± 0.07	3.94 ± 0.14
Thymus			
Absolute	0.054 ± 0.003	0.049 ± 0.004	0.051 ± 0.005
Relative	2.04 ± 0.13	1.87 ± 0.16	1.96 ± 0.20
Female			
n	5	5	4
Necropsy body wt	21.2 ± 0.4	21.9 ± 0.5	22.4 ± 0.1
Heart			
Absolute	0.120 ± 0.003	0.118 ± 0.005	0.118 ± 0.002
Relative	5.66 ± 0.11	5.40 ± 0.14	5.28 ± 0.09
R. Kidney			
Absolute	0.169 ± 0.007	0.173 ± 0.002	0.173 ± 0.007
Relative	7.96 ± 0.22	7.90 ± 0.12	7.75 ± 0.32
Liver			
Absolute	1.073 ± 0.043	1.136 ± 0.025	1.209 ± 0.017*
Relative	50.51 ± 1.35	51.88 ± 0.69	54.12 ± 1.04
Lung			
Absolute	0.171 ± 0.007	0.162 ± 0.008	0.150 ± 0.009
Relative	8.07 ± 0.23	7.38 ± 0.25	6.72 ± 0.36*
Spleen			
Absolute	0.078 ± 0.003	0.078 ± 0.004	0.076 ± 0.004
Relative	3.67 ± 0.12	3.56 ± 0.09	3.40 ± 0.20
Thymus			
Absolute	0.062 ± 0.003	0.064 ± 0.005	0.064 ± 0.007
Relative	2.94 ± 0.14	2.91 ± 0.21	2.84 ± 0.29

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or 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). No data are available for the 250, 500, and 1,000 mg/kg groups due to 100% mortality.

TABLE D4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male					
n	10	10	10	8	9
Necropsy body wt	34.9 ± 0.7	34.9 ± 1.0	34.5 ± 0.6	34.8 ± 0.9	33.9 ± 0.9
Heart					
Absolute	0.142 ± 0.003	0.146 ± 0.003	0.146 ± 0.002	0.151 ± 0.003	0.145 ± 0.003
Relative	4.06 ± 0.06	4.19 ± 0.06	4.25 ± 0.05*	4.30 ± 0.10*	4.29 ± 0.06*
R. Kidney					
Absolute	0.284 ± 0.006	0.290 ± 0.008	0.285 ± 0.006	0.311 ± 0.008*	0.297 ± 0.009
Relative	8.13 ± 0.13	8.32 ± 0.17	8.28 ± 0.16	8.82 ± 0.15**	8.77 ± 0.16**
Liver					
Absolute	1.519 ± 0.042	1.553 ± 0.049	1.534 ± 0.049	1.599 ± 0.041	1.537 ± 0.036
Relative	43.44 ± 0.61	44.48 ± 0.57	44.47 ± 0.90	45.33 ± 0.50	45.39 ± 1.00
Lung					
Absolute	0.196 ± 0.011	0.199 ± 0.008	0.198 ± 0.014	0.188 ± 0.015	0.184 ± 0.006
Relative	5.63 ± 0.32	5.71 ± 0.17	5.78 ± 0.46	5.31 ± 0.34	5.43 ± 0.18
R. Testis					
Absolute	0.119 ± 0.005	0.113 ± 0.007	0.119 ± 0.003	0.120 ± 0.005	0.120 ± 0.004
Relative	3.39 ± 0.11	3.24 ± 0.21	3.46 ± 0.07	3.39 ± 0.12	3.54 ± 0.05
Thymus					
Absolute	0.063 ± 0.009	0.045 ± 0.006	0.044 ± 0.004	0.042 ± 0.005	0.044 ± 0.005
Relative	1.80 ± 0.25	1.31 ± 0.19	1.26 ± 0.11	1.19 ± 0.14	1.31 ± 0.14
Female					
n	10	10	10	10	9
Necropsy body wt	29.1 ± 1.0	29.9 ± 0.9	28.7 ± 0.9	29.2 ± 1.3	28.2 ± 0.9
Heart					
Absolute	0.125 ± 0.002	0.128 ± 0.003	0.122 ± 0.003	0.126 ± 0.003	0.124 ± 0.003
Relative	4.31 ± 0.12	4.31 ± 0.09	4.28 ± 0.11	4.37 ± 0.12	4.40 ± 0.08
R. Kidney					
Absolute	0.193 ± 0.003	0.190 ± 0.004	0.184 ± 0.004	0.192 ± 0.006	0.184 ± 0.006
Relative	6.70 ± 0.22	6.40 ± 0.18	6.44 ± 0.14	6.64 ± 0.17	6.52 ± 0.06
Liver					
Absolute	1.168 ± 0.022	1.223 ± 0.046	1.246 ± 0.034	1.249 ± 0.055	1.208 ± 0.039
Relative	40.47 ± 1.35	40.95 ± 0.81	43.57 ± 0.50	42.95 ± 1.21	42.81 ± 0.64
Lung					
Absolute	0.187 ± 0.004	0.196 ± 0.009	0.172 ± 0.005	0.176 ± 0.006	0.176 ± 0.006
Relative	6.48 ± 0.24	6.58 ± 0.27	6.02 ± 0.12	6.08 ± 0.16	6.25 ± 0.14
Thymus					
Absolute	0.051 ± 0.003	0.054 ± 0.003	0.048 ± 0.002	0.056 ± 0.005	0.050 ± 0.004
Relative	1.77 ± 0.09	1.79 ± 0.09	1.69 ± 0.10	1.90 ± 0.12	1.78 ± 0.16

