NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 438

TOXICOLOGY AND CARCINOGENESIS STUDIES OF BENZETHONIUM CHLORIDE

(CAS NO. 121-54-0)

IN F344/N RATS AND B6C3F$_1$ MICE

(DERMAL STUDIES)

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. 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 Technical 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. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

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NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
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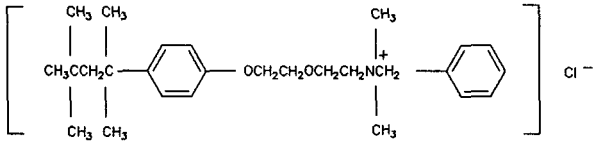
ABSTRACT



BENZETHONIUM CHLORIDE

CAS No. 121-54-0

Chemical Formula: $C_{27}H_{42}NO_2 \cdot Cl$ Molecular Weight: 448.1

Synonyms: Benzyldimethyl-*p*-(1,1,3,3-tetramethylbutyl) phenoxyethoxy-ethylammonium chloride; diisobutylphenoxyethoxy-ethyldimethyl benzyl ammonium chloride; *p*-tert-octylphenoxyethoxyethyldimethylbenzyl ammonium chloride

Trade names: Anti-germ 77, Antiseptol, BZT, Diapp, Disilyn, Hyamine, Hyamine 1622, Phemeride, Phemithyn, Polymine D, Quatrachlor, Solamine

Benzethonium chloride is used primarily in cosmetics for its antimicrobial and cationic surfactant properties. Benzethonium chloride was nominated by the National Cancer Institute to the NTP for study from a class study of chemicals used as biocides. The chemical was selected based on a suspicion of carcinogenicity and its known widespread human exposure. Male and female F344/N rats and B6C3F$_1$ mice were topically administered benzethonium chloride (greater than 98% pure) for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and cultured Chinese hamster ovary cells.

16-Day Study in Rats

Groups of five male and five female F344/N rats were topically administered 0, 6.3, 12.5, 25, 50, or 100 mg benzethonium chloride/kg body weight. Rats were administered a total of 12 doses in a fixed volume of 250 μL ethanol. All rats survived to the end of the study. The final mean body weights and body weight gains of rats administered 50 or 100 mg benzethonium chloride/kg body weight were significantly less than those of the controls. Clinical findings at necropsy included thickening or hardening of the skin at the site of application in all rats administered 50 or 100 mg/kg and in 25 mg/kg males. Lesions at the site of application appeared crusty or red-grey in color. Epithelial hyperplasia with or without inflammation occurred at the site of application in all groups of males and females administered benzethonium chloride.

16-Day Study in Mice

Groups of five male and five female B6C3F$_1$ mice were topically administered 0, 6.3, 12.5, 25, 50, or 100 mg benzethonium chloride/kg body weight. Mice were administered a total of 12 doses in a fixed volume of 100 μL ethanol. One 100 mg/kg male mouse died on day 4 of the study. Final mean body weights of all groups of males and females were similar to those of the controls. Clinical findings included mild irritation at the site of application in 50 and 100 mg/kg males and females and in 25 mg/kg males. Epithelial hyperplasia with or without inflammation occurred at the site of application in all groups of males and females administered benzethonium chloride.

13-WEEK STUDY IN RATS

Groups of 10 male and 10 female F344/N rats were topically administered 0, 1.56, 3.13, 6.25, 12.5, or 25 mg benzethonium chloride/kg body weight, 5 days per week for 13 weeks. Doses were administered in ethanol at a volume not exceeding 300 μL. All rats survived to the end of the study. The final mean body weight and body weight gain of 25 mg/kg males were significantly lower than those of the controls. The final mean body weights of all other groups of males and of all groups of females were similar to those of the controls. Clinical findings included irritation at the site of application in groups administered 3.13 mg/kg or greater. There were no differences in absolute or relative organ weights considered to be related to chemical administration. Epithelial hyperplasia was observed at the site of application in all groups of males and females administered benzethonium chloride. Additionally, inflammation and ulceration were observed at the site of application in males and females administered 3.13 mg/kg or greater. Based on the lesions observed in the 13-week study, benzethonium chloride dose levels selected for the 2-year dermal study in male and female rats were 0.15, 0.5, and 1.5 mg/kg.

13-WEEK STUDY IN MICE

Groups of 10 male and 10 female B6C3F$_1$ mice were topically administered 0, 1.56, 3.13, 6.25, 12.5, or 25 mg benzethonium chloride/kg body weight, 5 days per week for 13 weeks. Doses were administered in ethanol at a volume not exceeding 100 μL. All mice survived to the end of the study. The final mean body weights of all dosed groups of males and females were similar to those of the controls; the mean body weight gain of 25 mg/kg males was significantly less than that of the controls. Males administered 6.25, 12.5, or 25 mg/kg developed irritation, thickening of the skin, scales, and/or discoloration at the site of application, as did female mice administered 12.5 or 25 mg/kg. Increased incidences of epithelial hyperplasia and inflammation were observed at the site of application in all groups of males and females administered benzethonium chloride. Based on the lesions observed in the 13-week study, benzethonium chloride dose levels selected for the 2-year dermal study in mice were 0.15, 0.5, and 1.5 mg/kg.

2-YEAR STUDY IN RATS

Groups of 60 male and 60 female F344/N rats were topically administered 0, 0.15, 0.5, or 1.5 mg benzethonium chloride/kg body weight 5 days per week for 103 weeks. Doses were administered in ethanol, and dose volumes were adjusted weekly according to the average body weights of the groups. As many as nine rats per group were evaluated after 15 months of chemical administration.

Survival, Body Weights, and Clinical Findings

Survival of dosed rats was similar to that of the controls throughout the study. Mean body weights of all dosed groups of males and females were similar to those of the controls throughout the study. Reddening of the skin was observed at the site of application in all dosed groups of males and females. There were no other clinical findings considered to be related to chemical administration.

Pathology Findings

There were no increased incidences of neoplasms in dosed male or female rats that were attributed directly to the administration of benzethonium chloride. Increased incidences of epithelial hyperplasia, sebaceous gland hyperplasia, and ulcers were observed at the site of application in dosed females. The incidence of epithelial hyperplasia was increased in 0.5 and 1.5 mg/kg males.

2-YEAR STUDY IN MICE

Groups of 60 male and 60 female B6C3F$_1$ mice were topically administered 0, 0.15, 0.5, or 1.5 mg benzethonium chloride/kg body weight 5 days per week for 103 weeks. Doses were administered in ethanol, and dose volumes were adjusted weekly according to the average body weights of the groups. As many as 10 mice per group were evaluated after 15 months of chemical administration.

Survival, Body Weights, and Clinical Findings

Survival of dosed mice was similar to that of the controls throughout the study. Mean body weights of all dosed groups of males and females were similar to those of the controls throughout the study. Reddening of the skin was observed at the site of

application in all dosed groups of males and in 0.15 mg/kg females. There were no other clinical findings attributed to chemical administration.

Pathology Findings

There were no increased incidences of neoplasms in dosed males or females that were related to administration of benzethonium chloride. Increased incidences of epithelial hyperplasia were observed at the site of application in dosed males and females.

GENETIC TOXICOLOGY

Benzethonium chloride was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 and did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells. All tests were conducted with and without S9 metabolic activation enzymes.

CONCLUSIONS

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity** of benzethonium chloride in male or female F344/N rats receiving 0.15, 0.5, or 1.5 mg/kg. There was *no evidence of carcinogenic activity* in male or female $B6C3F_1$ mice receiving 0.15, 0.5, or 1.5 mg/kg.

Exposure of rats and mice to benzethonium chloride by dermal application in ethanol for 2 years resulted in epithelial hyperplasia in male and female rats and mice and sebaceous gland hyperplasia and ulcers in female rats at the site of application.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Benzethonium Chloride

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 0.15, 0.5, or 1.5 mg/kg in not more than 296 µL of acetone	0, 0.15, 0.5, or 1.5 mg/kg in not more than 317 µL of acetone	0, 0.15, 0.5, or 1.5 mg/kg in not more than 131 µL of acetone	0, 0.15, 0.5, or 1.5 mg/kg in not more than 131 µL of acetone
Body weights	Dosed groups similar to controls	Dosed groups similar to controls	Dosed groups similar to controls	Dosed groups similar to controls
2-Year survival rates	15/52, 11/52, 9/55, 16/56	24/51, 33/53, 26/51, 24/53	43/50, 38/50, 42/50, 39/50	38/52, 34/53, 31/48, 34/54
Nonneoplastic effects	Skin (site of application): epithelial hyperplasia (1/52, 0/52, 4/55, 12/56)	Skin (site of application): epithelial hyperplasia (2/51, 2/53, 6/51, 32/53); sebaceous gland hyperplasia (1/51, 2/53, 6/51, 30/53); ulcer (0/51, 1/53, 3/51, 19/53)	Skin (site of application): epithelial hyperplasia (2/50, 7/50, 16/50, 23/50)	Skin (site of application): epithelial hyperplasia (3/52, 7/53, 6/48, 22/54)
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Negative in strains TA98, TA100, TA1535, and TA1537 with and without S9			
Sister chromatid exchanges				
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Chromosomal aberrations				
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on benzethonium chloride on June 21, 1994, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On June 21, 1994, the draft Technical Report on the toxicology and carcinogenesis studies of benzethonium chloride received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, N.C.

Dr. J.R. Bucher, NIEHS, introduced the toxicology and carcinogenesis studies of benzethonium chloride by discussing the uses of the chemical and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related nonneoplastic lesions in rats and mice. The proposed conclusions were *no evidence of carcinogenic activity* of benzethonium chloride in male and female F344/N rats and in male and female B6C3F$_1$ mice. Dermal exposure of rats and mice to benzethonium chloride in ethanol for 2 years resulted in epithelial hyperplasia in male and female rats and mice and sebaceous gland hyperplasia and ulcers in female rats at the site of application.

Dr. Bailey, a principal reviewer, agreed with the proposed conclusions and believed the dose levels selected were adequate to evaluate the carcinogenic potential of the chemical in rats and mice.

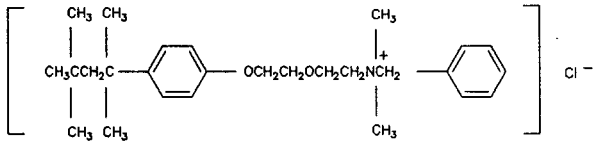
Dr. Vodicnik, the second principal reviewer, also agreed with the proposed conclusions. She questioned part of the rationale for study of the chemical (i.e., "suspicion of carcinogenicity"). She said that the statement was based on results of a dated, isolated study in which commercial grade benzethonium chloride was administered subcutaneously to rats, and noted that the localized sarcomas observed in the study were typical of those resulting from repeated irritation. Dr. Vodicnik said sufficient rationale for study was the widespread human exposure and lack of adequate testing. Dr. Bucher noted that some human carcinogens (e.g., nickel compounds) are very difficult to show as being carcinogenic in animal studies by other than an injection route. He thought it an appropriate response by the NTP to do this study by the dermal route to clarify whether there was, in fact, any suspicion of carcinogenicity. Dr. Vodicnik added that part of her concern had to do with the lack of characterization of the test material and impurities in the earlier study.

Dr. Reddy, the third principal reviewer, also agreed with the proposed conclusions, but believed the high dose administered to male rats and mice in the 2-year studies could have been greater. Dr. Bucher agreed. Dr. Reddy asked whether it would be appropriate to modify future dermal study protocols to examine the potential promoting effect of compounds such as benzethonium chloride. Dr. Bucher asked the subcommittee to comment on the value of promotion studies.

Dr. Ward said that he agreed with the rationale for study, adding that the results of the studies on benzethonium chloride provide more evidence that chronic irritation alone does not cause neoplasms. Dr. Ryan asked for clarification of a statement that sebaceous gland carcinomas in treated male rats were consistent with the spectrum of neoplasms found in adjacent control skin from treated and untreated animals. Dr. Bucher said that these lesions in treated animals were similar to those observed in control animals, suggesting that these neoplasms were not associated with chemical exposure. Dr. Klaassen asked why there was a tenfold difference between the lowest and highest doses administered in this study, adding that the difference is generally fourfold. Dr. D.S. Marsman, NIEHS, said that between the 16-day and 13-week studies, there appeared to be more lesions developing at lower dose levels; thus, a wider dose range in the 2-year studies could have allowed for continuance of this observation. Dr. Miller commented that in view of the widespread human exposure in skin products, it would be useful to relate the doses applied to typical concentrations in consumer products. Dr. Bucher agreed.

Dr. Bailey moved that the Technical Report on benzethonium chloride be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, *no evidence of carcinogenic activity*. Dr. Vodicnik seconded the motion, which was accepted unanimously with 11 votes.

INTRODUCTION



BENZETHONIUM CHLORIDE

CAS No. 121-54-0

Chemical Formula: $C_{27}H_{42}NO_2 \cdot Cl$ Molecular Weight: 448.1

Synonyms: Benzyldimethyl-*p*-(1,1,3,3-tetramethylbutyl) phenoxyethoxy-ethylammonium chloride, diisobutylphenoxyethoxy-ethyldimethyl benzyl ammonium chloride, *p*-tert-octylphenoxyethoxyethyldimethylbenzyl ammonium chloride
Trade names: Anti-germ 77, Antiseptol, BZT, Diapp, Disilyn, Hyamine, Hyamine 1622, Phemeride, Phemithyn, Polymine D, Quatrachlor, Solamine

CHEMICAL AND PHYSICAL PROPERTIES

Benzethonium chloride is a white to colorless, nearly odorless, bitter tasting crystalline substance. It is soluble in water, alcohols, glycols, acetone, benzene, and other organic solvents (*Merck Index*, 1976; CIR, 1985). Details of the synthesis of the compound are proprietary information. Commercial preparations typically contain numerous impurities including unreacted starting materials and side-reaction products. Impurities may include benzyl chloride, benzal chloride, benzyl alcohol, benzaldehyde, and benzylamine derivatives (CIR, 1982).

PRODUCTION, USE, AND HUMAN EXPOSURE

Benzethonium chloride is used primarily in cosmetics for its antimicrobial and cationic surfactant properties. Concentrations below 1% (with most below 0.1%) are typically found in baby bath, eye makeup, personal hygiene, fragrance, hair, shaving, skin, and suntan preparations (CIR, 1982). The material is also widely used in disinfectants, cationic detergents, and preservatives, including uses in fabric softening, ore flotation, corrosion inhibition, paper processing, and in pharmaceuticals and vaccines (CIR, 1982). Benzethonium chloride is used in a variety of over-the-counter drug products and is permitted at maximum concentrations of 0.01% in preparations for ophthalmic uses, and at 0.02% for other topical uses (FDA, 1980a). Mouth rinses containing benzethonium chloride at 0.075% to 0.1% have been shown to reduce plaque accumulation (Volpe *et al.*, 1969; Compton and Beagrie, 1975; Tanzer *et al.*, 1979; Gaffar *et al.*, 1980), but yellow-brown teeth and tongue discolorations are sometimes noted. Benzethonium chloride has also been incorporated into polymerized methyl methacrylate used in contact lenses (Mote *et al.*, 1969). Benzethonium chloride at 0.2% is spermicidal (Paniagua *et al.*, 1961). Assuming complete chloride absorption from a product containing 1% of the chemical by weight, the dose to a 70-kg human would be 0.14 mg/kg per gram of product used. This is similar to the lowest dose used in the present 2-year studies.

Approximately 29,000 people are potentially exposed occupationally to benzethonium chloride annually, and consumer exposure has been estimated to be approximately 3.8 million people per year in the United States (SRI, 1984).

Although quaternary ammonium compounds such as benzethonium chloride have been reported to have significant antimicrobial properties, their rather limited spectrum of action (primarily affecting gram-positive bacteria) and a tendency to be inactivated by a large number of substances have caused their antimicrobial effectiveness to be questioned (FDA, 1980b). The primary germicidal action of benzethonium chloride has been attributed to an ability to disrupt cell membrane permeability (AMA, 1980). Concentrations of benzethonium chloride required to effect germicidal activity are reported to range from 0.005% to 0.01% (Christensen, 1963). Benzethonium chloride also has been shown to inhibit a number of proteolytic enzymes, and this inhibition can occur at concentrations below that needed to produce significant losses in bacterial viability (Stedman *et al.*, 1957; CIR, 1982). The compound also shows some inhibitory action on acetylcholinesterase (Jackson and Aprison, 1966).

ABSORPTION, DISPOSITION, METABOLISM, AND EXCRETION

Experimental Animals

The extent and rate of absorption following single and repeated dermal doses and the pattern of tissue distribution and route of elimination of [^{14}C]-benzethonium chloride were studied in Fischer 344/N rats (NTP, 1988). The kinetics of distribution and excretion following an intravenous administration were also determined. From intravenous studies, an elimination half-life of radiolabel from blood was 110 minutes, the volume of distribution was 5.5 L/kg body weight, and the total clearance was 14.8 mL per minute per kg body weight. Twenty-four hours after a single intravenous dose, approximately 50% of the radiolabel was found in feces, and 2% in urine. In dermal studies, after application of 0.15 or 1.5 mg/kg [^{14}C]-labeled compound to the skin under a non-occlusive patch, peak elimination of the radiolabel in urine and feces was observed at 24 to 48 hours (urine) or 48 to 72 hours (feces). Total urinary excretion was 1% to 2% of the applied dose, and fecal excretion accounted for about 45% of the dose. Radiolabel was below the detection limit in blood and most tissues during the study, but low levels were measured in the liver, and some residual radiolabel could be accounted for in the epidermis at the site of application. This could not be washed away. When similar studies were performed with repeated daily dermal dosing, the total amount of radiolabel excreted up to 10 days following the last dose was about 25%, suggesting some accumulation with repeated dermal administration. However, following these studies it was discovered that urinary excretion was likely underestimated due to adherence of benzethonium chloride to the glass walls of the metabolism cages (NTP, 1988).

Humans

No information on the absorption, distribution, metabolism, or excretion of benzethonium chloride in humans was found in the literature.

TOXICITY

Experimental Animals

Acute oral LD_{50} values for benzethonium chloride in rats (strain unspecified or Charles River CD) and in Charles River CD-1 mice range from 368 to 665 mg/kg (CIR, 1982). When benzethonium chloride was administered subcutaneously to Fischer 344/N rats, the LD_{50} was reported as 119 mg/kg (Mason *et al.*, 1971), and intravenous administration resulted in LD_{50} values of 19 mg/kg (rats) and 35 mg/kg (mice) in unspecified strains (CIR, 1982).

In a study designed to evaluate the ability of benzethonium chloride to inactivate influenza virus, co-administration of the virus with the chemical by intranasal instillation to mice was shown to reduce mortality due to the virus. Effective concentrations were in the range of 0.00625% to 0.0125%. Exposure of mice to 0.05 mL of 0.25% benzethonium chloride and greater doses was toxic, causing lobular consolidation, pneumonia, and death (Klein and Stevens, 1945).

Dermal administration of 0.1% benzethonium chloride to the clipped skin of rabbits caused no irritation or systemic toxicity over the course of a 4-week study in which a single daily dose was administered 5 days per week (Finnegan and Dienna, 1954). However, Homburger (1968) found severe local blistering and more moderate lesions at doses of 35 to 280 mg/kg benzethonium chloride (in tricaprylin) following a single dermal application to the unshaved backs of C57BL/6 male mice. Benzethonium chloride is irritating to the rabbit eye at concentrations of 0.03% and greater (Finnegan and Dienna, 1954).

In studies of the primary irritancy and sensitization potential of benzethonium chloride in female $B6C3F_1$ mice, concentrations greater than 10% (in 95% ethanol) were found to be irritating and 20% was selected as the challenge concentration to be used in the sensitization test. For this study, sensitization doses of 1%, 3%, and 10% were applied to the shaved and dermabraded dorsal surface daily for 5 days. Mice were challenged 7 days following the last treatment by application of 20% benzethonium chloride to the left ear; no evidence of contact hypersensitivity was noted (NTP, 1989).

Benzethonium chloride was administered by gavage to pregnant rats (strain not specified) on days 6 through 15 of gestation. At the highest dose of 35.6 mg per kg body weight per day, maternal weight gain was decreased, and delayed ossification was seen in the fetuses. The fetal effects were attributed to maternal toxicity (Gilman and DeSalva, 1979).

Humans

Benzethonium chloride has been extensively studied for dermal irritant and sensitizing properties both alone and in various commercial formulations. Most results of these studies were negative; however, case reports of contact sensitization have been described (CIR, 1982). Conjunctival reactions consisting of hyperemia, edema, capillary dilation, lacrimation, and desquamation of the conjunctival epithelium were frequently reported in tests of benzethonium chloride incorporated into ocular wetting solutions (Swan, 1944). Irritation is also a common reaction to vaginal aerosol foam contraceptives containing benzethonium chloride (CIR, 1982).

Benzethonium chloride is cytotoxic to human cells. Several cultured human cell lines were reported killed by 10 μg/mL concentrations, and 1 μg/mL inhibited their growth (Kuwahara *et al.*, 1976).

There are no other studies in the literature concerning the toxicity of benzethonium chloride in humans, nor is there any information concerning toxicity to the human immune, nervous, or reproductive systems.

CARCINOGENICITY

Experimental Animals

Benzethonium chloride has been evaluated for carcinogenicity in several species and by several routes of administration. The studies have been generally deficient in length of exposure time or in number of animals tested to be considered an adequate assessment of the carcinogenic potential of benzethonium chloride.

No gross or microscopic lesions attributed to chemical administration were observed when nine dogs were exposed to 5, 100, or 500 ppm benzethonium chloride in Purina Dog Chow for 1 year (Finnegan and Dienna, 1954). Purina Dog Chow mixed with 0, 50, 200, 1,000, 2,500, or 5,000 ppm benzethonium chloride was given to groups of 10 male and 10 female rats (strain unspecified) for 2 years (Finnegan and Dienna, 1954). Mortality was increased at 5,000 ppm, and thinning of the cecal wall and cecal distension at the three highest concentrations were the only abnormalities found.

Benzethonium chloride was administered by subcutaneous injection at doses of 0, 0.1, 0.3, 1.0, and 3.0 mg/kg to groups of 60, 10, 20, 30, or 40 Fischer 344/N male and female rats, respectively. Doses were administered twice per week for 1 year. The rats were kept an additional 6 months without dosing. Body weights were reduced by as much as 20% in the highest dose groups. Twenty-six of the 200 rats receiving benzethonium chloride developed sarcomas at the site of injection; no control rats developed sarcomas at the site of injection. The incidences of sarcomas were dose related and were attributed to chemical administration. The neoplasms that were observed were described as typical fibrosarcomas arising from mesenchymal cells within an area of granulomatous reaction (Mason *et al.*, 1971).

Benzethonium chloride has been studied in a short-term mouse lung adenoma assay (Homburger, 1968). A single dose of 0.35 mg of the chemical was injected into the tail vein of 50 CF-1 and 50 A/Jax female mice. Twenty additional CF-1 female mice were administered monthly injections for 7 months, at

which time all mice were evaluated. No increase in the incidence of pulmonary adenomas was found.

Humans

No information on the carcinogenicity of benzethonium chloride in humans was found in the literature.

GENETIC TOXICITY

Genotoxicity data for benzethonium chloride are limited to bacterial mutation and DNA damage tests. The chemical was not mutagenic, with or without Aroclor-induced S9 metabolic activation enzymes, in any of several strains of *Salmonella typhimurium* (De Flora *et al.*, 1984a,b; Zeiger *et al.*, 1987). However, it was reported to induce DNA damage in several strains of repair-deficient *Escherichia coli* (De Flora *et al.*, 1984b).

STUDY RATIONALE

Benzethonium chloride was nominated by the National Cancer Institute to the NTP for study from a class study of chemicals used as biocides (Johnson *et al.*, 1984). The chemical was selected based on a suspicion of carcinogenicity and its known widespread human exposure. The dermal route was recommended because of its predominant use in cosmetics and other topical products. The NTP has performed 16-day, 13-week, and 2-year studies in F344/N rats and B6C3F$_1$ mice by the dermal route (this report), and has studied the dermal absorption, distribution, and excretion of the chemical in the F344/N rat (NTP, 1988). Additional studies evaluated the potential irritant and contact hypersensitivity properties of benzethonium chloride in female B6C3F$_1$ mice (NTP, 1989), and the chemical's genetic toxicity in *Salmonella typhimurium* and cultured Chinese hamster ovary cells.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF BENZETHONIUM CHLORIDE

United States Pharmacopeia (USP) grade benzethonium chloride was obtained from Rohm and Haas (Philadelphia, PA) in one lot (W0061), which was used throughout the 16-day, 13-week, and 2-year dermal studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). Reports on analyses performed in support of the benzethonium chloride studies are on file at the National Institute of Environmental Health Sciences (NIEHS). The methods and results of these studies are detailed in Appendix G.

The chemical, a white powder, was identified as benzethonium chloride by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity of the chemical was determined by elemental analyses, Karl Fischer water analysis, functional group titration, thin-layer chromatography, and high-performance liquid chromatography. Elemental analyses for carbon, hydrogen, nitrogen, and chlorine were in agreement with the theoretical values for benzethonium chloride. Functional group titration indicated a purity of 98.5% ± 0.5%. Karl Fischer water analysis indicated 0.6% ± 0.3% water. Thin-layer chromatography indicated a major spot and a trace impurity. High-performance liquid chromatography detected a major peak and no impurities greater than or equal to 0.1% of the major peak area. The overall purity was determined to be greater than 98%.

The complete battery of USP analyses was performed by the analytical chemistry laboratory as a supplement to the chemical characterization of benzethonium chloride. This lot of benzethonium chloride met the specifications of all analyses required by the USP XX.

Stability studies were performed by the analytical chemistry laboratory using high-performance liquid chromatography. These studies indicated that benzethonium chloride was stable as a bulk chemical for at least 2 weeks when stored protected from light at temperatures up to 60° C. At the study laboratory, the bulk chemical was stored at room temperature protected from light. The stability of the chemical was monitored periodically using high-performance liquid chromatography. No degradation of the bulk chemical was observed.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Dose formulations were prepared once for the 16-day studies and every 2 weeks for the 13-week and 2-year studies by mixing benzethonium chloride and 95% ethanol (USP grade) to give the required concentrations (Table G1). Dose formulation stability studies were performed by the analytical chemistry laboratory using high-performance liquid chromatography. The stability of the benzethonium chloride dose formulation was confirmed for at least 3 weeks at room temperature when stored protected from light, and for at least 3 hours when exposed to light and air.

Periodic analyses of the dose formulations of benzethonium chloride sampled from the dose preparation laboratory as well as from the animal room were conducted by the study laboratory and the analytical chemistry laboratory using ultraviolet spectroscopy. During the 16-day studies, the dose formulations were analyzed once (Table G2); during the 13-week studies, the dose formulations were analyzed three times (Table G3); and during the 2-year studies, the dose formulations were analyzed approximately every 2 months (Table G4). All of the dose formulations from the 16-day and 13-week studies were found to be within 10% of the target concentrations. In the 2-year studies, 98% (159/163) of the dose formulations analyzed were within 10% of the target concentrations. Results of the periodic referee analyses performed by the analytical chemistry laboratory were in agreement with the results obtained by the study laboratory (Table G5).

16-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). On receipt, the rats and mice were 25 to 31 days old. Animals were quarantined for 11 days (rats) or 12 days (mice). Groups of five male and five female rats and mice were topically administered 0, 6.3, 12.5, 25, 50, or 100 mg benzethonium chloride/ per kg body weight. Doses were administered in ethanol at fixed volumes of 250 μL (rats) or 100 μL (mice). Doses were applied 5 days per week to the dorsal interscapular areas of the animals; the site of application was clipped three times during the studies. Animals were administered a total of 12 doses; animals were dosed for 2 (rats) or 3 (mice) consecutive days prior to necropsy, and the last doses were applied less than 24 hours before necropsy. Feed and water were available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded on dosing days. The animals were weighed initially, on day 10, and at the end of the study. Details of the study design and animal maintenance are summarized in Table 1.

A necropsy was performed on all animals. The brain, heart, right kidney, liver, lung, right testis (rats), and thymus were weighed. For all animals, skin at the site of application and from undosed sites was examined histopathologically.

13-WEEK STUDIES

The 13-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to benzethonium chloride and to determine the appropriate doses to be used in the 2-year studies.

Male and female F344/N and B6C3F$_1$ mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). On receipt, the rats were 25 to 31 days old and mice were 31 days old. Animals were quarantined for 27 days (mice) or 28 days (rats) before chemical administration began. Before the start of the studies, five male and five female rats and mice were selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female control rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix I).

Groups of 10 male and 10 female rats and mice were topically administered 0, 1.56, 3.13, 6.25, 12.5, or 25 mg benzethonium chloride/kg body weight. Doses were administered in ethanol and dose volumes were adjusted weekly according to the average body weights of the dosed groups. Dose volumes did not exceed 300 μL for rats or 100 μL for mice. Doses were applied 5 days per week to the dorsal interscapular areas of the animals; the site of application was clipped weekly during the studies. Feed and water were available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded weekly for rats and mice. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 13-week studies, a necropsy was performed on all animals. The brain, 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, imbedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin. A complete histopathologic evaluation was performed on all rats and mice in the control and 25 mg/kg groups. Table 1 lists the tissues and organs routinely examined. In addition, sections of treated and untreated skin from rats and mice in all dose groups were examined microscopically.

2-YEAR STUDIES

Study Design

Groups of 60 male and 60 female rats and mice were topically administered 0, 0.15, 0.5, or 1.5 mg benzethonium chloride/kg body weight. Doses were administered in ethanol, and dose volumes were adjusted weekly according to the average body weights of the dosed groups. Throughout the study, dose volumes ranged from 63 to 296 μL (male rats), 95 to 317 μL (female rats), or 50 to 131 μL (mice). Doses were applied 5 days per week to the dorsal interscapular areas of the animals; the site of application was clipped approximately once per week during the studies. As many as 10 male and 10 female rats and mice were evaluated at 15 months for histopathology and organ weights.

Source and Specification of Animals

Male and female F344/N and B6C3F$_1$ mice were obtained from Taconic Farms, Inc. (Germantown, NY) for use in the 2-year studies. Rats and mice were quarantined for 11 days before the beginning of the studies. Six male and six female rats and six male and eight female mice were selected for parasite evaluation and gross observation of disease. Serology samples were selected for viral screening. Rats were approximately 45 days old and mice were approximately 40 days old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix I).

Animal Maintenance

Rats and mice were housed individually. Feed and water were available *ad libitum*. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are provided in Table 1. Information on feed composition and contaminants is provided in Appendix H.

Clinical Examinations and Pathology

All animals were observed twice daily for moribundity and mortality. Clinical findings were recorded monthly and body weights were recorded weekly through week 10, once during week 12, and monthly thereafter.

A complete necropsy was performed on all rats and mice. At the 15-month interim evaluation, the left kidney, right kidney, and liver of rats and mice were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. Complete histopathologic examinations were performed on skin from the site of application and control skin in all animals. Otherwise, complete histopathologic examinations were performed only on controls and 1.5 mg/kg animals. For all paired organs (i.e., adrenal gland, kidney, and ovary), samples from each organ are examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The microscope 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 evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist reviewed sections of treated and untreated skin.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and any other tissues for which a disagreement in diagnosis between the laboratory and quality assessment pathologists existed. Representative histopathology slides containing examples of skin lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologist, or lesions of general interest were presented by the chair to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes or missexed were censored from the survival analyses; animals dying from natural causes were not censored.

Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test

to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 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. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, and D3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm, i.e., the Kaplan-Meier estimate of the neoplasm incidence that would have been observed at the end of the study in the absence of mortality from all other competing risks (Kaplan and Meier, 1958).

Analysis of Neoplasm Incidences

The majority of neoplasms in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if the fit of the model was not significantly enhanced. The neoplasm incidences of exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984).

Other methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These methods include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal neoplasms, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of neoplasm-bearing animals.

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected tests were used in the analysis of neoplasm incidence, and reported P values are one sided. The procedures described in the preceding paragraphs were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, refer to Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test was used, a procedure based on the overall proportion of affected animals.

Analysis of Continuous Variables

Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). 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). Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973).

Quality Assurance Methods

The 13-week and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, so all comments had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of benzethonium chloride was assessed by testing the ability of the chemical to induce mutations in *Salmonella typhimurium* and sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells. The protocols and results for these studies are given in Appendix E.

The genetic toxicity studies of benzethonium chloride are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the structure and responses of the chemical in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemically induced DNA damage and to predict carcinogenicity in animals, based on the electrophilic theory of chemical carcinogenesis and the somatic mutation theory (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests do not correlate well with rodent carcinogenicity (Tennant *et al.*, 1987; Zeiger *et al.*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is currently the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens were rodent carcinogens), and that there is no complementarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests is not yet defined.

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Benzethonium Chloride

16-Day Studies	13-Week Studies	2-Year Studies
Study Laboratory		
Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species		
Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$
Animal Source		
Simonsen Laboratories, Inc. (Gilroy, CA)	Simonsen Laboratories, Inc. (Gilroy, CA)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies		
Rats: 11 days Mice: 12 days	Rats: 28 days Mice: 27 days	11 days
Average Age When Studies Began		
Rats: 36-42 days Mice: 37-43 days	Rats: 53-60 days Mice: 58 days	Rats: 45 days Mice: 40 days
Date of First Dose		
Rats: 17 December 1984 Mice: 18 December 1984	Rats: 2 or 3 May 1985 Mice: 1 May 1985	Rats: 15 June 1987 Mice: 22 June 1987
Duration of Dosing		
16 days (5 days/week, excluding holidays)	91 days (5 days/week, excluding holidays)	103 weeks (5 days/week, excluding holidays)
Date of Last Dose		
Rats: 1 January 1985 Mice: 2 January 1985	Rats: 31 July or 1 August 1985 Mice: 29 or 30 July 1985	Rats: 2 June 1989 Mice: 9 June 1989
Necropsy Dates		
Rats: 2 January 1985 Mice: 3 January 1985	Rats: 1 or 2 August 1985 Mice: 30 or 31 July 1985	Rats: 12-14 June 1989 Mice: 19-23 June 1989
Average Age at Necropsy		
Rats: 53-59 days Mice: 54-60 days	Rats: 144-152 days Mice: 149 days	Rats: 111 weeks Mice: 110 weeks
Size of Study Groups		
5 males and 5 females	10 males and 10 females	60 males and 60 females
Method of Distribution		
Animals randomized from weight classes using a computer-generated list of random numbers	Same as 16-day studies	Same as 16-day studies
Animals per Cage		
1	1	1

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Benzethonium Chloride
(continued)

16-Day Studies	13-Week Studies	2-Year Studies
Method of Animal Identification		
Toe clip	Rats: toe clip and cage card Mice: toe mark	Toe clip
Diet		
NIH-07 open formula pellet diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as 16-day studies	Same as 16-day studies
Water Distribution		
Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 16-day studies	Same as 16-day studies
Cages		
Polycarbonate (Lab Products, Inc., Garfield, NJ), changed once weekly	Same as 16-day studies	Same as 16-day studies
Bedding		
BetaChip heat-treated hardwood chips (Northeastern Products, Inc., Warrensburg, NY), changed once weekly	BetaChip heat-treated hardwood chips (Northeastern Products, Inc., Warrensburg, NY), changed twice weekly	BetaChip heat-treated hardwood chips (Northeastern Products, Inc., Warrensburg, NY) through the week of 22 May 1988; SaniChip hardwood bedding (P.J. Murphy Forest Products, Corp., Montville, NJ) from the week of 22 May 1988 until the end of the study; changed once weekly
Cage Filters		
Spun-bonded polyester (DuPont 2024) (Snow Filtration, Cincinnati, OH), changed once every 2 weeks	Same as 16-day studies	Same as 16-day studies
Racks		
Stainless steel (Lab Products, Inc., Garfield, NJ), changed once every 2 weeks	Same as 16-day studies	Same as 16-day studies
Animal Room Environment		
Temperature: 21°-24° C Relative humidity: 35%-65% Fluorescent light: 12 hours/day Room air: 15 changes/hour	Temperature: 21°-24° C Relative humidity: 35%-65% Fluorescent light: 12 hours/day Room air: 15 changes/hour	Temperature: 19°-24° C Relative humidity: 32%-74% Fluorescent light: 12 hours/day Room air: 10 changes/hour

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Benzethonium Chloride
(continued)

16-Day Studies	13-Week Studies	2-Year Studies
Doses		
Rats: 0, 6.3, 12.5, 25, 50, or 100 mg/kg in 250 µL of ethanol Mice: 0, 6.3, 12.5, 25, 50, or 100 mg/kg in 100 µL of ethanol	Rats: 0, 1.56, 3.13, 6.25, 12.5, or 25 mg/kg in not more than 300 µL of ethanol Mice: 0, 1.56, 3.13, 6.25, 12.5, or 25 mg/kg in not more than 100 µL of ethanol	Rats: 0, 0.15, 0.5, or 1.5 mg/kg in not more than 296 µL (males) or 317 µL (females) of ethanol Mice: 0, 0.15, 0.5, or 1.5 mg/kg in not more than 131 µL of ethanol
Type and Frequency of Observation		
Observed twice daily; animals were weighed initially, on day 10, and at the end of the studies; clinical observations recorded on dosing days	Observed twice daily; body weights and clinical observations recorded weekly	Observed twice daily; body weights recorded weekly through week 10, once during week 12, and monthly thereafter; clinical observations recorded monthly
Method of Sacrifice		
CO_2 asphyxiation	CO_2 asphyxiation	CO_2 asphyxiation
Necropsy		
A necropsy was performed on all animals. Organs weighed were brain, heart, right kidney, liver, lung, right testis (rats), and thymus.	A necropsy was performed on all animals. Organs weighed were brain, heart, right kidney, liver, lung, right testis, and thymus.	A necropsy was performed on all animals. Organs weighed at the 15-month interim evaluation were left kidney, right kidney, and liver.
Histopathology		
Histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, skin samples from the site of application and other sites were examined	Complete histopathology was performed on all control and 25 mg/kg rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral or preputial gland, esophagus, femur (including marrow), gallbladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung, mammary gland, mandibular or mesenteric lymph node, nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, spinal cord and sciatic nerve, spleen, stomach, testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, skin samples (from the site of application and from unexposed sites) from all dose groups were examined microscopically	Complete histopathology was performed on all control and 1.5 mg/kg rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral or preputial gland, esophagus, femur (including marrow), gallbladder (mice), harderian gland (mice) heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung, mammary gland, mandibular or mesenteric lymph node, nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, spinal cord and sciatic nerve, spleen, stomach, testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, skin samples (from the site of application and from unexposed sites) from all dose groups were examined microscopically

RESULTS

RATS

16-DAY STUDY

All rats survived to the end of the study (Table 2). The final mean body weights and weight gains of males and females administered 50 or 100 mg benzethonium chloride/kg body weight were significantly less than those of the controls. At necropsy, thickening or hardening of the skin at the site of application was observed in all rats administered 50 or 100 mg/kg and in 25 mg/kg males. Lesions at the site of application appeared crusty or red-grey in color.

Organ weights appeared generally appropriate for body weight in the various dose groups of males and females with the exception of thymus weights, both relative and absolute, which were decreased in 100 mg/kg males and females and in 50 mg/kg females (Table F1).

Histopathologic lesions of the skin varied from minimal epithelial hyperplasia to severe necrotizing, ulcerative lesions penetrating the epidermis to involve the underlying dermis and subcutaneous tissues. The proliferative lesions of the skin at the site of application are collectively referred to as epithelial hyperplasia. Epithelial hyperplasia consisted of a spectrum of epidermal proliferative lesions (in order of greater to lesser frequency): acanthosis, hyperkeratosis, basal cell hyperplasia, and/or increase

TABLE 2
Survival and Body Weights of Rats in the 16-Day Dermal Study of Benzethonium Chloride

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	97 ± 4	167 ± 7	70 ± 4	
6.3	5/5	96 ± 3	165 ± 5	69 ± 2	98
12.5	5/5	95 ± 3	162 ± 7	67 ± 4	97
25	5/5	94 ± 4	154 ± 4	59 ± 3*	92
50	5/5	93 ± 4	140 ± 6**	48 ± 3**	84
100	5/5	94 ± 4	127 ± 6**	33 ± 3**	76
Female					
0	5/5	86 ± 25	123 ± 3	37 ± 30	
6.3	5/5	85 ± 39	120 ± 3	35 ± 10	98
12.5	5/5	86 ± 37	124 ± 2	37 ± 16	101
25	5/5	83 ± 34	116 ± 3	33 ± 27	94
50	5/5	84 ± 37	109 ± 5*	26 ± 35**	89
100	5/5	83 ± 37	108 ± 6*	25 ± 33**	88

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.
** $P \leq 0.01$
[a] Number of animals surviving at 16 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

in the stratum granulosum. Often concomitant with epithelial hyperplasia, and in particular with increasing dose, was evidence of the following lesions: parakeratosis, sebaceous gland hyperplasia, chronic inflammation, necrosis, and/or ulceration. Sebaceous gland hyperplasia is often considered a component of epithelial hyperplasia but is described separately (along with inflammation, necrosis, and/or ulceration) for interpretive purposes. Sebaceous gland hyperplasia was defined to include both an apparent increase either in the number of cells within a gland or in the number of glands observed per unit area.

Ulcerative, necrotizing inflammation of the epidermis of marked severity was a consistent feature in 50 and 100 mg/kg males and females. Chronic inflammation of mild to moderate severity in the dermis and subcutaneous tissues was also generally present in these animals. Similar lesions of lesser severity were also observed in 25 mg/kg male and female rats. In 6.3 and 12.5 mg/kg males and females, the predominant lesion consisted of minimal to mild epithelial hyperplasia (primarily hyperkeratosis) with minimal evidence of inflammation of the epidermis and/or dermis. Epithelial hyperplasia was observed in three males and two females administered 6.3 mg/kg and in four males and two females administered 12.5 mg/kg.

Dose Selection Rationale: The severity of the skin lesions and the lower body weights observed in 50 and 100 mg/kg males and females precluded the use of doses greater than 25 mg/kg. Adaptation of the skin may occur following chronic topical administration of an apparent irritant, and thus a high dose of 25 mg/kg was selected for the 13-week study in male and female rats.

13-WEEK STUDY

All rats survived to the end of the study (Table 3). The final mean body weight and weight gain of 25 mg/kg males were significantly lower than those of the controls. The final mean body weights of all other groups of males and of all groups of females were similar to those of the controls. Clinical findings included crusting, apparent thickening, and reddening of the skin (irritation) at the site of application in groups administered 3.13 mg/kg or greater.

The absolute and relative thymus weights of 25 mg/kg males were significantly less than those of the controls (Table F2); the absolute and relative right kidney weights of 25 mg/kg females were significantly greater than those of the controls.

In groups of rats receiving 6.25 mg/kg or greater, histopathologic lesions of the skin varied from epithelial hyperplasia and inflammation of mild to moderate severity to necrotizing, ulcerative lesions penetrating the epidermis to involve the underlying dermis and subcutaneous tissues (Table 4). Similar lesions of lesser (minimal) severity were observed in 3.13 mg/kg males and females. In 1.56 mg/kg males and females, minimal epithelial hyperplasia and chronic inflammation of the epidermis occurred in up to half of the animals.

In the bone marrow, hypercellularity of the myeloid fraction was noted in 25 mg/kg male and female rats. This observation was considered a secondary response, indicative of the extent and duration of the inflammation at 25 mg/kg, and was not evaluated at lower doses.

TABLE 3
Survival and Body Weights of Rats in the 13-Week Dermal Study of Benzethonium Chloride

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	177 ± 6	320 ± 6	143 ± 3	
1.56	10/10	178 ± 6	319 ± 6	141 ± 5	100
3.13	10/10	180 ± 5	327 ± 6	147 ± 4	102
6.25	10/10	176 ± 5	315 ± 6	139 ± 5	98
12.5	10/10	177 ± 5	312 ± 4	135 ± 4	97
25	10/10	176 ± 5	288 ± 6**	112 ± 3**	90
Female					
0	10/10	119 ± 2	180 ± 3	61 ± 3	
1.56	10/10	121 ± 2	181 ± 3	59 ± 2	100
3.13	10/10	119 ± 2	176 ± 2	57 ± 1	98
6.25	10/10	121 ± 2	177 ± 3	56 ± 2	98
12.5	10/10	120 ± 2	177 ± 3	57 ± 2	98
25	10/10	120 ± 2	176 ± 3	56 ± 2	98

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

TABLE 4
Incidences of Nonneoplastic Lesions of the Skin in Rats in the 13-Week Dermal Study of Benzethonium Chloride

Dose	Vehicle Control	1.56 mg/kg	3.13 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg
Male						
Skin (Site of Application)[a]	10	10	10	10	10	10
Epithelial Hyperplasia[b]	0	4* (1.0)[c]	9** (1.1)	10** (1.7)	10** (2.6)	10** (3.0)
Inflammation, Chronic	0	2 (1.0)	7** (1.1)	9** (1.7)	10** (2.5)	10** (3.5)
Necrosis	0	0	1 (1.0)	2 (1.0)	6** (1.2)	9** (1.9)
Ulceration	0	0	2 (1.0)	4* (1.5)	8** (1.9)	10** (2.5)
Female						
Skin (Site of Application)	10	10	10	9	10	10
Epithelial Hyperplasia	0	5* (1.0)	9** (1.4)	9** (1.6)	10** (1.9)	10** (3.0)
Inflammation, Chronic	0	4* (1.0)	10** (1.5)	7** (1.6)	10** (1.7)	10** (3.2)
Necrosis	0	0	1 (1.0)	3 (1.3)	5* (1.0)	8** (1.4)
Ulceration	0	0	5* (1.8)	3 (1.7)	1 (1.0)	10** (2.0)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test.
** $P \leq 0.01$
[a] Number of animals with skin examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals (1=minimal; 2=mild; 3=moderate; 4=marked)

Dose Selection Rationale: The frequency of epithelial hyperplasia, chronic inflammation, necrosis, and/or ulcerations in males and females administered 3.13 mg/kg or greater, combined with the apparent progression of skin lesions (rather than adaptation) between the 16-day and 13-week studies (more animals and lower dose groups were affected), suggested 3.13 mg/kg may be excessive for a chronic study. Thus, a high dose of 1.5 mg/kg was selected for the 2-year study in male and female rats. Concern over the potential for progression of the skin lesions led to a larger than typical dose spacing. The doses selected covered a tenfold range from 0.15 to 1.5 mg/kg per day.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 5 and in the Kaplan-Meier survival curves (Figure 1). Survival rates of all dosed groups of males and females were similar to those of the controls.