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' 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).

APPENDIX E
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE E1
Summary of Reproductive Tissue Evaluations for Male Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	338 ± 9	335 ± 8	340 ± 5	311 ± 9*
L. Cauda epididymis	0.1660 ± 0.0076	0.1633 ± 0.0063	0.1607 ± 0.0056	0.1582 ± 0.0078
L. Epididymis	0.4933 ± 0.0157	0.4944 ± 0.0084	0.4973 ± 0.0140	0.4828 ± 0.0149
L. Testis	1.5400 ± 0.0487	1.5126 ± 0.0259	1.5180 ± 0.0316	1.4909 ± 0.0441
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.15 ± 0.42	9.51 ± 0.38	9.12 ± 0.54	9.36 ± 0.35
Spermatid heads (10^7/testis)	13.98 ± 0.56	14.35 ± 0.49	13.84 ± 0.83	13.90 ± 0.49
Spermatid count (mean/10^{-4} mL suspension)	69.90 ± 2.78	71.75 ± 2.45	69.18 ± 4.16	69.48 ± 2.46
Epididymal spermatozoal measurements				
Motility (%)	84.66 ± 0.43[b]	83.63 ± 0.47	83.32 ± 0.47	83.27 ± 0.40
Concentration (10^6/g cauda epididymal tissue)	427 ± 19	454 ± 21	460 ± 17	409 ± 36

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).
[b] n=8

TABLE E2
Estrous Cycle Characterization for Female Rats in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
n	10	9	10	8
Necropsy body wt (g)	190 ± 3	193 ± 3	192 ± 4	187 ± 4
Estrous cycle length (days)	4.75 ± 0.13	4.44 ± 0.15[b]	4.80 ± 0.17	4.94 ± 0.26
Estrous stages[c] (% of cycle)				
Diestrus	42.5	36.1	38.3	37.5
Proestrus	14.2	20.4	17.5	18.8
Estrus	25.0	23.1	25.8	22.9
Metestrus	18.3	20.4	18.3	20.8

[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (necropsy body weight) or Dunn's test (estrous cycle length).
[b] Estrous cycle was longer than 12 days or unclear in one of nine animals.
[c] Evidence shows that females administered 25 mg/kg differ significantly (Wilk's Criterion, $P \leq 0.05$) from the vehicle control females in the relative length of time spent in the estrous stages. Dosed females spent more time in proestrus and less time in diestrus than the vehicle control females.

TABLE E3
Summary of Reproductive Tissue Evaluations for Male Mice in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
n	10	10	9	9
Weights (g)				
Necropsy body wt	34.9 ± 0.7	34.5 ± 0.6	35.5 ± 0.7	33.9 ± 0.9
L. Cauda epididymis	0.0175 ± 0.0008	0.0167 ± 0.0010	0.0173 ± 0.0010	0.0174 ± 0.0009
L. Epididymis	0.0503 ± 0.0016	0.0470 ± 0.0016	0.0514 ± 0.0020	0.0486 ± 0.0015
L. Testis	0.1106 ± 0.0044	0.1150 ± 0.0033	0.1180 ± 0.0052	0.1184 ± 0.0034
Spermatid measurements				
Spermatid heads (10^7/g testis)	15.90 ± 0.34	14.83 ± 0.44	14.25 ± 0.35*	15.10 ± 0.49
Spermatid heads (10^7/testis)	1.76 ± 0.09	1.70 ± 0.05	1.69 ± 0.09	1.78 ± 0.07
Spermatid count (mean/10^{-4} mL suspension)	55.10 ± 2.76	53.05 ± 1.47	52.64 ± 2.75	55.78 ± 2.13
Epididymal spermatozoal measurements				
Motility (%)	88.75 ± 0.31	88.42 ± 0.52[b]	88.92 ± 0.54	87.82 ± 0.40
Concentration (10^6/g cauda epididymal tissue)	914 ± 55	925 ± 71	796 ± 117	856 ± 57