Body Weights and Clinical Findings

Mean body weights of all dosed groups of males and females were similar to those of the controls throughout the study (Figure 2, Tables 6 and 7), and the final mean body weights of all dosed groups of males and females were also similar to those of the controls. There were no significant differences in liver or kidney weights in males or females evaluated at 15 months (Table F3). Reddening of the skin was observed at the site of application in all dosed groups of males and females; crusts were observed in 0.5 mg/kg males and in 1.5 mg/kg females.

TABLE 5
Survival of Rats in the 2-Year Dermal Study of Benzethonium Chloride

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Male				
Animals initially in study	60	60	60	60
15-Month interim evaluation[a]	8	8	5	4
Accidental death[a]	0	0	1	0
Moribund	25	34	34	25
Natural deaths	12	7	11	15
Animals surviving to study termination	15	11	9[e]	16
Percent probability of survival at end of study[b]	29	21	17	29
Mean survival (days)[c]	627	605	599	615
Survival analysis[d]	P=0.892N	P=0.286	P=0.129	P=0.802
Female				
Animals initially in study	60	60	60	60
15-Month interim evaluation[a]	9	7	9	7
Moribund	13	11	13	13
Natural deaths	14	9	12	16
Animals surviving to study termination	24	33	26	24
Percent probability of survival at end of study	47	63	51	46
Mean survival days	638	652	641	630
Survival analysis	P=0.256	P=0.232N	P=0.854N	P=0.728

[a] Censored from survival analyses
[b] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or a lower mortality in a dose group is indicated by N.
[e] Includes one animal that died during the last week of the study

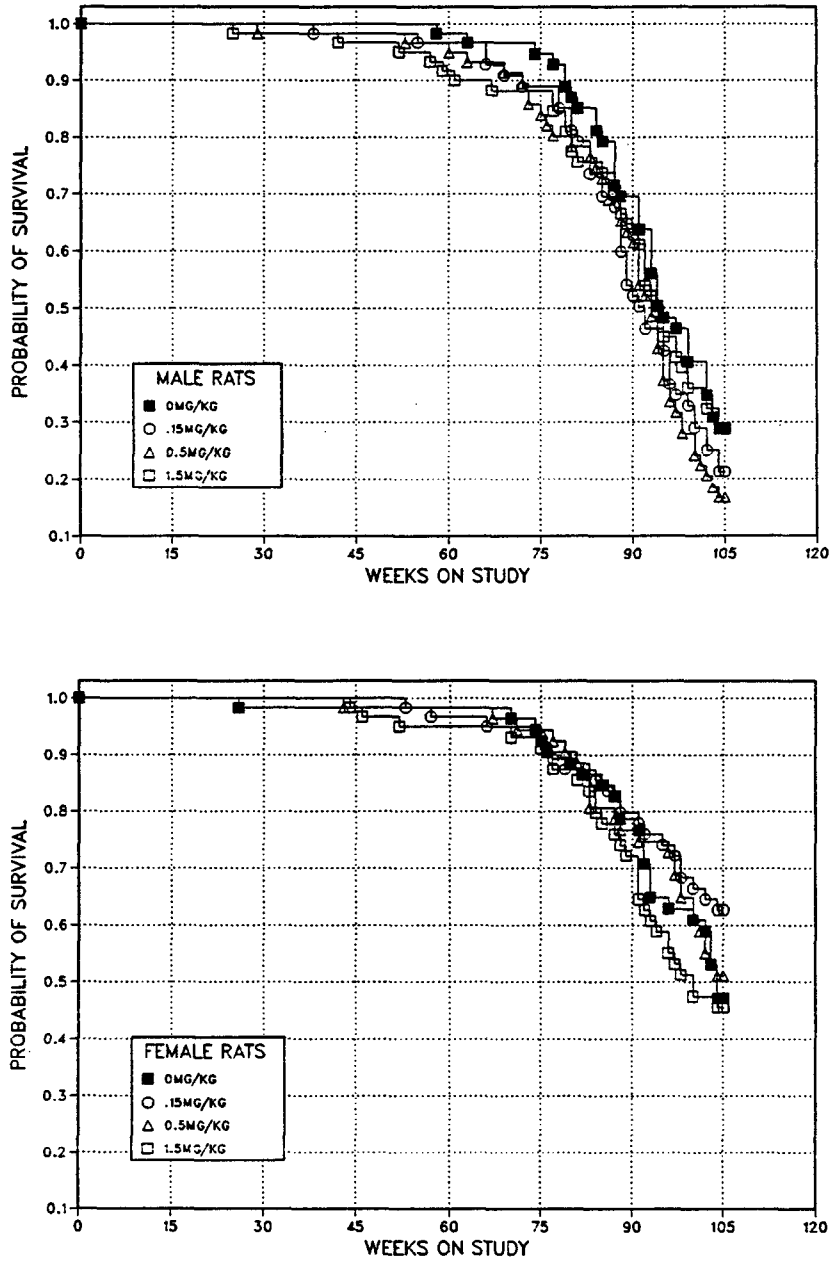


FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Administered Benzethonium Chloride Topically for 2 Years

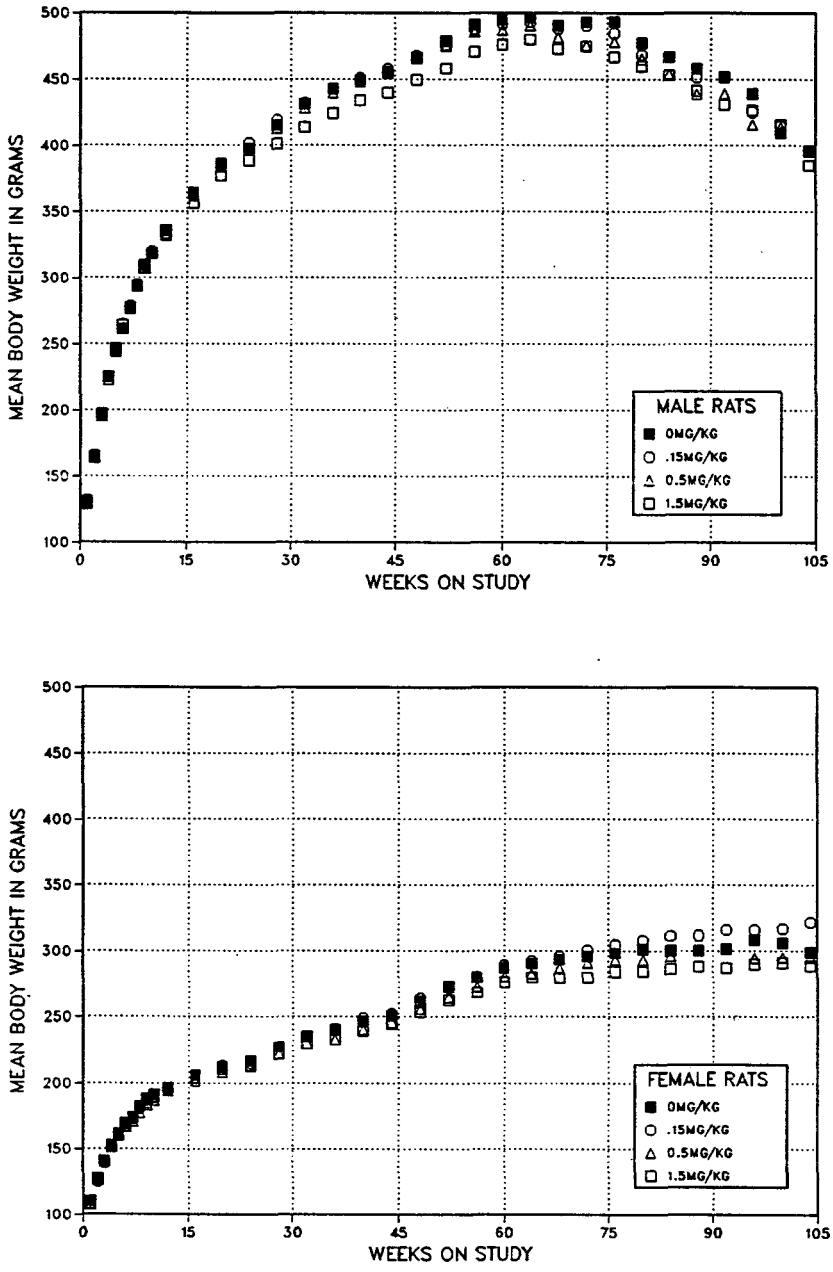


FIGURE 2
Growth Curves for Male and Female Rats Administered Benzethonium Chloride Topically for 2 Years

TABLE 6
Mean Body Weights and Survival of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride

Weeks on Study	Vehicle Control		0.15 mg/kg			0.5 mg/kg			1.5 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	131	60	132	101	60	129	98	60	130	100	60
2	165	60	166	100	60	164	99	60	165	100	60
3	197	60	197	100	60	196	99	60	197	100	60
4	225	60	225	100	60	223	99	60	225	100	60
5	245	60	246	100	60	244	100	59	247	101	60
6	261	60	265	101	60	262	100	59	264	101	60
7	278	60	279	100	60	277	100	59	277	100	60
8	294	60	295	100	60	294	100	59	293	100	60
9	309	60	310	100	60	308	100	59	307	99	60
10	318	60	320	101	60	318	100	59	318	100	60
12	336	60	336	100	60	333	99	59	334	99	60
16	364	60	363	100	60	362	99	59	357	98	60
20	386	60	387	100	60	384	99	59	377	98	60
24	398	60	402	101	60	398	100	59	389	98	60
28	415	60	419	101	60	413	100	59	401	97	59
32	431	60	432	100	60	428	99	58	414	96	59
36	443	60	443	100	60	440	99	58	424	96	59
40	449	60	451	100	59	448	100	58	434	97	59
44	456	60	458	100	59	455	100	58	440	96	58
48	466	60	467	100	59	466	100	58	450	96	58
52	479	60	475	99	59	475	99	58	458	96	58
56	491	60	487	99	58	486	99	57	471	96	57
60	495	59	492	99	58	487	98	57	476	96	55
64	497	58	493	99	58	490	99	55	480	97	54
68[a]	490	50	488	100	48	482	98	50	473	96	49
72	493	50	491	100	47	476	97	49	475	96	49
76	494	49	485	98	46	478	97	44	467	95	49
80	478	46	468	98	44	465	98	43	460	96	45
84	467	44	466	100	38	454	97	41	453	97	42
88	458	37	451	99	35	439	96	37	441	96	39
92	452	33	452	100	25	439	97	29	431	95	34
96	439	25	425	97	22	416	95	20	427	97	25
100	410	21	415	101	17	414	101	15	416	102	20
104	396	16	396	100	13	396	100	10	385	97	18
Mean for weeks											
1-13	251		252	100		250	100		251	100	
14-52	429		430	100		427	100		414	97	
53-104	466		462	99		456	98		450	97	

[a] Interim evaluation occurred during week 66.

TABLE 7
Mean Body Weights and Survival of Female Rats in the 2-Year Dermal Study of Benzethonium Chloride

Weeks on Study	Vehicle Control Av. Wt. (g)	Vehicle Control No. of Survivors	0.15 mg/kg Av. Wt. (g)	0.15 mg/kg Wt. (% of controls)	0.15 mg/kg No. of Survivors	0.5 mg/kg Av. Wt. (g)	0.5 mg/kg Wt. (% of controls)	0.5 mg/kg No. of Survivors	1.5 mg/kg Av. Wt. (g)	1.5 mg/kg Wt. (% of controls)	1.5 mg/kg No. of Survivors
1	110	60	108	98	60	109	99	60	108	98	60
2	127	60	125	98	60	126	99	60	127	100	60
3	141	60	139	98	60	140	99	60	141	100	60
4	152	60	152	100	60	151	99	60	153	100	60
5	162	60	161	100	60	160	99	60	162	100	60
6	170	60	167	99	60	167	98	60	169	100	60
7	175	60	173	99	60	171	98	60	174	100	60
8	183	60	181	99	60	178	97	60	182	100	60
9	188	60	185	98	60	184	98	60	187	99	60
10	191	60	189	99	60	187	98	60	190	99	60
12	197	60	195	99	60	194	99	60	196	100	60
16	206	60	204	99	60	202	98	60	204	99	60
20	212	60	213	100	60	209	98	60	211	99	60
24	217	60	216	99	60	213	98	60	214	99	60
28	228	59	228	100	60	223	98	60	223	98	60
32	236	59	236	100	60	230	98	60	230	98	60
36	241	59	241	100	60	233	97	60	233	97	60
40	247	59	249	101	60	240	97	60	240	97	60
44	251	59	253	101	60	246	98	59	245	98	59
48	262	59	264	101	60	256	98	59	254	97	58
52	273	59	272	100	60	265	97	59	263	96	57
56	280	59	280	100	59	273	97	59	268	96	57
60	287	59	289	101	58	281	98	59	276	96	57
64	291	59	292	101	58	283	97	59	280	96	57
68[a]	294	50	296	101	50	287	98	49	280	95	50
72	296	49	301	102	50	291	98	48	280	95	49
76	299	46	305	102	49	293	98	48	284	95	48
80	302	45	308	102	46	293	97	46	285	94	46
84	300	44	311	104	45	296	99	41	286	95	44
88	301	41	312	104	44	300	100	39	288	96	40
92	302	39	316	105	40	302	100	38	287	95	34
96	309	33	316	103	39	295	96	38	290	94	30
100	306	32	317	104	36	295	96	32	291	95	27
104	299	26	322	108	34	295	99	27	289	97	25
Mean for weeks											
1-13	163		161	99		161	99		163	100	
14-52	237		238	100		232	98		232	98	
53-104	297		305	103		291	98		283	95	

[a] Interim evaluation occurred during week 66.

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions in the skin. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for male rats and Appendix B for female rats.

No neoplasms observed in male or female rats were considered to be chemical related. In particular, no skin-associated neoplasms were attributed to treatment with benzethonium chloride. Two incidences of keratoacanthoma and one of sebaceous gland carcinoma were observed in treated males (Table 8). These neoplasms were consistent with the spectrum of neoplasms found in adjacent control skin of treated and untreated animals (Table A1). No neoplasms were found in females at the site of application (Table B1).

Treatment-related nonneoplastic lesions were observed at the site of application and varied from minimal evidence of epithelial hyperplasia with or without evidence of sebaceous gland hyperplasia to more advanced cases of mild to moderate epithelial hyperplasia accompanied by evidence of ulceration. Sebaceous gland hyperplasia was commonly observed in 1.5 mg/kg females and to a lesser extent in 0.5 mg/kg females at both the 15-month interim evaluation and the end of the 2-year study. With rare exceptions, sebaceous gland hyperplasia was associated with cases of epithelial hyperplasia and generally was a coexistent finding with the more severely affected cases of epithelial hyperplasia involving inflammation and/or ulceration (Plates 1 and 2). Microscopic evidence of epidermal ulceration was observed in one 1.5 mg/kg male and in four 1.5 mg/kg females at the 15-month interim evaluation. While this lesion was not observed in males at the end of the study, epidermal ulceration was noted in females, particularly at 1.5 mg/kg (Table 8).

TABLE 8
Incidences of Skin Neoplasms and Nonneoplastic Lesions in Rats in the 2-Year Dermal Study of Benzethonium Chloride

Dose	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Male				
15-Month Interim Evaluation				
Skin (Site of Application)[a]	8	8	5	4
Epithelial Hyperplasia[b,c]	0	0	3* (1.3)[d]	4** (2.3)
Sebaceous Gland, Hyperplasia	0	0	2 (2.0)	2 (1.5)
Ulceration	0	0	0	1 (1.0)
2-Year Study				
Skin (Site of Application)	52	52	55	56
Epithelial Hyperplasia	1 (1.0)	0	4 (1.0)	12** (1.6)
Sebaceous Gland, Hyperplasia	0	0	2 (1.5)	2 (1.5)
Ulceration	0	0	0	0
Keratoacanthoma[e]	0	0	1	1
Sebaceous Gland, Carcinoma	0	0	1	0
Female				
15-Month Interim Evaluation				
Skin (Site of Application)	9	7	9	7
Epithelial Hyperplasia	0	1 (3.0)	2 (2.0)	6** (2.7)
Sebaceous Gland, Hyperplasia	0	1 (2.0)	1 (2.0)	6** (2.0)
Ulceration	0	1 (2.0)	1 (2.0)	4* (2.3)
2-Year Study				
Skin (Site of Application)	51	53	51	53
Epithelial Hyperplasia	2 (2.0)	2 (3.5)	6 (3.0)	32** (2.7)
Sebaceous Gland, Hyperplasia	1 (1.0)	2 (2.5)	6 (1.7)	30** (1.9)
Ulceration	0	1 (3.0)	3 (3.3)	19** (1.8)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test (15-month interim evaluation) or the logistic regression test (2-year study).
** $P \leq 0.01$
[a] Number of animals with skin examined microscopically
[b] Number of animals with lesion
[c] Epithelial hyperplasia was originally diagnosed as acanthosis and/or hyperkeratosis.
[d] Average severity grade of lesions in affected animals (1=minimal; 2=mild; 3=moderate; 4=marked)
[e] All neoplasms found at the site of application are included for completeness. The incidences of neoplasms were not statistically significant, nor were any neoplasms considered to be related to chemical treatment.

MICE

16-DAY STUDY

One 100 mg/kg male mouse died on day 4 of the study; all other mice survived to the end of the study (Table 9). The mean body weight gains of males administered 25, 50, or 100 mg benzethonium chloride/kg body weight were significantly greater than that of the controls; final mean body weights of all groups of dosed males and females were similar to those of the controls. Mild crusting, scaling, and reddening of the skin at the site of application were observed in 50 and 100 mg/kg male and female groups and in 25 mg/kg males.

The absolute heart weights of 100 mg/kg males and females and the relative heart weight of 100 mg/kg females were significantly greater than those of the controls (Table F4). The absolute and relative thymus weights of 100 mg/kg females were significantly decreased.

Necrotizing inflammation of the epidermis (moderate to marked in males, and mild to moderate in females) with inflammatory involvement of the underlying dermis and subcutaneous tissues was a consistent feature in 100 mg/kg male and female mice. Although of lesser severity (minimal to mild), this spectrum of lesions of the dermis and epidermis was also observed in three 50 mg/kg males and two 50 mg/kg females. The predominant lesion in 12.5 and 25 mg/kg males and females and in 6.3 mg/kg females consisted of minimal to mild epithelial hyperplasia with minimal evidence of inflammatory involvement of the epidermis extending into the dermis in some animals. Minimal epithelial hyperplasia without evidence of inflammation was observed in 6.25 mg/kg male mice. Histopathologic lesions of the skin were not observed in control males; however, minimal epithelial hyperplasia was noted in two control females.

TABLE 9
Survival and Body Weights of Mice in the 16-Day Dermal Study of Benzethonium Chloride

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	23.9 ± 0.7	25.1 ± 0.4	1.2 ± 0.7	
6.3	5/5	24.1 ± 0.6	25.3 ± 0.6	1.2 ± 0.1	101
12.5	5/5	23.9 ± 0.5	25.9 ± 0.4	1.9 ± 0.2	103
25	5/5	23.4 ± 0.7	26.5 ± 0.5	3.0 ± 0.4*	105
50	5/5	23.9 ± 0.7	26.4 ± 0.7	2.5 ± 0.2*	105
100	4/5[c]	23.7 ± 0.7	26.5 ± 0.5	2.4 ± 0.2*	105
Female					
0	5/5	18.0 ± 0.3	21.2 ± 0.5	3.2 ± 0.2	
6.3	5/5	18.0 ± 0.2	21.6 ± 0.4	3.5 ± 0.3	102
12.5	5/5	18.0 ± 0.3	21.0 ± 0.4	3.1 ± 0.4	99
25	5/5	18.1 ± 0.6	21.3 ± 0.3	3.2 ± 0.4	101
50	5/5	17.8 ± 0.3	21.1 ± 0.3	3.3 ± 0.0	99
100	5/5	17.8 ± 0.3	20.9 ± 0.5	3.1 ± 0.3	99

* Significantly different ($P \leq 0.05$) from the control group by Williams' or 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.
[c] Day of death: 4

Dose Selection Rationale: Because of the severity of the skin lesions in mice administered 50 mg/kg or greater, 25 mg/kg was selected as the high dose for the 13-week study in male and female mice.

13-WEEK STUDY

All mice survived to the end of the study (Table 10). The final mean body weights of all dosed groups of males and females were similar to those of the controls; the mean weight gain of 25 mg/kg males was significantly less than that of the controls. Males administered 6.25, 12.5, or 25 mg benzethonium chloride/kg body weight developed crusting, thickening of the skin, scales, and reddening of the skin at the site of application, as did female mice administered 12.5 or 25 mg/kg.

The absolute and relative right kidney and liver weights of 12.5 and 25 mg/kg males were slightly greater than those of the controls (Table F5).

Histopathologic lesions of the skin at the site of application varied from a minimal epithelial hyperplasia with chronic inflammation to mild epithelial hyperplasia with chronic inflammation of the epidermis and focal necrosis of the epithelium and involvement of the underlying dermis and subcutaneous tissues (Table 11). Chronic inflammation and necrosis of the epidermis of minimal to mild severity was observed in 25 mg/kg males. Necrosis was a feature of the spectrum of lesions observed in one 6.25 mg/kg male and in two 25 mg/kg females. All dosed groups of male and female mice exhibited evidence of chemical treatment; epithelial hyperplasia (with or without inflammation) was observed in nine males and nine females administered 1.56 mg/kg.

TABLE 10
Survival and Body Weights of Mice in the 13-Week Dermal Study of Benzethonium Chloride

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	23.9 ± 0.6	32.7 ± 1.1	8.9 ± 0.8	
1.56	10/10	23.8 ± 0.5	31.9 ± 0.8	8.1 ± 0.7	97
3.13	10/10	23.7 ± 0.5	32.2 ± 1.0	8.5 ± 0.6	98
6.25	10/10	23.8 ± 0.3	31.8 ± 0.5	7.9 ± 0.5	97
12.5	10/10	23.6 ± 0.4	31.3 ± 0.9	7.7 ± 0.5	96
25	10/10	24.0 ± 0.5	30.6 ± 0.7	6.6 ± 0.5*	93
Female					
0	10/10	18.8 ± 0.3	26.2 ± 0.8	7.4 ± 0.7	
1.56	10/10	18.7 ± 0.2	26.4 ± 0.6	7.7 ± 0.5	101
3.13	10/10	19.4 ± 0.4	27.0 ± 0.6	7.6 ± 0.4	103
6.25	10/10	18.9 ± 0.2	26.8 ± 0.6	7.9 ± 0.6	102
12.5	10/10	19.4 ± 0.4	25.8 ± 0.6	6.4 ± 0.4	98
25	10/10	19.4 ± 0.5	26.4 ± 0.9	7.0 ± 0.5	101

* Significantly different ($P \leq 0.05$) from the control group by Williams' or 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.

TABLE 11
Incidences of Nonneoplastic Lesions of the Skin in Mice in the 13-Week Dermal Study of Benzethonium Chloride

Dose	Vehicle Control	1.56 mg/kg	3.12 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg
Male						
Skin (Site of Application)[a]	10	10	10	10	10	10
Epithelial Hyperplasia[b]	0	9** (1.0)[c]	8** (1.0)	9** (1.1)	9** (1.6)	10** (2.2)
Inflammation, Chronic	0	2 (1.0)	3 (1.0)	6** (1.0)	9** (1.2)	10** (2.0)
Necrosis	0	0	0	1 (1.0)	1 (1.0)	5* (1.6)
Female						
Skin (Site of Application)	10	10	10	10	10	10
Epithelial Hyperplasia	0	9** (1.0)	10** (1.0)	10** (1.0)	10** (1.5)	10** (2.0)
Inflammation, Chronic	1 (1.0)	6* (1.0)	8** (1.0)	8** (1.1)	10** (1.6)	10** (1.9)
Necrosis	0	0	0	0	0	2 (1.0)

* Significantly different (P≤0.05) from the control group by the Fisher exact test.
** P≤0.01
[a] Number of animals with skin examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals (1=minimal; 2=mild; 3=moderate; 4=marked)

Dose Selection Rationale: Although the skin lesions observed in mice were not as severe as those observed in the 13-week rat study, there was concern over the frequency of inflammatory involvement in male and particularly female mice at the lowest dose tested, 1.56 mg/kg. Inflammation at the site of application and evidence of progression of lesions between the 16-day and 13-week studies (e.g., necrosis and inflammation observed in male and female mice in the lower dose groups) led to the selection of a high dose of 1.5 mg/kg for the 2-year study.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 12 and in the Kaplan-Meier survival curves (Figure 3). Survival rates of all dosed groups of males and females were similar to those of the controls.

Body Weights and Clinical Findings

Mean body weights of all dosed groups of males and females were similar to those of the controls during the study (Figure 4, Tables 13 and 14). Final mean body weights of all dosed male and female groups were also similar to those of the controls. There were no marked changes in liver or kidney weights of dosed mice at the 15-month interim evaluation (Table F6). Reddening of the skin was observed at the site of application in all dosed groups of males and in 0.15 mg/kg females; crusts were observed in 0.5 mg/kg females.

TABLE 12
Survival of Mice in the 2-Year Dermal Study of Benzethonium Chloride

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Male				
Animals initially in study	60	60	60	60
15-Month interim evaluation[a]	10	9	9	10
Missexed[a]	0	1	1	0
Moribund	3	8	4	9
Natural deaths	4	4	4	2
Animals surviving to study termination	43	38	42	39
Percent probability of survival at end of study[b]	86	76	84	78
Mean survival (days)[c]	674	675	678	672
Survival analysis[d]	P=0.671	P=0.320	P=0.990	P=0.449
Female				
Animals initially in study	60	60	60	60
15-Month interim evaluation[a]	8	7	10	6
Accidental death[a]	1	0	0	0
Missexed[a]	0	0	2	0
Moribund	10	4	8	13
Natural deaths	3	15	9	7
Animals surviving to study termination	38	34	31	34
Percent probability of survival at end of study	75	65	65	64
Mean survival days	669	640	660	646
Survival analysis	P=0.406	P=0.222	P=0.373	P=0.226

[a] Censored from survival analyses
[b] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns.

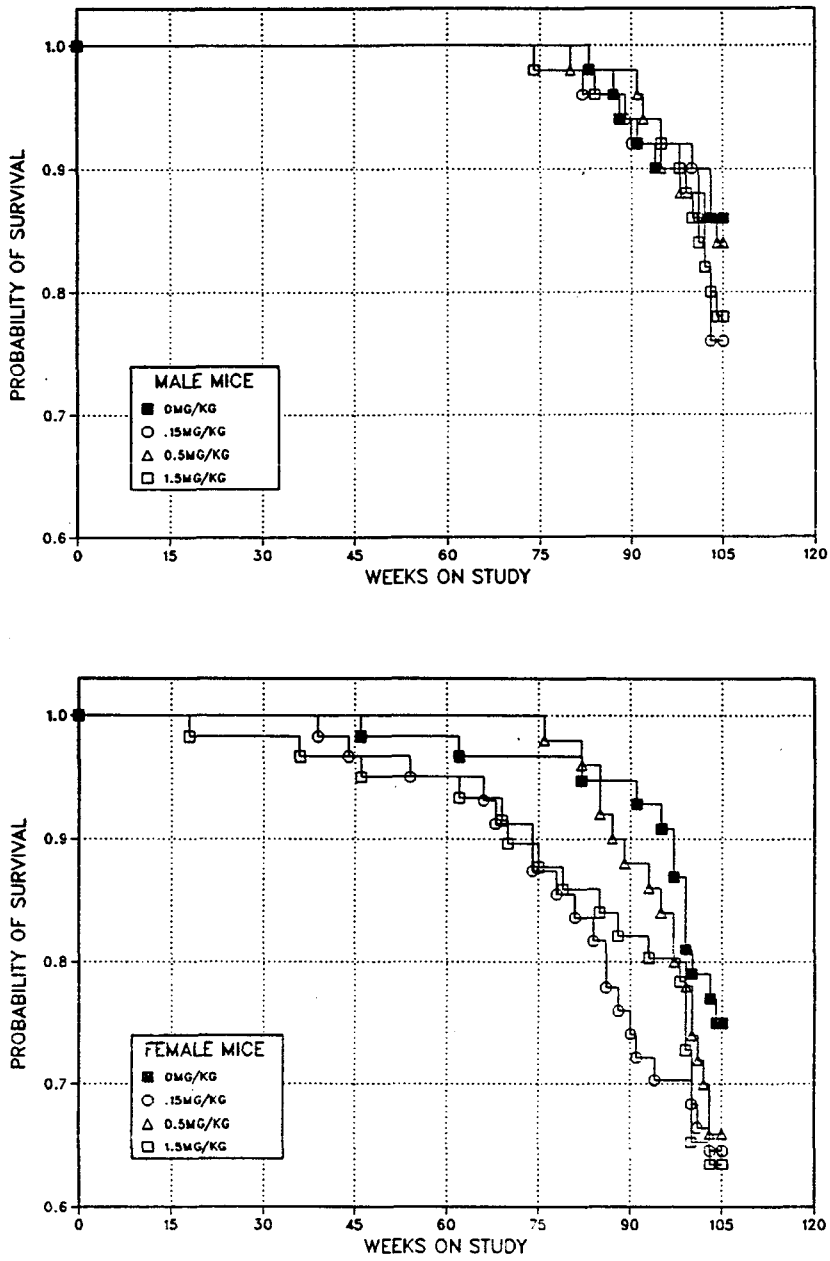


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Mice Administered Benzethonium Chloride Topically for 2 Years

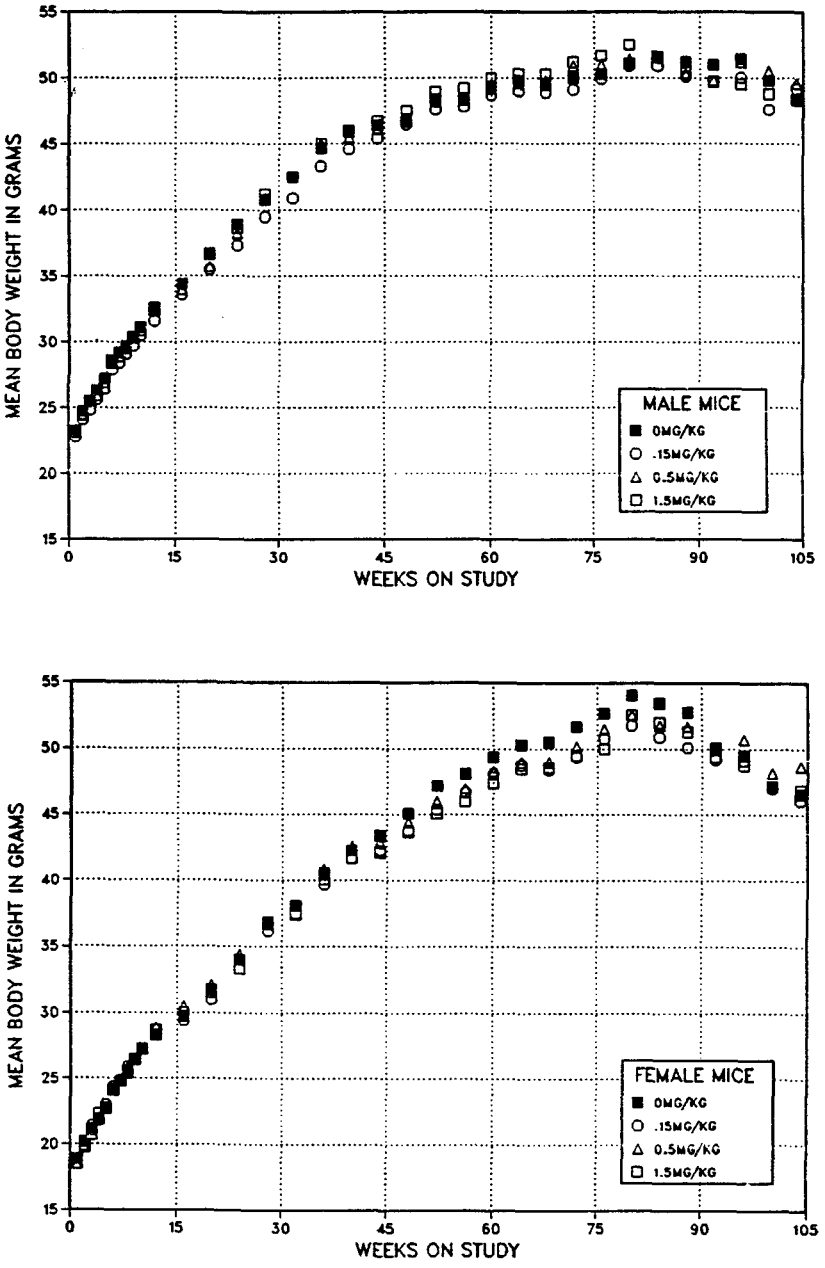


FIGURE 4
Growth Curves for Male and Female Mice Administered Benzethonium Chloride Topically for 2 Years

TABLE 13
Mean Body Weights and Survival of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride

Weeks on Study	Vehicle Control		0.15 mg/kg			0.5 mg/kg			1.5 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.1	60	22.8	99	60	23.3	101	60	23.2	100	60
2	24.7	60	24.1	98	60	24.5	99	60	24.4	99	60
3	25.5	60	24.8	97	60	25.5	100	60	25.5	100	60
4	26.3	60	25.6	97	60	25.9	99	60	25.9	99	60
5	27.2	60	26.4	97	60	26.9	99	60	26.9	99	60
6	28.4	60	27.9	98	60	28.5	100	60	28.6	101	60
7	29.2	60	28.4	97	60	28.9	99	60	28.9	99	60
8	29.6	60	29.1	98	60	29.5	100	60	29.7	100	60
9	30.3	60	29.7	98	60	30.3	100	60	30.4	100	60
10	31.1	60	30.5	98	60	31.1	100	60	30.9	99	60
12	32.4	60	31.6	98	60	32.5	100	60	32.6	101	60
16	34.4	60	33.6	98	60	34.0	99	60	34.4	100	60
20	36.6	60	35.5	97	60	35.7	98	60	36.7	100	60
24	38.9	60	37.3	96	60	38.2	98	60	38.6	99	60
28	40.8	60	39.5	97	60	40.9	100	60	41.2	101	60
32	42.5	60	40.9	96	60	42.5	100	60	42.5	100	60
36	44.7	60	43.3	97	60	45.0	101	60	45.0	101	60
40	46.0	60	44.6	97	60	45.5	99	60	46.0	100	60
44	46.4	60	45.4	98	60	46.1	99	60	46.7	101	60
48	46.9	60	46.5	99	60	46.7	100	60	47.5	101	60
52	48.4	60	47.6	98	60	48.2	100	60	48.9	101	60
56	48.4	60	47.8	99	60	48.3	100	60	49.2	102	60
60	49.4	60	48.7	99	60	49.2	100	60	50.0	101	60
64	49.8	60	49.0	98	60	49.6	100	60	50.3	101	60
68[a]	49.5	50	49.0	99	51	49.8	101	51	50.3	102	50
72	50.0	50	49.1	98	51	50.9	102	51	51.2	102	50
76	50.3	50	49.9	99	50	51.0	101	51	51.7	103	49
80	51.1	50	50.9	100	50	51.4	101	51	52.5	103	49
84	51.5	49	50.9	99	49	51.5	100	50	51.6	100	49
88	51.2	48	50.1	98	49	50.3	98	50	50.4	98	48
92	51.0	46	49.7	98	47	49.8	98	48	49.7	98	47
96	51.4	45	50.0	97	47	51.2	100	46	49.5	96	46
100	49.8	45	47.6	96	47	50.5	101	45	48.8	98	43
104	48.3	43	49.2	102	39	49.6	103	44	48.5	100	40
Mean for weeks											
1-13	28.0		27.4	98		27.9	100		27.9	100	
14-52	42.6		41.4	97		42.3	99		42.8	100	
53-104	50.1		49.4	99		50.2	100		50.3	100	

[a] Interim evaluation occurred during week 66.

TABLE 14
Mean Body Weights and Survival of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride

Weeks on Study	Vehicle Control Av. Wt. (g)	Vehicle Control No. of Survivors	0.15 mg/kg Av. Wt. (g)	0.15 mg/kg Wt. (% of controls)	0.15 mg/kg No. of Survivors	0.5 mg/kg Av. Wt. (g)	0.5 mg/kg Wt. (% of controls)	0.5 mg/kg No. of Survivors	1.5 mg/kg Av. Wt. (g)	1.5 mg/kg Wt. (% of controls)	1.5 mg/kg No. of Survivors
1	18.9	60	18.5	98	60	18.5	98	60	18.5	98	60
2	20.2	60	19.9	99	60	19.8	98	60	19.9	99	60
3	21.2	60	21.4	101	60	21.0	99	60	20.7	98	60
4	21.9	60	21.8	100	60	22.0	101	60	22.3	102	60
5	22.8	60	23.0	101	60	22.7	100	60	22.8	100	60
6	24.1	60	24.4	101	60	24.1	100	60	24.2	100	60
7	24.8	60	24.9	100	60	24.9	100	60	24.8	100	60
8	25.4	60	25.9	102	60	25.9	102	60	25.6	101	60
9	26.5	60	26.4	100	60	26.4	100	60	26.4	100	60
10	27.2	60	27.3	100	60	27.3	100	60	27.3	100	60
12	28.3	60	28.7	101	60	28.9	102	60	28.7	101	60
16	29.7	60	29.4	99	60	30.5	103	60	30.0	101	60
20	31.7	60	31.0	98	60	32.1	101	60	31.5	99	59
24	34.0	60	34.0	100	60	34.4	101	60	33.3	98	59
28	36.9	60	36.2	98	60	36.9	100	60	36.7	100	59
32	38.1	60	37.5	98	60	38.2	100	60	37.4	98	59
36	40.6	60	39.7	98	60	40.9	101	60	40.1	99	59
40	42.3	60	41.7	99	59	42.6	101	60	41.7	99	58
44	43.4	60	42.3	98	59	42.9	99	60	42.1	97	58
48	45.1	59	43.8	97	58	44.4	98	60	43.7	97	57
52	47.2	59	45.4	96	58	46.0	98	60	45.1	96	57
56	48.1	59	46.7	97	57	47.0	98	60	46.0	96	57
60	49.4	59	48.1	97	57	48.3	98	60	47.4	96	57
64	50.3	58	48.8	97	57	49.0	97	60	48.5	96	56
68[a]	50.5	50	48.4	96	48	49.0	97	50	48.6	96	50
72	51.7	50	49.4	96	48	50.2	97	50	49.5	96	48
76	52.7	50	50.8	96	46	51.5	98	49	50.0	95	47
80	54.1	50	51.8	96	45	52.6	97	49	52.6	97	46
84	53.5	49	50.9	95	44	51.8	97	48	52.0	97	46
88	52.8	49	50.1	95	41	51.7	98	45	51.3	97	45
92	50.1	47	49.2	98	38	50.1	100	44	49.4	99	44
96	49.5	46	49.1	99	37	50.7	102	42	48.8	99	43
100	47.2	41	47.0	100	37	48.2	102	39	47.2	100	38
104	46.6	38	46.1	99	34	48.7	105	33	46.9	101	34
Mean for weeks											
1-13	23.8		23.8	100		23.8	100		23.7	100	
14-52	38.9		38.1	98		38.9	100		38.2	98	
53-104	50.5		49.0	97		49.9	99		49.1	97	

[a] Interim evaluation occurred during week 66.

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions in the skin. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix C for male mice and Appendix D for female mice.

No neoplasms were observed that were considered related to chemical treatment, including no significant increases in incidences of neoplasms at the site of application. Two subcutaneous neoplasms were found in control females at the site of application: a hemangioma and a sarcoma. In addition, one subcutaneous sarcoma was found in a 1.5 mg/kg female (Tables 15 and D1). No neoplasms were found in male mice at the site of application (Table C1), and the spectrum of neoplasms found in control skin of treated male and female mice was consistent with similar neoplasms found in vehicle controls.

The predominant treatment-related histopathologic nonneoplastic lesion observed at the site of application was epithelial hyperplasia of minimal to mild severity (Tables 15, C4, and D4). Epithelial hyperplasia was commonly observed in 1.5 mg/kg male and female mice at the 15-month interim evaluation. Similarly at the end of the study, a dose-response increase in the incidence of epithelial hyperplasia was observed in male and female mice (Plates 3 and 4). Unlike the female rat, the progression of the skin lesion with development of ulceration was not observed in male or female mice in this study.

GENETIC TOXICOLOGY

Benzethonium chloride (0.01 to 100 μg/plate) was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 (Table E1). All tests were performed with a preincubation protocol, with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9. In cytogenetic tests with cultured Chinese hamster ovary cells, benzethonium chloride did not induce sister chromatid exchanges (Table E2) or chromosomal aberrations (Table E3) with or without S9. Although increases in chromosomal aberrations were observed in each of the two trials conducted, the increases were not statistically significant or dose related. No cell cycle delay was observed in either cytogenetic test.

TABLE 15
Incidences of Skin Neoplasms and Nonneoplastic Lesions in Mice in the 2-Year Dermal Study of Benzethonium Chloride

Dose	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Male				
15-Month Interim Evaluation				
Skin (Site of Application)[a]	10	9	9	10
Epithelial Hyperplasia[b,c]	0	0	2 (1.0)[d]	10** (1.2)
2-Year Study				
Skin (Site of Application)	50	50	50	50
Epithelial Hyperplasia	2 (1.5)	7 (1.1)	16** (1.6)	23** (1.2)
Inflammation, Chronic	0	0	0	2 (1.5)
Sebaceous Gland, Hyperplasia	0	0	1 (1.0)	0
Ulceration	1 (2.0)	1 (2.0)	4 (2.5)	2 (2.0)
Female				
15-Month Interim Evaluation				
Skin (Site of Application)	8	7	10	6
Epithelial Hyperplasia	0	0	3 (1.0)	4* (1.3)
2-Year Study				
Skin (Site of Application)	52	52	48	53
Epithelial Hyperplasia	3 (1.3)	7 (1.3)	6 (2.2)	22** (1.3)
Inflammation, Chronic	1 (1.0)	2 (1.0)	0	0
Sebaceous Gland, Hyperplasia	0	0	1 (3.0)	0
Ulceration	0	0	2 (3.0)	0
Subcutaneous Tissue, Hemangioma[e]	1	0	0	0
Subcutaneous Tissue, Sarcoma	1	0	0	1

* Significantly different (P≤0.05) from the control group by the Fisher exact test (15-month interim evaluation) or the logistic regression test (2-year study).
** P≤0.01
[a] Number of animals with skin examined microscopically
[b] Number of animals with lesion
[c] Epithelial hyperplasia was originally diagnosed as acanthosis and/or hyperkeratosis.
[d] Average severity grade of lesions in affected animals (1=minimal; 2=mild; 3=moderate; 4=marked)
[e] All neoplasms found at the site of application are included for completeness. The incidences of neoplasms were not statistically significant, nor were any neoplasms considered to be related to chemical treatment.