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid heads per testis, spermatid count, and epididymal spermatozoal measurements).
[b] n=9

TABLE E4
Estrous Cycle Characterization for Female Mice in the 13-Week Gavage Study of Benzyltrimethylammonium Chloride[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
n	10	10	10	9
Necropsy body wt (g)	29.1 ± 1.0	28.7 ± 0.9	29.2 ± 1.3	28.2 ± 0.9
Estrous cycle length (days)	4.00 ± 0.00[b]	4.30 ± 0.13	4.61 ± 0.44[c]	4.17 ± 0.12
Estrous stages (% of cycle)				
Diestrus	40.0	32.5	36.7	27.8
Proestrus	16.7	17.5	15.8	17.6
Estrus	22.5	29.2	26.7	30.6
Metestrus	20.8	20.8	20.8	24.1

[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (necropsy body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in 3 of 10 animals.
[c] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

APPENDIX F
GENETIC TOXICOLOGY

TABLE F1
Mutagenicity of Benzyltrimethylammonium Chloride in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] −S9 Trial 1	−S9 Trial 2	+ hamster S9 10%	+ hamster S9 30%	+rat S9 10%	+rat S9 30%
TA100	0	122 ± 8.2	122 ± 9.2	116 ± 7.0	119 ± 11.0	84 ± 6.6	147 ± 9.6
	100	119 ± 12.7	128 ± 3.7	102 ± 1.2	130 ± 4.9	105 ± 7.1	128 ± 7.1
	333	115 ± 6.9	118 ± 14.2	110 ± 3.5	115 ± 9.0	89 ± 6.9	128 ± 3.4
	1,000	116 ± 11.7	116 ± 8.2	107 ± 8.1	113 ± 5.7	104 ± 8.7	136 ± 2.5
	3,333	121 ± 9.0[c]	122 ± 2.4	99 ± 3.0	135 ± 2.9[c]	102 ± 8.7	138 ± 3.8
	10,000	65 ± 3.7[c]	104 ± 4.4[c]	103 ± 13.5[c]	109 ± 6.4[c]	91 ± 7.8	113 ± 7.2[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		1,389 ± 22.6	1,485 ± 4.8	1,125 ± 21.5	2,231 ± 35.7	1,883 ± 33.0	702 ± 16.7
TA1535	0	31 ± 3.0	29 ± 0.9	9 ± 0.9	14 ± 0.9	9 ± 1.7	12 ± 2.3
	100	29 ± 0.7	30 ± 1.0	11 ± 3.8	8 ± 1.0	10 ± 1.9	13 ± 0.0
	333	25 ± 0.9	26 ± 4.4	13 ± 2.3	12 ± 1.5	10 ± 1.5	15 ± 2.0
	1,000	28 ± 1.5	37 ± 2.2	9 ± 1.2	10 ± 1.7	11 ± 0.7	11 ± 0.7
	3,333	28 ± 3.0[c]	27 ± 2.7	9 ± 0.9	12 ± 3.5	9 ± 3.8	13 ± 1.2
	10,000	22 ± 4.8[c]	21 ± 1.5[c]	9 ± 1.5[c]	12 ± 0.6	11 ± 1.5[c]	11 ± 2.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,051 ± 8.7	1,095 ± 18.9	127 ± 9.0	323 ± 17.8	117 ± 5.0	130 ± 13.9
TA97	0	104 ± 8.4	121 ± 3.5	140 ± 4.1	170 ± 8.8	113 ± 9.7	181 ± 5.5
	100	113 ± 7.0	110 ± 7.4	143 ± 0.3	158 ± 8.8	126 ± 12.1	181 ± 4.2
	333	99 ± 0.9	114 ± 3.6	144 ± 8.0	156 ± 1.9	124 ± 3.5	155 ± 9.2
	1,000	112 ± 8.3	128 ± 5.5	139 ± 6.9	165 ± 6.1	133 ± 5.3	168 ± 11.1
	3,333	95 ± 2.1[c]	128 ± 2.9	130 ± 7.1	152 ± 6.0[c]	129 ± 2.2	167 ± 6.4
	10,000	92 ± 5.2[c]	100 ± 5.6[c]	131 ± 2.2[c]	172 ± 7.8[c]	110 ± 4.6[c]	174 ± 12.8
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,626 ± 161.0	722 ± 9.3	511 ± 17.2	1,452 ± 23.1	763 ± 36.3	691 ± 13.3
TA98	0	19 ± 4.1	16 ± 0.6	39 ± 2.6	35 ± 2.7	32 ± 5.5	31 ± 5.8
	100	14 ± 1.5	14 ± 1.7	37 ± 3.4	27 ± 4.4	31 ± 1.2	29 ± 0.9
	333	16 ± 2.1	19 ± 4.1	32 ± 3.2	31 ± 2.8	30 ± 1.3	34 ± 2.6
	1,000	17 ± 2.0	17 ± 2.4	36 ± 2.1	34 ± 1.2	35 ± 1.7	30 ± 5.8
	3,333	19 ± 1.2[c]	16 ± 1.5	33 ± 5.5	30 ± 3.5	38 ± 2.2	35 ± 2.2
	10,000	14 ± 1.0[c]	19 ± 3.5[c]	34 ± 1.2	28 ± 1.8	31 ± 3.8	36 ± 1.7[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,246 ± 69.5	1,848 ± 57.8	1,205 ± 26.9	2,210 ± 28.6	1,401 ± 30.6	659 ± 32.9