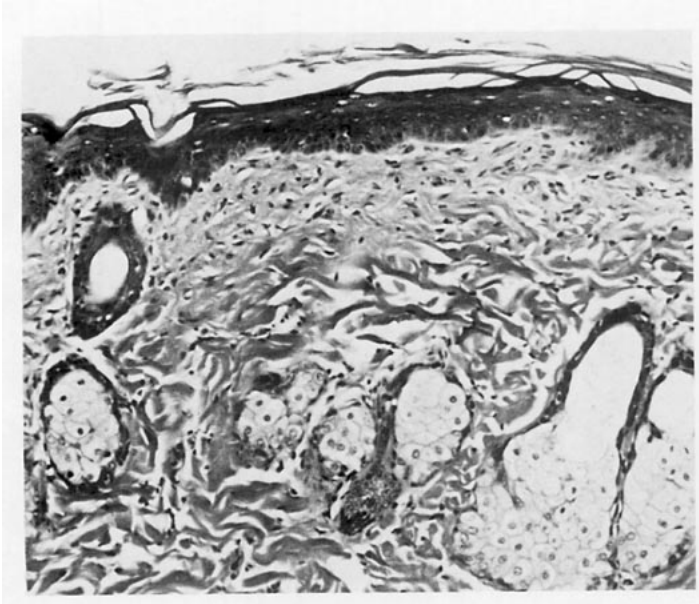


PLATE 1
Skin of a female F344/N rat administered 1.5 mg/kg benzethonium chloride for 2 years by dermal application. Note the mild to moderate degree of epithelial hyperplasia and sebaceous gland hyperplasia compared to the vehicle control in Plate 2. H&E; 40×

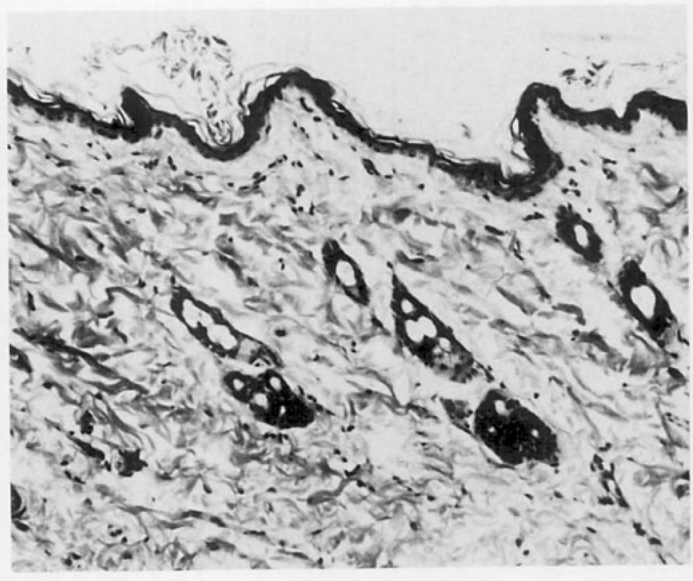


PLATE 2
Skin of a vehicle control (ethanol) female F344/N rat for comparison with skin of a treated rat shown in Plate 1. H&E; 40×

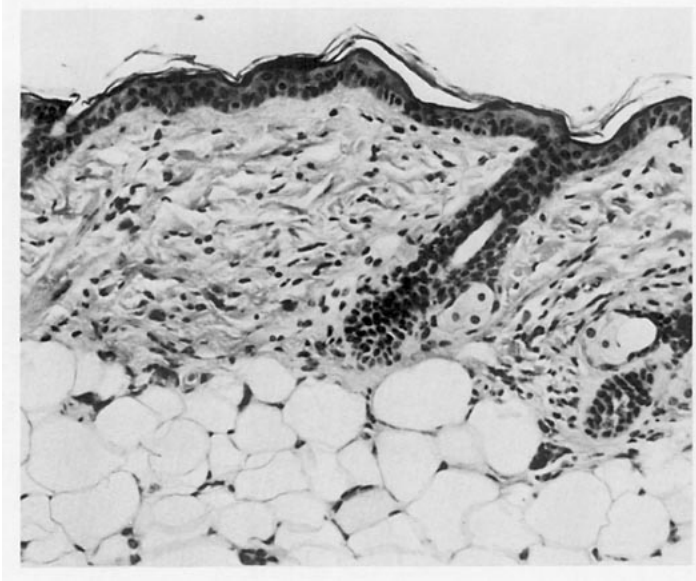


PLATE 3
Skin of a female B6C3F$_1$ mouse administered 1.5 mg/kg benzethonium chloride for 2 years by dermal application. Note the minimal degree of epithelial hyperplasia compared to vehicle control in Plate 4. H&E; 50×

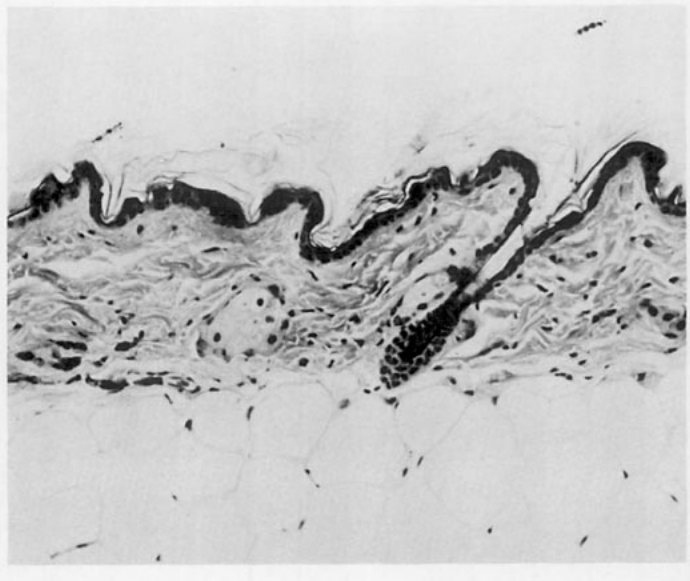


PLATE 4
Skin of a vehicle control (ethanol) female B6C3F$_1$ mouse for comparison with skin of a treated mouse shown in Plate 3. H&E; 50×

DISCUSSION AND CONCLUSIONS

The toxicity and carcinogenicity studies described in this report were performed because benzethonium chloride is found in a variety of over-the-counter products resulting in widespread human exposure and because of a report of possible carcinogenicity of benzethonium chloride in mice receiving subcutaneous injections of the chemical (Mason *et al.*, 1971). Quaternary ammonium compounds such as benzethonium chloride have antimicrobial properties (CIR, 1982), and some have cholinergic agonist or antagonist actions (Hume and Holland, 1965; Strycker and Long, 1969).

Although these studies were performed using dermal exposure, the results of absorption and disposition studies (NTP, 1988) suggested uptake of at least half of 0.15 to 1.5 mg/kg [^{14}C]-labeled doses of benzethonium chloride from the skin when applied under a nonocclusive patch. Thus, unless significant chemical breakdown occurs on the skin, systemic effects of a toxic or pharmacologic nature would be expected to be demonstrated in these studies.

In the 16-day studies, there were no clinical signs suggestive of systemic cholinergic effects; however, one male mouse receiving 100 mg/kg died after 4 days. There is no reported LD_{50} in mice for dermally applied benzethonium chloride. Homberger (1968) administered as much as 280 mg/kg in a single application to unshaved backs of C57BL/6 male mice without lethal effects. The intravenous LD_{50} for mice is reported to be from 20 to 35 mg/kg (CIR, 1982), so it is possible that repeated dermal application of 100 mg/kg could account for the one observed death. Body weight gains of treated rats were decreased at the higher doses, and this was probably related to the rather severe skin lesions produced at these dose levels.

There were no deaths or evidence of systemic toxicity in the 13-week studies. The high doses used in these studies were one fourth those used in the 16-day studies, and body weight gains were only decreased in high-dose male rats and mice. Again, this was likely secondary to the skin lesions produced by exposure to benzethonium chloride during the studies. Hypercellularity of the bone marrow was diagnosed in 25 mg/kg rats. This was considered a secondary manifestation of the inflammation occurring at the site of application.

There was little histopathology attributed to exposure to benzethonium chloride in the short- or long-term studies in rats or mice other than a spectrum of skin lesions at the site of application. These lesions were characteristic of lesions observed with a variety of skin irritants and included components of epithelial hyperplasia and inflammation. The lesions were in general dependent on dose and, to a lesser extent, time. However, some skin healing and/or adaptation did occur, as illustrated by the fact that a larger percentage of the treated animals had epithelial hyperplasia at the end of the 13-week studies than at the end of the 2-year studies. In contrast to the results of the 2-year studies in male rats and male and female mice, skin lesions in treated female rats appeared to progress in severity during the 2-year study, with significant evidence of ulceration present in treated females from this study. This is an atypical finding in that the response of male and female rats and mice to skin irritants has been very similar in other NTP studies (NTP, 1993, 1995a,b).

There was no evidence of treatment-related increased incidences of neoplasms at any site in rats or mice. Skin neoplasms occurring at the site of application included keratoacanthomas in one 0.5 mg/kg male rat and in one 1.5 mg/kg male rat and one sebaceous gland carcinoma in one 0.5 mg/kg male rat. There was no evidence of an increased incidence of skin neoplasms in the treated groups when skin neoplasms that occurred at the site of application were combined with those occurring away from the site of application. In addition, the results of genetic toxicity studies indicated that benzethonium chloride is not mutagenic or otherwise genotoxic. Thus, there would appear to be little concern that repeated exposure to benzethonium chloride at these concentrations is carcinogenic.

As indicated previously, benzethonium chloride at doses of 3.125 mg/kg and greater in the rat caused dermatotoxicity in the 13-week study, as evidenced by epithelial hyperplasia, inflammatory infiltration, and ulceration of the epidermis. At higher doses, this ulceration and inflammation became more severe and involved the deeper dermal and subcutaneous tissues. The irritant property of benzethonium chloride has been observed previously in studies in which dilute solutions of the chemical were administered twice weekly into the subcutaneous tissue of rats (Mason *et al.*, 1971). At doses of 1 and 3 mg/kg, a high number of animals had severe granulomatous reactions at the injection site. By week 50, 8 of 60 rats administered 1 mg/kg and 16 of 80 rats administered 3 mg/kg developed locally invasive, non-metastatic sarcomas (primarily fibrosarcomas); none were present in the 50 controls. While clearly a treatment-related effect, it is difficult to evaluate the degree to which the marked inflammatory component may have contributed to the increase in the incidence of neoplasms. Full-thickness damage or "wounding" of the skin of mice has been shown to induce epithelial hyperplasia, inflammation of the dermis and epidermis, and the marked proliferation of granulation tissue. In long-term initiation/promotion studies the "wounding" has been shown to be a highly effective skin neoplasm promotion regimen (Argyris, 1989). 12-*O*-Tetradecanoyl-phorbol-13-acetate (TPA), often considered the positive control in skin neoplasm promotion studies, is known to induce a low incidence of skin neoplasms when administered alone at doses which also induce skin irritation (NTP, 1995b,c). Following initiation with dimethylbenz(a)anthracene (DMBA) the most effective doses of TPA for skin neoplasm promotion are also those doses that result in epithelial erosion, inflammation, and regenerative epithelial hyperplasia (Argyris, 1989). Recent skin initiation/promotion studies with TPA have shown that the strains of mice most sensitive to neoplasm promotion were also those that were significantly more sensitive to the irritancy of the chemical, as evidenced by a marked inflammatory reaction (NTP, 1995c). Lower doses of TPA or lesser epidermal damage such as repeated mild abrasion of the skin surface with fine sandpaper, although adequate to induce some epithelial hyperplasia, were not shown to induce a deeper inflammatory component or to be sufficient to promote neoplasms following similar skin neoplasm initiation with DMBA (Argyris, 1989). Although epithelial hyperplasia was observed in male and female rats and mice in the present studies and ulcerative lesions were observed in female rats, it is unknown whether higher doses capable of inducing more severe, necrotizing inflammatory skin lesions as were observed in the study by Mason *et al.* (1971) may have resulted in increases in skin-associated neoplasia.

CONCLUSIONS

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity** of benzethonium chloride in male or female F344/N rats receiving 0.15, 0.5, or 1.5 mg/kg. There was *no evidence of carcinogenic activity* in male or female $B6C3F_1$ mice receiving 0.15, 0.5, or 1.5 mg/kg.

Exposure of rats and mice to benzethonium chloride by dermal application in ethanol for 2 years resulted in epithelial hyperplasia in male and female rats and mice and sebaceous gland hyperplasia and ulcers in female rats at the site of application.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR DERMAL STUDY OF BENZETHONIUM CHLORIDE

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	8	8	5	4
Early deaths				
Accidental death			1	
Moribund	25	34	34	25
Natural deaths	12	7	11	15
Survivors				
Died last week of study			1	
Terminal sacrifice	15	11	8	16
Animals examined microscopically	60	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Stomach, forestomach	(1)			
Squamous cell papilloma	1 (100%)			
Endocrine System				
Pituitary gland	(8)			(4)
Pars distalis, adenoma	3 (38%)			2 (50%)
Thyroid gland	(8)			(4)
C-cell, adenoma				2 (50%)
Genital System				
Testes	(8)			(4)
Bilateral, interstitial cell, adenoma	1 (13%)			
Interstitial cell, adenoma	2 (25%)			
Integumentary System				
Skin, control	(8)	(8)	(5)	(4)
Skin, site of application	(8)	(8)	(5)	(4)
Systems Examined With No Neoplasms Observed				
Cardiovascular System				
General Body System				
Hematopoietic System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study				
Alimentary System				
Intestine large, colon				(1)
Adenoma				1 (100%)
Intestine large, cecum	(1)			
Intestine small, duodenum				(3)
Intestine small, jejunum	(1)			(1)
Adenocarcinoma				1 (100%)
Adenoma	1 (100%)			
Liver	(52)			(56)
Histiocytic sarcoma	1 (2%)			
Mesentery	(8)			(8)
Lipoma	1 (13%)			
Fat, histiocytic sarcoma	1 (13%)			
Pancreas	(52)			(56)
Pharynx	(1)			
Palate, squamous cell papilloma	1 (100%)			
Salivary glands	(52)			(56)
Sarcoma	1 (2%)			
Tooth	(2)			
Gingiva, squamous cell carcinoma	1 (50%)			
Cardiovascular System				
Heart	(51)			(56)
Endocrine System				
Adrenal cortex	(28)			(24)
Adenoma	1 (4%)			
Squamous cell carcinoma, metastatic, lung				1 (4%)
Adrenal medulla	(27)			(29)
Pheochromocytoma malignant				1 (3%)
Pheochromocytoma benign	3 (11%)			4 (14%)
Bilateral, pheochromocytoma benign	2 (7%)			3 (10%)
Islets, pancreatic	(52)			(56)
Adenoma	1 (2%)			3 (5%)
Parathyroid gland	(50)			(54)
Adenoma	1 (2%)			
Pituitary gland	(52)			(56)
Pars distalis, adenoma	31 (60%)			23 (41%)
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(51)			(56)
C-cell, adenoma	6 (12%)			7 (13%)
C-cell, adenoma, multiple	1 (2%)			
Follicular cell, adenoma	1 (2%)			2 (4%)
General Body System				
None				

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Genital System				
Epididymis	(52)			(56)
Preputial gland	(52)			(56)
Adenoma				1 (2%)
Carcinoma	1 (2%)			
Bilateral, carcinoma	1 (2%)			
Prostate	(52)			(56)
Seminal vesicle	(52)			(56)
Testes	(52)			(56)
Bilateral, interstitial cell, adenoma	23 (44%)			30 (54%)
Interstitial cell, adenoma	19 (37%)			10 (18%)
Hematopoietic System				
Bone marrow	(52)			(56)
Femoral, histiocytic sarcoma	1 (2%)			
Lymph node	(7)			(14)
Mediastinal, histiocytic sarcoma	1 (14%)			
Renal, histiocytic sarcoma	1 (14%)			
Thoracic, squamous cell carcinoma, metastatic, lung				1 (7%)
Lymph node, mandibular	(13)			(17)
Hemangiosarcoma	1 (8%)			
Lymph node, mesenteric	(8)			(14)
Spleen	(52)			(56)
Hemangiosarcoma	1 (2%)			
Histiocytic sarcoma	2 (4%)			1 (2%)
Thymus	(44)			(47)
Squamous cell carcinoma, metastatic, lung				1 (2%)
Integumentary System				
Mammary gland	(49)			(50)
Adenoma	1 (2%)			
Fibroadenoma	1 (2%)			3 (6%)
Fibroma	3 (6%)			1 (2%)
Histiocytic sarcoma	1 (2%)			
Skin, control	(52)	(52)	(55)	(56)
Basal cell adenoma			1 (2%)	
Basosquamous tumor benign			1 (2%)	
Histiocytic sarcoma	1 (2%)			
Keratoacanthoma	1 (2%)		3 (5%)	
Squamous cell papilloma	1 (2%)			1 (2%)
Sebaceous gland, lip, carcinoma	1 (2%)			
Subcutaneous tissue, fibroma			1 (2%)	1 (2%)
Subcutaneous tissue, fibrosarcoma		1 (2%)		
Subcutaneous tissue, fibrous histiocytoma		1 (2%)		
Subcutaneous tissue, histiocytic sarcoma				1 (2%)
Subcutaneous tissue, neurofibroma	1 (2%)			
Skin, site of application-no mass	(52)	(52)	(55)	(56)
Subcutaneous tissue, histiocytic sarcoma	1 (2%)	1 (2%)		1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Integumentary System (continued)				
Skin, site of application-mass	(5)		(3)	(4)
Keratoacanthoma			1 (33%)	1 (25%)
Sebaceous gland, carcinoma			1 (33%)	
Subcutaneous tissue, histiocytic sarcoma	1 (20%)		1 (33%)	
Musculoskeletal System				
Bone	(52)			(56)
Maxilla, osteosarcoma	1 (2%)			
Nervous System				
Brain	(52)			(56)
Astrocytoma NOS				1 (2%)
Respiratory System				
Lung	(51)			(56)
Histiocytic sarcoma	1 (2%)			
Squamous cell carcinoma				1 (2%)
Special Senses System				
Ear	(3)			
Squamous cell papilloma	3 (100%)			
Zymbal's gland	(2)			
Carcinoma	2 (100%)			
Urinary System				
Kidney	(52)			(56)
Transitional epithelium, carcinoma	1 (2%)			
Urinary bladder	(52)			(56)
Transitional epithelium, papilloma				1 (2%)
Systemic Lesions				
Multiple organs[b]	(52)	(52)	(55)	(56)
Histiocytic sarcoma	3 (6%)	1 (2%)	1 (2%)	3 (5%)
Leukemia mononuclear	23 (44%)			30 (54%)
Mesothelioma benign				1 (2%)
Mesothelioma malignant	1 (2%)			1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]				
15-Month interim evaluation	5			3
2-Year study	52	3	8	54
Total primary neoplasms				
15-Month interim evaluation	7			4
2-Year study	142	3	9	131
Total animals with benign neoplasms				
15-Month interim evaluation	5			3
2-Year study	50		7	51
Total benign neoplasms				
15-Month interim evaluation	7			4
2-Year study	104		7	93
Total animals with malignant neoplasms				
2-Year study	30	3	2	33
Total malignant neoplasms				
2-Year study	38	3	2	37
Total animals with metastatic neoplasms				
2-Year study				1
Total metastatic neoplasms				
2-Year study				3
Total animals with uncertain neoplasms - benign or malignant				
2-Year study				1
Total uncertain neoplasms				
2-Year study				1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control

Number of Days on Study	4 0 6	4 3 7	5 1 3	5 3 3	5 4 9	5 5 1	5 5 6	5 6 4	5 8 3	5 8 5	5 9 2	6 0 3	6 0 6	6 0 6	6 0 6	6 1 2	6 3 2	6 3 4	6 3 5	6 4 7	6 4 7	6 4 8	6 5 0	6 5 3	6 5 3
Carcass ID Number	0 3 2	0 4 6	0 0 6	0 0 4	0 4 1	0 4 9	0 1 8	0 6 0	0 5 6	0 5 4	0 3 6	0 0 5	0 0 9	0 1 3	0 1 6	0 5 0	0 3 4	0 5 3	0 4 8	0 1 9	0 5 8	0 5 5	0 0 2	0 1 1	0 1 3
Alimentary System																									
Esophagus	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum											+														
Intestine small, jejunum																									
Adenoma																									
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Mesentery			+				+				+									+	+				
Lipoma																					X				
Fat, histiocytic sarcoma			X																						
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx																									
Palate, squamous cell papilloma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma											X														
Stomach, forestomach												+			+									+	
Stomach, glandular					+		+					+													+
Tongue																								+	
Tooth																								+	
Gingiva, squamous cell carcinoma																								X	
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex							+		+		+		+	+	+	+	+	+	+						
Adenoma																									
Adrenal medulla						+	+	+	+			+	+		+	+	+	+				+	+		+
Pheochromocytoma benign																		X				X			
Bilateral, pheochromocytoma benign							X																		
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X							
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X	X		X			X							X	X		X		X	X	X	X	X		X
Pars intermedia, adenoma										X															
Thyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma										X															
C-cell, adenoma, multiple																						X			
Follicular cell, adenoma																									
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	6 5 5	6 5 9	6 7 9	6 8 9	6 9 1	6 9 1	7 0 9	7 0 9	7 1 4	7 1 6	7 1 6	7 2 6	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	0 2 0	0 2 8	0 2 6	0 3 7	0 2 2	0 4 5	0 2 7	0 4 7	0 4 0	0 3 8	0 5 9	0 0 1	0 0 8	0 1 0	0 1 2	0 1 4	0 1 7	0 2 3	0 2 5	0 3 0	0 3 3	0 3 5	0 4 2	0 4 3	0 4 4	0 5 1	0 5 2	Total Tissues/ Tumors
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine large, cecum																												1
Intestine small, jejunum																+												1
Adenoma																X												1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Histiocytic sarcoma																												1
Mesentery								+						+													+	8
Lipoma																												1
Fat, histiocytic sarcoma																												1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Pharynx	+																											1
Palate, squamous cell papilloma	X																											1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Sarcoma																												1
Stomach, forestomach			+		+	+	+			+							+											9
Stomach, glandular		+		+		+	+	+			+								+	+			+					13
Tongue																												1
Tooth											+																	2
Gingiva, squamous cell carcinoma																												1
Cardiovascular System																												
Blood vessel								+																				1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Endocrine System																												
Adrenal cortex	+	+		+	+		+	+		+	+	+			+	+	+	+	+	+		+	+		+		28
Adenoma											X																	1
Adrenal medulla	+	+		+		+				+	+		+	+	+				+		+	+	+			+		27
Pheochromocytoma benign															X													3
Bilateral, pheochromocytoma benign																					X							2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adenoma																												1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	50
Adenoma																										X		1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Pars distalis, adenoma		X	X	X	X				X	X	X	X	X		X		X			X	X	X	X	X	X		X	31
Pars intermedia, adenoma																												1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
C-cell, adenoma			X														X	X					X	X				6
C-cell, adenoma, multiple																												1
Follicular cell, adenoma			X																									1
General Body System																												
None																												

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	4 0 6	4 3 7	5 1 3	5 3 3	5 4 9	5 5 1	5 5 6	5 6 4	5 8 3	5 8 5	5 9 2	6 0 3	6 0 6	6 0 6	6 0 6	6 1 2	6 3 2	6 3 4	6 3 5	6 4 7	6 4 7	6 4 8	6 5 0	6 5 3	6 5 3
Carcass ID Number	0 3 2	0 4 6	0 0 6	0 0 4	0 4 1	0 4 9	0 1 8	0 6 0	0 5 6	0 5 4	0 3 6	0 0 5	0 0 9	0 1 3	0 1 6	0 5 0	0 3 4	0 5 3	0 4 8	0 1 9	0 5 8	0 5 5	0 0 2	0 1 1	0 3 1
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma		X																							
Bilateral, carcinoma																	X								
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma						X		X	X				X						X			X	X	X	
Interstitial cell, adenoma			X				X			X		X				X		X		X					X
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Femoral, histiocytic sarcoma			X																						
Lymph node			+		+		+			+	+					+							+		
Mediastinal, histiocytic sarcoma			X																						
Renal, histiocytic sarcoma			X																						
Lymph node, mandibular						+	+			+	+		+			+		+					+	+	
Hemangiosarcoma																									
Lymph node, mesenteric										+	+					+							+		
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																X									
Histiocytic sarcoma					X				X																
Thymus	+	+	M	+	M	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Adenoma																									
Fibroadenoma														X											
Fibroma																						X			
Histiocytic sarcoma			X																						
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Keratoacanthoma																									
Squamous cell papilloma																									
Sebaceous gland, lip, carcinoma									X																
Subcutaneous tissue, neurofibroma																									
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, histiocytic sarcoma			X																						
Skin, site of application-mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, histiocytic sarcoma			X																						

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	6 5 5	6 5 9	6 7 9	6 8 9	6 9 1	6 9 1	7 0 9	7 0 9	7 1 4	7 1 6	7 1 6	7 2 6	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	0 2 0	0 2 8	0 2 6	0 3 7	0 2 2	0 4 5	0 2 7	0 4 7	0 4 0	0 3 8	0 5 9	0 0 1	0 0 8	0 1 0	0 1 2	0 1 4	0 1 7	0 2 3	0 2 5	0 3 0	0 3 3	0 3 5	0 4 2	0 4 3	0 4 4	0 5 1	0 5 2	Total Tissues/ Tumors
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Carcinoma																											1
Bilateral, carcinoma																											1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Bilateral, interstitial cell, adenoma		X				X	X	X	X				X	X	X			X				X	X	X	X	X	X	23
Interstitial cell, adenoma	X		X	X	X					X		X				X	X		X	X	X							19
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Femoral, histiocytic sarcoma																											1
Lymph node																											7
Mediastinal, histiocytic sarcoma																											1
Renal, histiocytic sarcoma																											1
Lymph node, mandibular	+			+							+					+											13
Hemangiosarcoma	X																										1
Lymph node, mesenteric	+			+							+			+													8
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Hemangiosarcoma																											1
Histiocytic sarcoma																											2
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	M	+	M	+	+	+	+	+	+	+	44
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																	X										1
Fibroadenoma																											1
Fibroma																		X							X		3
Histiocytic sarcoma																											1
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Histiocytic sarcoma																											1
Keratoacanthoma																						X					1
Squamous cell papilloma										X																	1
Sebaceous gland, lip, carcinoma																											1
Subcutaneous tissue, neurofibroma																						X					1
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Subcutaneous tissue, histiocytic sarcoma																											1
Skin, site of application-mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Subcutaneous tissue, histiocytic sarcoma																											1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	4 0 6	4 3 7	5 1 3	5 3 3	5 4 9	5 5 1	5 5 6	5 6 4	5 8 3	5 8 5	5 9 2	6 0 3	6 0 6	6 0 6	6 0 6	6 1 2	6 3 2	6 3 4	6 3 5	6 4 7	6 4 7	6 4 8	6 5 0	6 5 3	6 5 3
Carcass ID Number	0 3 2	0 4 6	0 0 6	0 0 4	0 4 1	0 4 9	0 1 8	0 6 0	0 5 6	0 5 4	0 3 6	0 0 5	0 0 9	0 1 3	0 1 6	0 5 0	0 3 4	0 5 3	0 4 8	0 1 9	0 5 8	0 5 5	0 0 2	0 1 1	0 3 1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maxilla, osteosarcoma																			X						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve											+	+			+										
Spinal cord											+	+			+										
Respiratory System																									
Lung	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Squamous cell papilloma																									
Eye																									
Zymbal's gland													+												
Carcinoma													X												
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, carcinoma								X																	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X		X				X																
Leukemia mononuclear					X	X	X		X	X	X	X		X		X		X	X	X			X	X	
Mesothelioma malignant																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	6 5 5	6 5 9	6 7 9	6 8 9	6 9 1	6 9 1	7 0 9	7 0 9	7 1 4	7 1 6	7 1 6	7 2 6	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	0 2 0	0 2 8	0 2 6	0 3 7	0 2 2	0 4 5	0 2 7	0 4 7	0 4 0	0 3 8	0 5 9	0 0 1	0 0 8	0 1 0	0 1 2	0 1 4	0 1 7	0 2 3	0 2 5	0 3 0	0 3 3	0 3 5	0 4 2	0 4 3	0 4 4	0 5 1	0 5 2	Total Tissues/ Tumors
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Maxilla, osteosarcoma																												1
Nervous System																												
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Peripheral nerve		+				+																						5
Spinal cord		+				+																						5
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Histiocytic sarcoma																												1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Special Senses System																												
Ear									+									+						+				3
Squamous cell papilloma									X									X						X				3
Eye			+											+														2
Zymbal's gland																						+						2
Carcinoma																						X						2
Urinary System																												
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Transitional epithelium, carcinoma																												1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Histiocytic sarcoma																												3
Leukemia mononuclear	X			X		X					X			X		X			X	X		X						23
Mesothelioma malignant								X																				1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: 0.15 mg/kg

Number of Days on Study	2	3	4	4	4	4	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6		
	6	7	5	6	7	9	4	4	5	5	6	7	7	7	9	9	0	1	1	1	1	2	2	2		
	4	9	7	1	9	9	1	2	6	6	4	6	8	9	1	3	6	0	2	2	6	0	1	3	6	
Carcass ID Number	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	1	1	1	1	1	0	
	6	6	7	6	8	9	6	8	0	1	6	9	9	6	8	9	9	1	7	1	1	2	1	1	9	
	7	5	0	2	8	3	6	4	9	7	1	6	4	3	2	1	7	5	7	4	8	0	2	0	0	
Alimentary System																										
None																										
Cardiovascular System																										
None																										
Endocrine System																										
None																										
General Body System																										
None																										
Genital System																										
None																										
Hematopoietic System																										
None																										
Integumentary System																										
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Subcutaneous tissue, fibrosarcoma																										
Subcutaneous tissue, fibrous histiocytoma			X																							
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Subcutaneous tissue, histiocytic sarcoma														X												
Musculoskeletal System																										
None																										
Nervous System																										
None																										
Respiratory System																										
None																										
Special Senses System																										
None																										
Urinary System																										
None																										
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Histiocytic sarcoma														X												

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: 0.15 mg/kg
(continued)

Number of Days on Study	6 3 2	6 3 8	6 3 9	6 5 9	6 5 9	6 6 8	6 7 0	6 7 1	6 7 5	6 9 1	6 9 8	6 9 8	7 1 0	7 1 2	7 2 2	7 2 3	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	0 7 8	0 7 5	0 8 0	1 0 6	1 0 8	0 9 9	0 8 3	0 8 4	1 0 9	0 8 9	0 6 9	0 7 3	1 0 2	0 6 8	1 9 6	0 8 5	0 6 6	0 7 4	0 7 1	0 7 2	0 8 1	0 9 8	1 0 1	1 0 3	1 0 5	1 0 7	1 1 1	1 1 3	Total Tissues/ Tumors
Alimentary System																												
None																												
Cardiovascular System																												
None																												
Endocrine System																												
None																												
General Body System																												
None																												
Genital System																												
None																												
Hematopoietic System																												
None																												
Integumentary System																												
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Subcutaneous tissue, fibrosarcoma																											X	1
Subcutaneous tissue, fibrous histiocytoma																												1
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Subcutaneous tissue, histiocytic sarcoma																												1
Musculoskeletal System																												
None																												
Nervous System																												
None																												
Respiratory System																												
None																												
Special Senses System																												
None																												
Urinary System																												
None																												
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Histiocytic sarcoma																												1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: 0.5 mg/kg

Number of Days on Study	0	1	3	4	4	4	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	
	2	9	6	1	3	8	0	0	1	2	2	3	5	7	8	8	9	0	1	1	2	2	3	3	3	4	
	5	9	7	6	7	3	0	5	1	3	6	7	6	5	7	9	9	1	3	3	1	6	2	2	5	5	3
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	6	6	4	2	3	4	2	5	3	3	6	6	2	7	4	4	3	6	5	8	6	5	3	7	5	5	2
	0	5	0	7	3	5	2	6	9	0	3	1	1	6	6	9	1	7	9	0	6	7	7	2	2	3	6
Alimentary System																											
None																											
Cardiovascular System																											
None																											
Endocrine System																											
None																											
General Body System																											
None																											
Genital System																											
None																											
Hematopoietic System																											
None																											
Integumentary System																											
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																											
Basosquamous tumor benign																										X	
Keratoacanthoma																											
Subcutaneous tissue, fibroma													X														
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin, site of application-mass																											
Keratoacanthoma																											
Sebaceous gland, carcinoma																											
Subcutaneous tissue, histiocytic sarcoma																											
Musculoskeletal System																											
None																											
Nervous System																											
None																											
Respiratory System																											
None																											
Special Senses System																											
None																											
Urinary System																											
None																											
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																											

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: 0.5 mg/kg
(continued)

Number of Days on Study	6 4 8	6 4 9	6 5 5	6 5 8	6 5 8	6 6 2	6 6 3	6 6 4	6 6 6	6 7 1	6 7 6	6 8 2	6 8 3	6 9 6	6 9 8	7 0 7	7 1 2	7 1 8	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 3 5	1 4 4	1 4 3	1 7 1	1 7 9	1 5 4	1 7 4	1 2 3	1 3 2	1 5 5	1 2 8	1 2 4	1 6 4	1 3 8	1 6 8	1 2 5	1 7 0	1 3 6	1 5 8	1 2 9	1 4 1	1 5 0	1 5 1	1 6 2	1 6 9	1 7 5	1 7 7	1 7 8	Total Tissues/ Tumors
Alimentary System																												
None																												
Cardiovascular System																												
None																												
Endocrine System																												
None																												
General Body System																												
None																												
Genital System																												
None																												
Hematopoietic System																												
None																												
Integumentary System																												
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	55
Basal cell adenoma														X														1
Basosquamous tumor benign																												1
Keratoacanthoma											X														X		X	3
Subcutaneous tissue, fibroma																												1
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	55
Skin, site of application-mass							+														+				+			3
Keratoacanthoma							X																					1
Sebaceous gland, carcinoma																					X							1
Subcutaneous tissue, histiocytic sarcoma																									X			1
Musculoskeletal System																												
None																												
Nervous System																												
None																												
Respiratory System																												
None																												
Special Senses System																												
None																												
Urinary System																												
None																												
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	55
Histiocytic sarcoma																									X			1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg

Number of Days on Study	1 7 0	2 9 1	3 6 4	3 9 6	4 0 8	4 2 5	4 6 5	5 3 3	5 3 7	5 4 9	5 5 1	5 5 6	5 5 7	5 6 2	5 9 4	6 0 5	6 0 9	6 1 3	6 1 3	6 1 9	6 2 6	6 3 5	6 3 9	6 3 9	6 4 3	6 4 4	6 4 9	6 5 5
Carcass ID Number	2 0 0 7	2 0 3 3	2 0 1 1	2 3 1 7	2 0 0 0	2 4 0 0	2 0 5 5	1 8 5 5	1 9 1 1	1 8 1 6	2 2 0 0	2 2 5 5	1 9 3 7	1 8 7 2	1 9 9 4	2 0 9 5	2 1 9 2	1 9 1 0	2 1 1 3	2 1 1 5	1 1 8 6	2 3 3 5	1 8 3 3	2 0 6 9	2 2 3 3	2 3 4 4	2 1 1 9	2 1 4 5
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon																												
Adenoma																												
Intestine small, duodenum																												
Intestine small, jejunum																												
Adenocarcinoma																												
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery																								+	+			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach							+	+	+	+						+	+											
Stomach, glandular								+	+		+			+													+	
Cardiovascular System																												
Blood vessel																												
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																												
Adrenal cortex									+					+				+		+	+		+		+		+	
Squamous cell carcinoma, metastatic, lung														X														
Adrenal medulla							+		+		+	+			+			+		+	+	+				+	+	
Pheochromocytoma malignant												X																
Pheochromocytoma benign																				X						X	X	
Bilateral, pheochromocytoma benign																												
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																												
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X	X	X									X	X	X					X	X	X	X	X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma							X															X						
Follicular cell, adenoma																								X				
General Body System																												
None																												
Genital System																												
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma										X																		
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma							X				X	X	X						X	X	X	X	X	X	X			
Interstitial cell, adenoma									X	X				X			X											X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	6 5 9	6 6 0	6 6 2	6 7 5	6 7 9	6 8 3	6 9 1	6 9 2	7 1 0	7 1 0	7 2 2	7 2 2	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	1 9 8	2 3 8	2 1 8	2 2 6	1 9 2	2 1 9	1 8 1	2 1 0	1 8 4	2 2 4	1 8 2	2 2 2	1 8 9	1 9 4	1 9 6	2 0 2	2 0 4	2 0 8	2 1 3	2 1 6	2 2 1	2 2 8	2 2 9	2 3 0	2 3 1	2 3 2	2 3 5	2 3 9	Total Tissues/ Tumors
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Intestine large, colon											+																	1
Adenoma											X																	1
Intestine small, duodenum	+												+	+														3
Intestine small, jejunum														+														1
Adenocarcinoma														X														1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Mesentery	+			+							+											+			+	+		8
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Stomach, forestomach					+	+	+			+	+	+				+												13
Stomach, glandular	+		+				+					+				+									+			11
Cardiovascular System																												
Blood vessel												+																1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Endocrine System																												
Adrenal cortex	+		+	+	+	+		+		+	+	+	+		+			+	+		+	+				+		24
Squamous cell carcinoma, metastatic, lung																												1
Adrenal medulla	+				+		+		+			+		+	+	+	+	+	+		+		+	+	+	+	+	29
Pheochromocytoma malignant																												1
Pheochromocytoma benign																					X							4
Bilateral, pheochromocytoma benign	X															X							X					3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Adenoma																	X			X							X	3
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Pars distalis, adenoma				X	X	X		X		X		X	X	X				X	X		X	X						23
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
C-cell, adenoma	X												X		X				X			X						7
Follicular cell, adenoma						X																						2
General Body System																												
None																												
Genital System																												
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Adenoma																												1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Bilateral, interstitial cell, adenoma		X	X				X	X	X	X	X	X			X	X	X	X			X	X		X	X	X	X	X	30
Interstitial cell, adenoma	X												X	X						X			X						10

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	1 7 0	2 9 1	3 6 4	3 9 6	4 0 8	4 2 5	4 6 5	5 3 3	5 3 7	5 4 9	5 5 1	5 5 6	5 5 7	5 6 2	5 9 4	6 0 5	6 0 9	6 1 3	6 1 3	6 1 9	6 2 6	6 3 5	6 3 9	6 3 9	6 4 3	6 4 4	6 4 9	6 5 5
Carcass ID Number	2 0 7	2 0 3	2 0 1	2 3 7	2 0 0	2 4 0	2 0 5	1 8 5	1 9 1	1 8 6	2 2 0	2 2 5	1 9 3	1 8 7	1 9 9	2 0 9	2 1 2	1 9 0	2 1 5	2 1 7	1 1 8	2 3 3	1 8 3	2 0 6	2 2 3	2 3 4	1 1 1	2 1 4
Hematopoietic System																												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+			+	+	+	+			+	+	+			+									
Thoracic, squamous cell carcinoma, metastatic, lung														X														
Lymph node, mandibular				+			+	+	+	+	+				+					+	+	+						
Lymph node, mesenteric				+			+	+	+	+	+				+			+										
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X															
Thymus	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Squamous cell carcinoma, metastatic, lung														X														
Integumentary System																												
Mammary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	M	+	+	+	M	+	+	+
Fibroadenoma																												
Fibroma																												
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																												
Subcutaneous tissue, fibroma																							X					
Subcutaneous tissue, histiocytic sarcoma																				X								
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, histiocytic sarcoma																												
Skin, site of application-mass																												
Keratoacanthoma																												
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																												
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma nos																										X		
Peripheral nerve																												
Spinal cord																												

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	659	660	662	675	779	783	891	912	1010	1100	1222	1229	1299	1299	1299	1299	1299	1299	1299	1299	1299	1299	1299	1299	1299	1299	1299	
Carcass ID Number	19 8	23 8	18 8	12 6	09 2	11 9	18 1	10 0	18 4	22 4	28 2	22 2	28 9	19 4	19 6	20 2	20 4	20 8	21 3	21 6	22 1	22 8	23 9	23 0	23 1	23 2	23 5	23 9	Total Tissues/ Tumors
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Lymph node	+								+						+	+							+						14
Thoracic, squamous cell carcinoma, metastatic, lung																													1
Lymph node, mandibular	+								+						+				+		+		+				+		17
Lymph node, mesenteric	+								+						+	+			+				+						14
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Histiocytic sarcoma																													1
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	M	M	M	47
Squamous cell carcinoma, metastatic, lung																													1
Integumentary System																													
Mammary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma											X		X					X											3
Fibroma						X																							1
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Squamous cell papilloma																						X							1
Subcutaneous tissue, fibroma																													1
Subcutaneous tissue, histiocytic sarcoma																													1
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Subcutaneous tissue, histiocytic sarcoma																			X										1
Skin, site of application-mass		+													+								+	+					4
Keratoacanthoma		X																											1
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Astrocytoma nos																													1
Peripheral nerve	+		+																										2
Spinal cord	+		+																										2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	1 7 0	2 9 1	3 6 4	3 9 6	4 0 8	4 2 5	4 6 5	5 3 3	5 3 7	5 4 9	5 5 1	5 5 6	5 5 7	5 6 2	5 9 4	6 0 5	6 0 9	6 1 3	6 1 3	6 1 9	6 2 6	6 3 5	6 3 9	6 3 9	6 4 3	6 4 4	6 4 9	6 5 5
Carcass ID Number	2 0 7	2 0 3	2 0 1	2 3 7	2 0 0	2 4 0	2 0 5	1 8 5	1 9 1	1 8 6	2 2 0	2 2 5	1 9 3	1 8 7	1 9 9	2 0 9	2 1 2	1 9 0	2 1 5	2 1 7	1 8 8	2 3 3	1 8 3	2 0 6	2 2 3	2 3 4	1 1 1	2 1 4
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma														X														
Nose	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																												
Eye																												
Urinary System																												
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma																												
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X							X								
Leukemia mononuclear				X	X		X	X	X	X	X		X		X		X		X	X		X					X	
Mesothelioma benign			X																									
Mesothelioma malignant																								X				

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	6 5 9	6 6 0	6 6 2	6 7 5	6 7 9	6 8 3	6 9 1	6 9 2	7 1 0	7 1 0	7 2 2	7 2 2	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	1 9 8	2 3 8	2 1 8	2 2 6	1 9 2	2 1 9	1 8 1	2 1 0	1 8 4	2 2 4	1 8 2	2 2 2	1 8 9	1 9 4	1 9 6	2 0 2	2 0 4	2 0 8	2 1 3	2 1 6	2 2 1	2 2 8	2 3 9	2 3 0	2 3 1	2 3 2	2 3 5	2 3 9	Total Tissues/ Tumors
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Squamous cell carcinoma																													1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	55
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Special Senses System																													
Eye														+		+											+		3
Urinary System																													
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Transitional epithelium, papilloma								X																					1
Systemic Lesions																													
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
Histiocytic sarcoma																			X										3
Leukemia mononuclear	X	X	X						X		X			X	X	X		X	X		X		X			X	X	X	30
Mesothelioma benign																													1
Mesothelioma malignant																													1

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	5/52 (10%)	–[e]	–	7/56 (13%)
Adjusted rate[b]	20.3%			28.7%
Terminal rate[c]	2/15 (13%)			3/16 (19%)
First incidence (days)	556			619
Life table test[d]				P=0.401
Logistic regression test[d]				P=0.387
Fisher exact test[d]				P=0.434
Ear: Squamous Cell Papilloma				
Overall rate	3/52 (6%)	–	–	0/56 (0%)
Adjusted rate	17.9%			0.0%
Terminal rate	2/15 (13%)			0/16 (0%)
First incidence (days)	714			–[f]
Life table test				P=0.117N
Logistic regression test				P=0.115N
Fisher exact test				P=0.108N
Mammary Gland: Fibroma				
Overall rate	3/52 (6%)	–	–	1/56 (2%)
Adjusted rate	16.1%			4.3%
Terminal rate	2/15 (13%)			0/16 (0%)
First incidence (days)	648			683
Life table test				P=0.302N
Logistic regression test				P=0.306N
Fisher exact test				P=0.281N
Mammary Gland: Fibroadenoma				
Overall rate	1/52 (2%)	–	–	3/56 (5%)
Adjusted rate	2.5%			17.4%
Terminal rate	0/15 (0%)			2/16 (13%)
First incidence (days)	606			722
Life table test				P=0.329
Logistic regression test				P=0.301
Fisher exact test				P=0.338
Mammary Gland: Fibroma, Fibroadenoma, or Adenoma				
Overall rate	5/52 (10%)	–	–	4/56 (7%)
Adjusted rate	24.5%			21.0%
Terminal rate	3/15 (20%)			2/16 (13%)
First incidence (days)	606			683
Life table test				P=0.475N
Logistic regression test				P=0.505N
Fisher exact test				P=0.453N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Pancreatic Islets: Adenoma				
Overall rate	1/52 (2%)	–	–	3/56 (5%)
Adjusted rate	2.9%			18.8%
Terminal rate	0/15 (0%)			3/16 (19%)
First incidence (days)	634			729 (T)
Life table test				P=0.321
Logistic regression test				P=0.333
Fisher exact test				P=0.338
Pituitary Gland (Pars Distalis or Unspecified Site): Adenoma				
Overall rate	31/52 (60%)	–	–	23/56 (41%)
Adjusted rate	84.4%			65.0%
Terminal rate	10/15 (67%)			6/16 (38%)
First incidence (days)	406			408
Life table test				P=0.136N
Logistic regression test				P=0.085N
Fisher exact test				P=0.041N
Skin: Keratoacanthoma				
Overall rate	1/52 (2%)	0/52 (0%)	3/55 (5%)	0/56 (0%)
Adjusted rate	6.7%	0.0%	26.5%	0.0%
Terminal rate	1/15 (7%)	0/11 (0%)	2/9 (22%)	0/16 (0%)
First incidence (days)	729 (T)	–	676	–
Life table test	P=0.394N	P=0.562N	P=0.155	P=0.487N
Logistic regression test	P=0.416N	P=0.562N	P=0.187	P=0.487N
Cochran-Armitage test	P=0.425N			
Fisher exact test		P=0.500N	P=0.330	P=0.481N
Testes: Adenoma				
Overall rate	42/52 (81%)	–	–	40/56 (71%)
Adjusted rate	100.0%			97.3%
Terminal rate	15/15 (100%)			15/16 (94%)
First incidence (days)	513			465
Life table test				P=0.389N
Logistic regression test				P=0.422N
Fisher exact test				P=0.182N
Thyroid Gland (C-cell): Adenoma				
Overall rate	7/51 (14%)	–	–	7/56 (13%)
Adjusted rate	33.5%			31.2%
Terminal rate	4/15 (27%)			4/16 (25%)
First incidence (days)	585			465
Life table test				P=0.584N
Logistic regression test				P=0.584N
Fisher exact test				P=0.538N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
All Organs: Histiocytic Sarcoma				
Overall rate	3/52 (6%)	–	–	3/56 (5%)
Adjusted rate	6.2%			10.9%
Terminal rate	0/15 (0%)			1/16 (6%)
First incidence (days)	513			557
Life table test				P=0.657
Logistic regression test				P=0.560N
Fisher exact test				P=0.625N
All Organs: Mononuclear Cell Leukemia				
Overall rate	23/52 (44%)	–	–	30/56 (54%)
Adjusted rate	61.7%			82.0%
Terminal rate	5/15 (33%)			11/16 (69%)
First incidence (days)	549			396
Life table test				P=0.214
Logistic regression test				P=0.209
Fisher exact test				P=0.218
All Organs: Benign Neoplasms				
Overall rate	50/52 (96%)	–	–	52/56 (93%)
Adjusted rate	100.0%			100.0%
Terminal rate	15/15 (100%)			16/16 (100%)
First incidence (days)	406			364
Life table test				P=0.501
Logistic regression test				P=0.649
Fisher exact test				P=0.375N
All Organs: Malignant Neoplasms				
Overall rate	30/52 (58%)	–	–	33/56 (59%)
Adjusted rate	70.8%			83.4%
Terminal rate	6/15 (40%)			11/16 (69%)
First incidence (days)	437			396
Life table test				P=0.424
Logistic regression test				P=0.559
Fisher exact test				P=0.526

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	52/52 (100%)	–	–	54/56 (96%)
Adjusted rate	100.0%			100.0%
Terminal rate	15/15 (100%)			16/16 (100%)
First incidence (days)	406			364
Life table test				P=0.501
Logistic regression test				_g
Fisher exact test				P=0.267N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, pancreatic islets, pituitary gland, skin, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Organ was not examined at this dose level
[f] Not applicable; no neoplasms in animal group
[g] Value of statistic cannot be computed.