[a] Study was performed at Microbiological Associates, Inc. The detailed protocol and these data are presented in Zeiger *et al.* (1988). 0 μg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in 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 F2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Benzyltrimethylammonium Chloride by Gavage for 13 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	Pairwise P Value[c]
Male				
Water[d]		10	3.7 ± 0.6	
Benzyltrimethylammonium chloride	12.5	10	2.5 ± 0.5	0.937
	25	10	2.8 ± 0.6	0.868
	50	10	5.2 ± 0.9	0.056
	100	9	6.6 ± 1.1	0.003
			P≤0.001[e]	
Female				
Water		10	2.0 ± 0.3	
Benzyltrimethylammonium chloride	12.5	10	2.5 ± 0.6	0.228
	25	10	3.0 ± 0.3	0.078
	50	10	3.9 ± 0.3	0.007
	100	9	6.4 ± 0.6	0.000
			P≤0.001	

[a] Study was performed at Integrated Laboratory Systems. The detailed protocol is presented in MacGregor *et al*. (1990). NCE=normochromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison to solvent control; significant at P≤0.006 (ILS, 1990)
[d] Solvent control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

NTP TECHNICAL REPORTS ON TOXICITY STUDIES
PRINTED AS OF FEBRUARY 2000

TOX No.	Chemical
1	Hexachloro-1,3-butadiene
2	*n*-Hexane
3	Acetone
4	1,2-Dichloroethane
5	Cobalt Sulfate Heptahydrate
6	Pentachlorobenzene
7	1,2,4,5-Tetrachlorobenzene
8	D & C Yellow No. 11
9	*o*-Cresol, *m*-Cresol, and *p*-Cresol
10	Ethylbenzene
11	Antimony Potassium Tartrate
12	Castor Oil
13	Trinitrofluorenone
14	*p*-Chloro-α,α,α-trifluorotoluene
15	*t*-Butyl Perbenzoate
16	Glyphosate
17	Black Newsprint Ink
18	Methyl Ethyl Ketone Peroxide
19	Formic Acid
20	Diethanolamine
21	2-Hydroxy-4-methoxybenzophenone
22	N, N-Dimethylformamide
23	*o*-Nitrotoluene, *m*-Nitrotoluene, and *p*-Nitrotoluene
24	1,6-Hexanediamine
25	Glutaraldehyde
26	Ethylene Glycol Ethers
27	Riddelliine
28	Tetrachlorophthalic Anhydride
29	Cupric Sulfate
30	Dibutyl Phthalate
31	Isoprene
32	Methylene Bis(thiocyanate)
33	2-Chloronitrobenzene and 4-Chloronitrobenzene
34	1-Nitropyrene
35	Chemical Mixture of 25 Groundwater Contaminants
36	Pesticide/Fertilizer Mixtures
37	Sodium Cyanide
38	Sodium Selenate and Sodium Selenite
39	Cadmium Oxide
40	β-Bromo-β-nitrostyrene
42	1,3-Diphenylguanidine
43	*o*-, *m*-, and *p*-Chloroaniline
44	*o*-Nitrotoluene and *o*-Toluidine Hydrochloride
45	Halogenated Ethanes
50	Cyclohexanone Oxime
51	Methyl Ethyl Ketoxime
52	Urethane
53	*t*-Butyl Alcohol
54	1,4-Butanediol
58	60-Hz Magnetic Fields
59	Chloral Hydrate
65	3,3′,4,4′-Tetrachloroazobenzene
66	3,3′,4,4′-Tetrachloroazoxybenzene

National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
P.O. Box 12233, MD K2-05
Durham, NC 27709
Tel: 984-287-3211
ntpwebrequest@niehs.nih.gov

https://ntp.niehs.nih.gov

ISSN 2378-8992