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	8	8	5	4
Early deaths				
Accidental death			1	
Moribund	25	34	34	25
Natural deaths	12	7	11	15
Survivors				
Died last week of study			1	
Terminal sacrifice	15	11	8	16
Animals examined microscopically	60	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Intestine small, jejunum	(1)			
Inflammation, chronic	1 (100%)			
Liver	(8)			(4)
Hepatodiaphragmatic nodule	3 (38%)			
Inflammation, chronic active	3 (38%)			2 (50%)
Bile duct, hyperplasia	3 (38%)			2 (50%)
Hepatocyte, vacuolization cytoplasmic	5 (63%)			4 (100%)
Pancreas	(8)			(4)
Acinus, atrophy	5 (63%)			2 (50%)
Cardiovascular System				
Heart	(8)			(4)
Degeneration, chronic	8 (100%)			3 (75%)
Endocrine System				
Adrenal cortex	(1)			
Hyperplasia	1 (100%)			
Pituitary gland	(8)			(4)
Pars distalis, cyst	1 (13%)			
Pars distalis, hyperplasia				1 (25%)
Genital System				
Epididymis	(8)			(4)
Aspermia				1 (25%)
Preputial gland	(8)			(4)
Inflammation, chronic active	5 (63%)			2 (50%)
Prostate	(8)			(4)
Inflammation, chronic active	4 (50%)			2 (50%)
Testes	(8)			(4)
Germinal epithelium, degeneration				1 (25%)
Interstitial cell, hyperplasia	8 (100%)			3 (75%)

[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 Male Rats in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
15-Month Interim Evaluation (continued)				
Hematopoietic System				
Lymph node	(1)			
Pancreatic, angiectasis	1 (100%)			
Lymph node, mesenteric	(1)			
Angiectasis	1 (100%)			
Spleen	(8)			(4)
Fibrosis	1 (13%)			
Thymus	(8)			(4)
Depletion lymphoid	8 (100%)			3 (75%)
Integumentary System				
Mammary gland	(6)			(3)
Hyperplasia, cystic	3 (50%)			1 (33%)
Skin, control	(8)	(8)	(5)	(4)
Skin, site of application-no mass	(8)	(8)	(5)	(4)
Epithelial hyperplasia			3 (60%)	4 (100%)
Ulcer				1 (25%)
Sebaceous gland, hyperplasia			2 (40%)	2 (50%)
Respiratory System				
Lung	(8)			(4)
Alveolar epithelium, hyperplasia	1 (13%)			
Alveolar epithelium, hyperplasia, focal	1 (13%)			
Nose	(8)			(4)
Submucosa, inflammation, acute	1 (13%)			
Urinary System				
Kidney	(8)			(4)
Nephropathy, chronic	8 (100%)			4 (100%)
Cortex, cyst	1 (13%)			
Cortex, renal tubule, mineralization	1 (13%)			
Systems Examined With No Lesions Observed				
General Body System				
Musculoskeletal System				
Nervous System				
Special Senses System				
2-Year Study				
Alimentary System				
Intestine small, duodenum				(3)
Dysplasia				1 (33%)
Ulcer				1 (33%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Liver	(52)			(56)
Basophilic focus	6 (12%)			7 (13%)
Clear cell focus	3 (6%)			3 (5%)
Eosinophilic focus	4 (8%)			3 (5%)
Hematopoietic cell proliferation	1 (2%)			
Hepatodiaphragmatic nodule	3 (6%)			4 (7%)
Inflammation, chronic active				1 (2%)
Inflammation, granulomatous	2 (4%)			3 (5%)
Bile duct, cyst				1 (2%)
Bile duct, hyperplasia	34 (65%)			36 (64%)
Hepatocyte, degeneration, cystic	4 (8%)			9 (16%)
Hepatocyte, hyperplasia				1 (2%)
Hepatocyte, vacuolization cytoplasmic	17 (33%)			17 (30%)
Mesentery	(8)			(8)
Artery, inflammation, chronic active	1 (13%)			1 (13%)
Fat, hemorrhage	1 (13%)			
Fat, inflammation, chronic active	3 (38%)			6 (75%)
Pancreas	(52)			(56)
Acinus, atrophy	18 (35%)			24 (43%)
Artery, inflammation, chronic active	1 (2%)			1 (2%)
Stomach, forestomach	(9)			(13)
Acanthosis	4 (44%)			2 (15%)
Inflammation, chronic active	1 (11%)			2 (15%)
Mineralization	1 (11%)			2 (15%)
Ulcer	5 (56%)			8 (62%)
Stomach, glandular	(13)			(11)
Dysplasia	2 (15%)			
Hyperplasia				1 (9%)
Mineralization	5 (38%)			3 (27%)
Necrosis	1 (8%)			2 (18%)
Pigmentation, hemosiderin	1 (8%)			
Ulcer	4 (31%)			5 (45%)
Tongue	(1)			
Inflammation, chronic active	1 (100%)			
Tooth	(2)			
Peridontal tissue, inflammation, chronic active	1 (50%)			
Cardiovascular System				
Blood vessel	(1)			(1)
Aorta, mineralization	1 (100%)			1 (100%)
Heart	(51)			(56)
Degeneration, chronic	43 (84%)			47 (84%)
Mineralization	1 (2%)			2 (4%)
Atrium, thrombosis	6 (12%)			4 (7%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Endocrine System				
Adrenal cortex	(28)			(24)
Hyperplasia	12 (43%)			6 (25%)
Hypertrophy	2 (7%)			
Vacuolization cytoplasmic	22 (79%)			20 (83%)
Adrenal medulla	(27)			(29)
Hyperplasia	24 (89%)			21 (72%)
Islets, pancreatic	(52)			(56)
Hyperplasia	1 (2%)			1 (2%)
Parathyroid gland	(50)			(54)
Hyperplasia	37 (74%)			39 (72%)
Pituitary gland	(52)			(56)
Pars distalis, cyst	6 (12%)			
Pars distalis, hemorrhage				2 (4%)
Pars distalis, hyperplasia	13 (25%)			10 (18%)
Pars distalis, vacuolization cytoplasmic				2 (4%)
Thyroid gland	(51)			(56)
Ultimobranchial cyst				1 (2%)
C-cell, hyperplasia	12 (24%)			11 (20%)
Follicle, cyst				1 (2%)
Follicular cell, hyperplasia	1 (2%)			
General Body System				
None				
Genital System				
Epididymis	(52)			(56)
Aspermia				1 (2%)
Inflammation, granulomatous				1 (2%)
Preputial gland	(52)			(56)
Inflammation, chronic active	41 (79%)			36 (64%)
Duct, ectasia	1 (2%)			2 (4%)
Prostate	(52)			(56)
Hyperplasia				1 (2%)
Inflammation, chronic active	26 (50%)			24 (43%)
Inflammation, granulomatous				1 (2%)
Epithelium, hyperplasia	1 (2%)			
Seminal vesicle	(52)			(56)
Atrophy				1 (2%)
Inflammation, chronic active	1 (2%)			2 (4%)
Mineralization	1 (2%)			
Testes	(52)			(56)
Cyst				1 (2%)
Germinal epithelium, degeneration	16 (31%)			16 (29%)
Interstitial cell, hyperplasia	9 (17%)			10 (18%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Hematopoietic System				
Bone marrow	(52)			(56)
Femoral, myelofibrosis	4 (8%)			4 (7%)
Lymph node	(7)			(14)
Mediastinal, congestion				1 (7%)
Mediastinal, inflammation, chronic active				1 (7%)
Lymph node, mandibular	(13)			(17)
Hyperplasia, lymphoid	1 (8%)			
Infiltration cellular, plasma cell				1 (6%)
Lymph node, mesenteric	(8)			(14)
Hyperplasia, lymphoid				1 (7%)
Inflammation, chronic active				1 (7%)
Spleen	(52)			(56)
Fibrosis	13 (25%)			7 (13%)
Infarct				1 (2%)
Thrombosis				1 (2%)
Capsule, thrombosis				1 (2%)
Thymus	(44)			(47)
Depletion lymphoid	1 (2%)			4 (9%)
Integumentary System				
Mammary gland	(49)			(50)
Hyperplasia, cystic	47 (96%)			48 (96%)
Duct, cyst				1 (2%)
Skin, control	(52)	(52)	(55)	(56)
Cyst epithelial inclusion				1 (2%)
Dermis, inflammation, chronic		1 (2%)		
Epithelial hyperplasia	7 (13%)	5 (10%)	2 (4%)	3 (5%)
Hyperplasia, basal cell			1 (2%)	
Ulcer				1 (2%)
Skin, site of application-no mass	(52)	(52)	(55)	(56)
Cyst				1 (2%)
Epithelial hyperplasia	1 (2%)		4 (7%)	12 (21%)
Sebaceous gland, hyperplasia			2 (4%)	2 (4%)
Skin, site of application-mass	(5)		(3)	(4)
Cyst epithelial inclusion	1 (20%)			2 (50%)
Dermis, fibrosis	3 (60%)			1 (25%)
Dermis, mineralization	2 (40%)			
Musculoskeletal System				
Bone	(52)			(56)
Tarsal, fracture				1 (2%)
Nervous System				
Brain	(52)			(56)
Compression	14 (27%)			13 (23%)
Hemorrhage	1 (2%)			
Hydrocephalus	10 (19%)			6 (11%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Respiratory System				
Lung	(51)			(56)
Inflammation, chronic active	3 (6%)			2 (4%)
Inflammation, granulomatous				1 (2%)
Inflammation, suppurative	1 (2%)			
Mineralization	1 (2%)			2 (4%)
Nose	(52)			(55)
Lumen, hemorrhage				1 (2%)
Mucosa, inflammation, chronic active	6 (12%)			6 (11%)
Mucosa, ulcer				1 (2%)
Sinus, foreign body	6 (12%)			2 (4%)
Trachea	(51)			(56)
Submucosa, inflammation, chronic				1 (2%)
Special Senses System				
Eye	(2)			(3)
Lens, cataract	2 (100%)			2 (67%)
Retina, atrophy	2 (100%)			2 (67%)
Urinary System				
Kidney	(52)			(56)
Cyst				1 (2%)
Nephropathy, chronic	52 (100%)			56 (100%)
Urinary bladder	(52)			(56)
Inflammation, chronic active	1 (2%)			
Transitional epithelium, hyperplasia	1 (2%)			

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR DERMAL STUDY OF BENZETHONIUM CHLORIDE

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	9	7	9	7
Early deaths				
Moribund	13	11	13	13
Natural deaths	14	9	12	16
Survivors				
Terminal sacrifice	24	33	26	24
Animals examined microscopically	60	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Mesentery	(1)			(1)
Fat, liposarcoma	1 (100%)			
Endocrine System				
Pituitary gland	(9)			(7)
Pars distalis, adenoma	1 (11%)			1 (14%)
Genital System				
Clitoral gland	(9)			(7)
Adenoma	1 (11%)			
Integumentary System				
Skin, control	(9)	(7)	(9)	(7)
Subcutaneous tissue, lipoma	1 (11%)			
Skin, site of application	(9)	(7)	(9)	(7)
Systems Examined With No Neoplasms Observed				
Cardiovascular System				
General Body System				
Hematopoietic System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study				
Alimentary System				
Intestine small, jejunum	(1)			
Liver	(51)			(53)
Pancreas	(51)			(53)
Salivary glands	(51)			(53)
Cardiovascular System				
None				
Endocrine System				
Adrenal cortex	(19)			(21)
Adenoma				1 (5%)
Adrenal medulla	(10)			(2)
Pheochromocytoma benign	3 (30%)			
Bilateral, pheochromocytoma benign				1 (50%)
Islets, pancreatic	(51)			(53)
Carcinoma				1 (2%)
Pituitary gland	(51)			(53)
Pars distalis, adenoma	27 (53%)			27 (51%)
Pars distalis, adenoma, multiple	1 (2%)			
Pars distalis, carcinoma	2 (4%)			2 (4%)
Thyroid gland	(51)			(53)
Bilateral, C-cell, adenoma	1 (2%)			
C-cell, adenoma	10 (20%)			5 (9%)
C-cell, carcinoma	1 (2%)			
General Body System				
None				
Genital System				
Clitoral gland	(50)			(52)
Adenoma	3 (6%)			4 (8%)
Carcinoma				2 (4%)
Ovary	(51)			(53)
Uterus	(51)			(53)
Polyp stromal	3 (6%)			7 (13%)
Hematopoietic System				
Bone marrow	(51)			(52)
Lymph node	(5)			(1)
Lymph node, mandibular	(7)			(3)
Osteosarcoma, metastatic, bone				1 (33%)
Lymph node, mesenteric	(5)			(3)
Spleen	(51)			(53)
Thymus	(46)			(50)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Integumentary System				
Mammary gland	(51)			(53)
Adenoma	2 (4%)			2 (4%)
Adenoma, multiple	1 (2%)			
Carcinoma				1 (2%)
Fibroadenoma	14 (27%)			12 (23%)
Fibroadenoma, multiple	3 (6%)			5 (9%)
Fibroma				1 (2%)
Skin, control	(51)	(53)	(51)	(53)
Basosquamous tumor malignant				1 (2%)
Basosquamous tumor benign	1 (2%)			
Trichoepithelioma			1 (2%)	
Subcutaneous tissue, lipoma				1 (2%)
Subcutaneous tissue, neurofibrosarcoma			1 (2%)	
Skin, site of application-no mass	(51)	(53)	(51)	(53)
Musculoskeletal System				
Bone	(51)			(53)
Mandible, osteosarcoma				1 (2%)
Nervous System				
Brain	(51)			(53)
Carcinoma, metastatic, pituitary gland	2 (4%)			2 (4%)
Respiratory System				
Lung	(51)			(53)
Carcinoma, metastatic, thyroid gland	1 (2%)			
Nose	(51)			(53)
Nares, squamous cell papilloma	1 (2%)			
Vomeronasal organ, squamous cell carcinoma	1 (2%)			
Special Senses System				
None				
Urinary System				
Kidney	(51)			(53)
Urinary bladder	(51)			(53)
Systemic Lesions				
Multiple organs[b]	(51)	(53)	(51)	(53)
Leukemia mononuclear	18 (35%)			18 (34%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]				
15-Month interim evaluation	3			1
2-Year study	43		2	49
Total primary neoplasms				
15-Month interim evaluation	4			1
2-Year study	92		2	92
Total animals with benign neoplasms				
15-Month interim evaluation	3			1
2-Year study	38		1	41
Total benign neoplasms				
15-Month interim evaluation	3			1
2-Year study	70		1	66
Total animals with malignant neoplasms				
15-Month interim evaluation	1			
2-Year study	22		1	21
Total malignant neoplasms				
15-Month interim evaluation	1			
2-Year study	22		1	26
Total animals with metastatic neoplasms				
2-Year study	3			3
Total metastatic neoplasms				
2-Year study	3			3

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control

Number of Days on Study	1 7 6	4 8 5	5 1 5	5 1 9	5 2 6	5 5 4	5 7 2	5 9 5	6 0 3	6 1 0	6 1 2	6 3 3	6 4 2	6 4 2	6 4 4	6 4 5	6 4 8	6 4 9	6 6 7	6 9 6	7 0 9	7 1 8	7 1 9	7 2 0	7 2 2
Carcass ID Number	2 5 7	2 7 5	2 9 7	2 5 1	2 8 1	2 7 2	2 4 7	2 7 6	2 9 4	3 6 1	2 0 0	2 7 3	2 6 6	2 9 2	2 6 6	2 7 9	2 4 3	2 5 9	2 8 3	2 4 9	2 9 9	2 9 1	2 8 4	2 7 3	2 9 5
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum									+														+		
Intestine small, jejunum												+													
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery								+																+	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach		+		+					+													+			
Stomach, glandular		+																							
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex		+					+		+			+	+					+	+			+	+	+	+
Adrenal medulla												+	+					+					+		
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	M	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X	X	X	X	X							X		X		X	X	X	X				X
Pars distalis, adenoma, multiple																									
Pars distalis, carcinoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma			X				X												X				X	X	
C-cell, carcinoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Adenoma							X																		
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal		X																							
Vagina																							+		
Hematopoietic System																									
Blood									+												+				
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+								+	+												+		
Lymph node, mandibular		+								+	+	+					+					+			

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	7 2 4	7 2 4	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	2 4 2	2 5 3	2 4 1	2 4 4	2 4 5	2 4 6	2 5 2	2 5 5	2 5 6	2 5 8	2 6 4	2 6 5	2 6 8	2 6 9	2 7 1	2 7 4	2 7 7	2 7 8	2 8 0	2 8 2	2 8 5	2 8 6	2 8 7	2 8 9	2 9 0	2 9 3	Total Tissues/ Tumors
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine small, duodenum																											2
Intestine small, jejunum																											1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Mesentery				+																							3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Stomach, forestomach	+																	+									6
Stomach, glandular																											1
Cardiovascular System																											
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Endocrine System																											
Adrenal cortex	+		+	+		+			+		+				+					+							19
Adrenal medulla						+														+	+	+		+	+		10
Pheochromocytoma benign						X														X	X						3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Pars distalis, adenoma	X			X		X	X	X							X		X	X	X	X	X	X	X	X	X		27
Pars distalis, adenoma, multiple														X													1
Pars distalis, carcinoma		X																								X	2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Bilateral, C-cell, adenoma																			X								1
C-cell, adenoma							X				X											X		X	X		10
C-cell, carcinoma																								X			1
General Body System																											
None																											
Genital System																											
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma								X														X					3
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Polyp stromal				X												X											3
Vagina																											1
Hematopoietic System																											
Blood																											2
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymph node																+											5
Lymph node, mandibular	+																										7

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	1 7 6	4 8 5	5 1 5	5 1 9	5 2 6	5 5 4	5 7 2	5 9 5	6 0 3	6 1 0	6 1 2	6 3 3	6 4 2	6 4 2	6 4 4	6 4 5	6 4 8	6 4 9	6 6 7	6 9 6	7 0 9	7 1 8	7 1 9	7 2 0	7 2 2
Carcass ID Number	2 5 7	2 7 5	2 9 7	2 5 1	2 8 1	2 7 2	2 4 7	2 7 6	2 9 4	2 6 1	3 0 0	2 7 0	2 6 3	2 9 6	2 6 2	2 7 9	2 4 3	2 5 9	2 8 3	2 4 9	2 9 9	2 9 1	2 8 4	2 7 3	2 9 5
Hematopoietic System (continued)																									
Lymph node, mesenteric		+								+	+	+										+			
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	M
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																	X								
Adenoma, multiple																									
Fibroadenoma							X											X			X	X	X	X	X
Fibroadenoma, multiple																									
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basosquamous tumor benign																									X
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, pituitary gland																									
Peripheral nerve									+																
Spinal cord									+																
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nares, squamous cell papilloma																X									
Vomeronasal organ, squamous cell carcinoma									X																
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye						+						+	+												
Harderian gland	+																								
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X								X	X	X					X		X	X	X	X	X	X	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	7 2 4	7 2 4	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	2 4 2	2 5 3	2 4 1	2 4 4	2 4 5	2 4 6	2 5 2	2 5 5	2 5 6	2 5 8	2 6 4	2 6 5	2 6 8	2 6 9	2 7 1	2 7 4	2 7 7	2 7 8	2 8 0	2 8 2	2 8 5	2 8 6	2 8 7	2 8 9	2 9 0	2 9 3	Total Tissues/ Tumors
Hematopoietic System (continued)																											
Lymph node, mesenteric																											5
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	46
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Adenoma																				X							2
Adenoma, multiple		X																									1
Fibroadenoma					X									X			X	X		X			X			X	14
Fibroadenoma, multiple							X								X										X		3
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Basosquamous tumor benign																											1
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Carcinoma, metastatic, pituitary gland		X																								X	2
Peripheral nerve																											1
Spinal cord																											1
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Carcinoma, metastatic, thyroid gland																								X			1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Nares, squamous cell papilloma																											1
Vomeronasal organ, squamous cell carcinoma																											1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Special Senses System																											
Eye								+				+										+					6
Harderian gland																											1
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Leukemia mononuclear	X				X	X	X						X			X							X				18

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Dermal Study of Benzethonium Chloride: 0.15 mg/kg

Number of Days on Study	3	3	4	5	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7
	6	9	5	1	3	3	5	8	0	1	1	3	3	5	7	8	8	9	1	2	3	3	3	3	3
	6	3	6	9	0	8	1	2	2	2	2	3	8	9	7	0	4	6	0	7	0	0	0	0	0
Carcass ID Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	3	3	2	6	0	2	0	4	0	3	4	3	2	1	4	4	5	0	0	5	0	0	0	1	1
	7	2	0	0	5	6	2	2	3	8	5	9	1	6	9	1	5	8	1	3	6	7	9	0	1
Alimentary System																									
None																									
Cardiovascular System																									
None																									
Endocrine System																									
None																									
General Body System																									
None																									
Genital System																									
None																									
Hematopoietic System																									
None																									
Integumentary System																									
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
None																									
Nervous System																									
None																									
Respiratory System																									
None																									
Special Senses System																									
None																									
Urinary System																									
None																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Dermal Study of Benzethonium Chloride: 0.15 mg/kg
(continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	3 1 2	3 1 3	3 1 4	3 1 5	3 1 8	3 1 9	3 2 2	3 2 3	3 2 4	3 2 5	3 2 7	3 2 9	3 3 0	3 3 1	3 3 3	3 3 4	3 3 6	3 4 0	3 4 3	3 4 6	3 4 7	3 5 1	3 5 2	3 5 4	3 5 6	3 5 7	3 5 8	3 5 9	Total Tissues/ Tumors
Alimentary System																													
None																													
Cardiovascular System																													
None																													
Endocrine System																													
None																													
General Body System																													
None																													
Genital System																													
None																													
Hematopoietic System																													
None																													
Integumentary System																													
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Musculoskeletal System																													
None																													
Nervous System																													
None																													
Respiratory System																													
None																													
Special Senses System																													
None																													
Urinary System																													
None																													
Systemic Lesions																													
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Dermal Study of Benzethonium Chloride: 0.5 mg/kg

Number of Days on Study	2	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7		
	9	6	9	3	4	6	7	7	7	8	0	1	3	6	7	7	8	8	9	9	0	0	1	2	2
	7	5	2	3	9	5	8	9	9	1	6	0	2	8	8	9	5	6	4	8	4	8	2	0	5
Carcass ID Number	3	4	4	3	3	4	4	3	4	3	3	3	3	4	3	3	4	3	3	3	4	3	3	4	4
	7	0	0	7	6	1	0	6	0	9	8	7	7	1	7	6	0	6	9	9	1	7	8	0	1
	6	3	2	0	2	1	0	5	8	0	3	8	5	6	2	1	4	8	9	6	0	7	4	9	7
Alimentary System																									
None																									
Cardiovascular System																									
None																									
Endocrine System																									
None																									
General Body System																									
None																									
Genital System																									
None																									
Hematopoietic System																									
None																									
Integumentary System																									
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trichoepithelioma																									
Subcutaneous tissue, neurofibrosarcoma																									
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
None																									
Nervous System																									
None																									
Respiratory System																									
None																									
Special Senses System																									
None																									
Urinary System																									
None																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Dermal Study of Benzethonium Chloride: 0.5 mg/kg
(continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	3 6 3	3 6 4	3 6 6	3 7 4	3 7 9	3 8 0	3 8 1	3 8 2	3 8 5	3 8 6	3 9 1	3 9 2	3 9 3	3 9 4	3 9 5	3 9 7	3 9 8	4 0 1	4 0 5	4 0 6	4 0 7	4 1 2	4 1 4	4 1 5	4 1 9	4 2 0	Total Tissues/ Tumors
Alimentary System																											
None																											
Cardiovascular System																											
None																											
Endocrine System																											
None																											
General Body System																											
None																											
Genital System																											
None																											
Hematopoietic System																											
None																											
Integumentary System																											
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Trichoepithelioma																						X					1
Subcutaneous tissue, neurofibrosarcoma																			X								1
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Musculoskeletal System																											
None																											
Nervous System																											
None																											
Respiratory System																											
None																											
Special Senses System																											
None																											
Urinary System																											
None																											
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg

Number of Days on Study	3 3 3 4 5 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 0 2 5 8 2 3 3 6 7 8 8 9 0 1 1 3 3 3 3 4 4 5 6 6 7 3 2 8 6 5 3 7 2 6 7 7 4 3 3 9 2 4 4 6 0 7 8 7 8 7
Carcass ID Number	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 5 6 8 5 4 6 6 7 6 2 4 6 7 7 4 5 3 6 6 4 3 5 5 2 7 2 0 0 7 5 2 4 9 8 8 6 1 2 7 7 1 5 3 9 1 9 6 0 2 3
Alimentary System	
Esophagus	+ +
Liver	+ +
Mesentery	
Pancreas	+ +
Salivary glands	+ +
Stomach, forestomach	+ + + + + +
Tongue	+
Cardiovascular System	
Heart	+ +
Endocrine System	
Adrenal cortex	+ + + + + +
Adenoma	X
Adrenal medulla	+
Bilateral, pheochromocytoma benign	X
Islets, pancreatic	+ +
Carcinoma	
Parathyroid gland	+ M + + M + + + + + + + + + + + + + + M + + + +
Pituitary gland	+ +
Pars distalis, adenoma	X X X X X X X X X X
Pars distalis, carcinoma	X
Thyroid gland	+ +
C-cell, adenoma	X
General Body System	
None	
Genital System	
Clitoral gland	+ M
Adenoma	
Carcinoma	
Ovary	+ +
Uterus	+ +
Polyp stromal	X X X
Hematopoietic System	
Bone marrow	+ + + + + + + M + + + + + + + + + + + + + + + +
Lymph node	
Lymph node, mandibular	+
Osteosarcoma, metastatic, bone	
Lymph node, mesenteric	+ + +
Spleen	+ +
Thymus	+ +

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	6 8 0	6 9 0	7 0 0	7 2 3	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	4 4 0	4 4 3	4 4 4	4 6 5	4 2 1	4 2 3	4 2 4	4 2 5	4 2 6	4 2 9	4 3 1	4 3 3	4 3 6	4 3 7	4 3 8	4 4 2	4 4 4	4 4 8	4 5 3	4 5 4	4 5 5	4 5 9	4 6 6	4 6 7	4 7 0	4 7 1	4 7 5	4 7 6	Total Tissue/ Tumors
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Mesentery				+														+					+					3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, forestomach				+																								8
Tongue																												1
Cardiovascular System																												
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Endocrine System																												
Adrenal cortex				+	+		+	+	+	+						+		+	+	+	+		+		+			21
Adenoma																												1
Adrenal medulla																				+								2
Bilateral, pheochromocytoma benign																												1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma		X																										1
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pars distalis, adenoma		X	X		X	X		X	X	X			X			X	X		X		X					X		27
Pars distalis, carcinoma				X																								2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
C-cell, adenoma															X		X			X		X						5
General Body System																												
None																												
Genital System																												
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adenoma																X			X			X				X		4
Carcinoma																				X			X					2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Polyp stromal							X												X			X				X		7
Hematopoietic System																												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Lymph node									+																			1
Lymph node, mandibular	+								+																			3
Osteosarcoma, metastatic, bone	X																											1
Lymph node, mesenteric																												3
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Thymus	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	3 0 3	3 2 2	3 5 8	4 8 6	5 2 5	5 3 3	5 3 7	5 6 2	5 7 6	5 8 7	5 8 7	5 9 4	6 0 3	6 1 3	6 1 9	6 3 2	6 3 4	6 3 4	6 3 6	6 4 0	6 4 7	6 5 8	6 6 7	6 6 8	6 7 7
Carcass ID Number	4 5 2	4 6 0	4 8 0	4 5 7	4 4 5	4 6 2	4 6 4	4 7 9	4 6 8	4 2 8	4 4 6	4 6 1	4 7 2	4 7 7	4 4 7	4 5 1	4 3 5	4 6 3	4 6 9	4 4 1	4 3 9	4 5 6	4 5 0	4 2 2	4 7 3
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																						X			
Carcinoma																									
Fibroadenoma					X	X									X						X				X
Fibroadenoma, multiple																									
Fibroma																									
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basosquamous tumor malignant																									
Subcutaneous tissue, lipoma																									
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mandible, osteosarcoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, pituitary gland								X																	
Peripheral nerve										+	+					+	+								
Spinal cord										+	+					+	+								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye										+								+							
Harderian gland																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X									X	X					X				X		X			

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	6 8 0	6 9 9	7 0 0	7 2 3	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	4 4 0	4 4 3	4 4 4	4 6 5	4 2 1	4 2 3	4 2 4	4 2 5	4 2 6	4 2 9	4 3 1	4 3 3	4 3 6	4 3 7	4 3 8	4 4 2	4 4 4	4 4 8	4 5 3	4 5 4	4 5 5	4 5 9	4 6 6	4 6 7	4 7 0	4 7 1	4 7 5	4 7 6	Total Tissues/ Tumors
Integumentary System																												
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma																				X									2
Carcinoma													X																1
Fibroadenoma							X	X	X					X		X				X								X	12
Fibroadenoma, multiple						X				X							X	X								X			5
Fibroma											X																		1
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Basosquamous tumor malignant												X																	1
Subcutaneous tissue, lipoma																									X				1
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Mandible, osteosarcoma	X																												1
Nervous System																												
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, pituitary gland				X																									2
Peripheral nerve																													4
Spinal cord																													4
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Special Senses System																												
Eye		+								+																			4
Harderian gland	+																												1
Urinary System																												
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Leukemia mononuclear		X		X	X		X		X				X	X		X		X		X		X		X					18

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of Benzethonium Chloride

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	3/51 (6%)	–[e]	–	1/53 (2%)
Adjusted rate[b]	12.5%			2.7%
Terminal rate[c]	3/24 (13%)			0/24 (0%)
First incidence (days)	729 (T)			634
Life table test[d]				P=0.310N
Logistic regression test[d]				P=0.325N
Fisher exact test[d]				P=0.294N
Clitoral Gland: Adenoma				
Overall rate	3/50 (6%)	–	–	4/52 (8%)
Adjusted rate	10.4%			16.7%
Terminal rate	2/24 (8%)			4/24 (17%)
First incidence (days)	572			729 (T)
Life table test				P=0.500
Logistic regression test				P=0.529
Fisher exact test				P=0.522
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	–	–	6/52 (12%)
Adjusted rate	10.4%			25.0%
Terminal rate	2/24 (8%)			6/24 (25%)
First incidence (days)	572			729 (T)
Life table test				P=0.237
Logistic regression test				P=0.257
Fisher exact test				P=0.264
Mammary Gland: Adenoma				
Overall rate	3/51 (6%)	–	–	2/53 (4%)
Adjusted rate	10.5%			7.2%
Terminal rate	1/24 (4%)			1/24 (4%)
First incidence (days)	648			658
Life table test				P=0.518N
Logistic regression test				P=0.518N
Fisher exact test				P=0.482N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	3/51 (6%)	–	–	3/53 (6%)
Adjusted rate	10.5%			11.2%
Terminal rate	1/24 (4%)			2/24 (8%)
First incidence (days)	648			658
Life table test				P=0.645
Logistic regression test				P=0.640
Fisher exact test				P=0.642N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Mammary Gland: Fibroadenoma				
Overall rate	17/51 (33%)	–	–	17/53 (32%)
Adjusted rate	53.6%			56.2%
Terminal rate	10/24 (42%)			12/24 (50%)
First incidence (days)	572			525
Life table test				P=0.545
Logistic regression test				P=0.517
Fisher exact test				P=0.529N
Mammary Gland: Fibroma, Fibroadenoma, or Adenoma				
Overall rate	19/51 (37%)	–	–	19/53 (36%)
Adjusted rate	56.6%			61.1%
Terminal rate	10/24 (42%)			13/24 (54%)
First incidence (days)	572			525
Life table test				P=0.535
Logistic regression test				P=0.514
Fisher exact test				P=0.522N
Mammary Gland: Fibroma, Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	19/51 (37%)	–	–	20/53 (38%)
Adjusted rate	56.6%			64.7%
Terminal rate	10/24 (42%)			14/24 (58%)
First incidence (days)	572			525
Life table test				P=0.458
Logistic regression test				P=0.424
Fisher exact test				P=0.560
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	28/51 (55%)	–	–	27/53 (51%)
Adjusted rate	73.9%			65.5%
Terminal rate	15/24 (63%)			11/24 (46%)
First incidence (days)	515			486
Life table test				P=0.550N
Logistic regression test				P=0.533N
Fisher exact test				P=0.418N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	30/51 (59%)	–	–	29/53 (55%)
Adjusted rate	77.8%			67.6%
Terminal rate	16/24 (67%)			11/24 (46%)
First incidence (days)	515			486
Life table test				P=0.552N
Logistic regression test				P=0.546N
Fisher exact test				P=0.411N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Thyroid Gland (C-cell): Adenoma				
Overall rate	11/51 (22%)	–	–	5/53 (9%)
Adjusted rate	35.1%			18.8%
Terminal rate	6/24 (25%)			4/24 (17%)
First incidence (days)	515			613
Life table test				P=0.099N
Logistic regression test				P=0.095N
Fisher exact test				P=0.074N
Uterus: Stromal Polyp				
Overall rate	3/51 (6%)	–	–	7/53 (13%)
Adjusted rate	10.2%			22.0%
Terminal rate	2/24 (8%)			4/24 (17%)
First incidence (days)	485			533
Life table test				P=0.166
Logistic regression test				P=0.181
Fisher exact test				P=0.176
All Organs: Mononuclear Cell Leukemia				
Overall rate	18/51 (35%)	–	–	18/53 (34%)
Adjusted rate	47.8%			53.8%
Terminal rate	6/24 (25%)			10/24 (42%)
First incidence (days)	485			303
Life table test				P=0.516
Logistic regression test				P=0.574N
Fisher exact test				P=0.525N
All Organs: Benign Neoplasms				
Overall rate	39/51 (76%)	–	–	42/53 (79%)
Adjusted rate	88.4%			93.0%
Terminal rate	19/24 (79%)			21/24 (88%)
First incidence (days)	485			486
Life table test				P=0.323
Logistic regression test				P=0.354
Fisher exact test				P=0.458
All Organs: Malignant Neoplasms				
Overall rate	23/51 (45%)	–	–	21/53 (40%)
Adjusted rate	59.2%			59.5%
Terminal rate	9/24 (38%)			11/24 (46%)
First incidence (days)	485			303
Life table test				P=0.494N
Logistic regression test				P=0.413N
Fisher exact test				P=0.357N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	43/51 (84%)	–	–	49/53 (92%)
Adjusted rate	91.4%			98.0%
Terminal rate	20/24 (83%)			23/24 (96%)
First incidence (days)	485			303
Life table test				P=0.191
Logistic regression test				P=0.105
Fisher exact test				P=0.161

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, clitoral gland, pituitary gland, thyroid gland, and uterus; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Fisher exact test compares directly the overall incidence rates. For all tests, a lower incidence in a dose group is indicated by N.
[e] Organ was not examined at this dose level

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	9	7	9	7
Early deaths				
Moribund	13	11	13	13
Natural deaths	14	9	12	16
Survivors				
Terminal sacrifice	24	33	26	24
Animals examined microscopically	60	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Liver	(9)			(7)
Hepatodiaphragmatic nodule	2 (22%)			3 (43%)
Hepatodiaphragmatic nodule, multiple	1 (11%)			
Inflammation, chronic active	2 (22%)			2 (29%)
Mesentery	(1)			(1)
Fat, inflammation, chronic active				1 (100%)
Pancreas	(9)			(7)
Acinus, atrophy				2 (29%)
Cardiovascular System				
Heart	(9)			(7)
Degeneration, chronic	1 (11%)			3 (43%)
Endocrine System				
Pituitary gland	(9)			(7)
Craniopharyngeal duct, pars distalis, cyst				1 (14%)
Pars distalis, cyst	4 (44%)			1 (14%)
Pars distalis, hyperplasia	3 (33%)			3 (43%)
Thyroid gland	(9)			(7)
Bilateral, ultimobranchial cyst				1 (14%)
C-cell, hyperplasia	1 (11%)			
Genital System				
Clitoral gland	(9)			(7)
Inflammation, chronic active	1 (11%)			
Duct, cyst	1 (11%)			
Ovary	(9)			(7)
Periovarian tissue, cyst	3 (33%)			3 (43%)
Uterus	(9)			(7)
Endometrium, hyperplasia, cystic, glandular				1 (14%)
Hematopoietic System				
Thymus	(8)			(6)
Angiectasis				1 (17%)
Depletion lymphoid	8 (100%)			6 (100%)

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

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
15-Month Interim Evaluation (continued)				
Integumentary System				
Mammary gland	(9)			(7)
Hyperplasia, cystic	8 (89%)			5 (71%)
Skin, control	(9)	(7)	(9)	(7)
Skin, site of application-no mass	(9)	(7)	(9)	(7)
Epithelial hyperplasia		1 (14%)	2 (22%)	6 (86%)
Erosion, focal				1 (14%)
Ulcer		1 (14%)	1 (11%)	4 (57%)
Sebaceous gland, hyperplasia		1 (14%)	1 (11%)	6 (86%)
Respiratory System				
Nose	(9)			(7)
Submucosa, inflammation, chronic	1 (11%)			
Urinary System				
Kidney	(9)			(7)
Mineralization	7 (78%)			7 (100%)
Nephropathy, chronic	3 (33%)			5 (71%)
Systems Examined With No Lesions Observed				
General Body System				
Musculoskeletal System				
Nervous System				
Special Senses System				
2-Year Study				
Alimentary System				
Intestine small, duodenum	(2)			
Ulcer	2 (100%)			
Liver	(51)			(53)
Angiectasis	2 (4%)			
Basophilic focus	26 (51%)			33 (62%)
Clear cell focus	2 (4%)			7 (13%)
Eosinophilic focus	13 (25%)			13 (25%)
Hepatodiaphragmatic nodule	7 (14%)			15 (28%)
Inflammation, granulomatous	8 (16%)			11 (21%)
Bile duct, hyperplasia	11 (22%)			13 (25%)
Hepatocyte, degeneration, cystic				2 (4%)
Hepatocyte, hypertrophy, focal				1 (2%)
Hepatocyte, necrosis	1 (2%)			
Hepatocyte, vacuolization cytoplasmic	7 (14%)			8 (15%)
Mesentery	(3)			(3)
Fat, inflammation, chronic active	3 (100%)			3 (100%)
Pancreas	(51)			(53)
Inflammation, chronic active				1 (2%)
Acinus, atrophy	15 (29%)			15 (28%)
Stomach, forestomach	(6)			(8)
Acanthosis				3 (38%)
Inflammation, chronic active				1 (13%)
Mineralization				1 (13%)
Ulcer	6 (100%)			5 (63%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Stomach, glandular	(1)			
Necrosis	1 (100%)			
Tongue				(1)
Cyst				1 (100%)
Cardiovascular System				
Heart	(51)			(53)
Degeneration, chronic	28 (55%)			30 (57%)
Atrium, thrombosis	3 (6%)			3 (6%)
Endocrine System				
Adrenal cortex	(19)			(21)
Degeneration, cystic	1 (5%)			1 (5%)
Hyperplasia	7 (37%)			12 (57%)
Adrenal cortex (continued)				
Hypertrophy				1 (5%)
Necrosis	2 (11%)			2 (10%)
Vacuolization cytoplasmic	8 (42%)			9 (43%)
Adrenal medulla	(10)			(2)
Hyperplasia	6 (60%)			1 (50%)
Parathyroid gland	(47)			(47)
Hyperplasia	32 (68%)			32 (68%)
Pituitary gland	(51)			(53)
Necrosis	1 (2%)			
Pars distalis, cyst	7 (14%)			11 (21%)
Pars distalis, hyperplasia	14 (27%)			17 (32%)
Thyroid gland	(51)			(53)
C-cell, hyperplasia	23 (45%)			17 (32%)
Follicular cell, hyperplasia				2 (4%)
General Body System				
None				
Genital System				
Clitoral gland	(50)			(52)
Hyperplasia	5 (10%)			4 (8%)
Inflammation, chronic active	1 (2%)			1 (2%)
Duct, ectasia	1 (2%)			2 (4%)
Ovary	(51)			(53)
Follicle, cyst	4 (8%)			7 (13%)
Periovarian tissue, cyst	5 (10%)			10 (19%)
Uterus	(51)			(53)
Endometrium, hyperplasia, cystic, glandular	3 (6%)			2 (4%)
Vagina	(1)			
Lumen, hemorrhage	1 (100%)			

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Hematopoietic System				
Blood	(2)			
Erythrocyte, atypia cellular	1 (50%)			
Bone marrow	(51)			(52)
Femoral, myelofibrosis	1 (2%)			
Lymph node, mandibular	(7)			(3)
Thrombosis	1 (14%)			
Lymph node, mesenteric	(5)			(3)
Angiectasis				2 (67%)
Spleen	(51)			(53)
Fibrosis	1 (2%)			2 (4%)
Hematopoietic cell proliferation	1 (2%)			2 (4%)
Thymus	(46)			(50)
Cyst				1 (2%)
Depletion lymphoid				3 (6%)
Integumentary System				
Mammary gland	(51)			(53)
Hyperplasia, cystic	50 (98%)			51 (96%)
Skin, control	(51)	(53)	(51)	(53)
Epithelial hyperplasia		3 (6%)	2 (4%)	
Sebaceous gland, hyperplasia		1 (2%)	1 (2%)	
Skin, site of application-no mass	(51)	(53)	(51)	(53)
Epithelial hyperplasia	2 (4%)	2 (4%)	6 (12%)	32 (60%)
Ulcer		1 (2%)	3 (6%)	19 (36%)
Sebaceous gland, hyperplasia	1 (2%)	2 (4%)	6 (12%)	30 (57%)
Musculoskeletal System				
Bone	(51)			(53)
Femur, hyperostosis	2 (4%)			2 (4%)
Femur, osteopetrosis				1 (2%)
Nervous System				
Brain	(51)			(53)
Compression	21 (41%)			17 (32%)
Hemorrhage	1 (2%)			
Hydrocephalus	8 (16%)			6 (11%)
Respiratory System				
Lung	(51)			(53)
Inflammation, chronic active	2 (4%)			2 (4%)
Alveolar epithelium, hyperplasia				1 (2%)
Perivascular, inflammation, chronic	1 (2%)			
Nose	(51)			(53)
Mucosa, inflammation, chronic active	4 (8%)			5 (9%)
Sinus, foreign body	3 (6%)			5 (9%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Special Senses System				
Eye	(6)			(4)
Phthisis bulbi	1 (17%)			
Lens, cataract	5 (83%)			4 (100%)
Retina, atrophy	5 (83%)			4 (100%)
Harderian gland	(1)			(1)
Inflammation, chronic active	1 (100%)			1 (100%)
Urinary System				
Kidney	(51)			(53)
Cyst	1 (2%)			
Mineralization	1 (2%)			2 (4%)
Nephropathy, chronic	45 (88%)			48 (91%)
Urinary bladder	(51)			(53)
Transitional epithelium, hyperplasia				1 (2%)

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR DERMAL STUDY OF BENZETHONIUM CHLORIDE

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	9	9	10
Early deaths				
Moribund	3	8	4	9
Natural deaths	4	4	4	2
Survivors				
Terminal sacrifice	43	38	42	39
Missexed		1	1	
Animals examined microscopically	60	59	59	60
15-Month Interim Evaluation				
Alimentary System				
Liver	(10)			(10)
Hepatocellular carcinoma	1 (10%)			2 (20%)
Hepatocellular carcinoma, multiple	1 (10%)			
Hepatocellular adenoma	4 (40%)			4 (40%)
Integumentary System				
Skin, control	(10)	(9)	(9)	(10)
Skin, site of application	(10)	(9)	(9)	(10)
Special Senses System				
Harderian gland				(1)
Adenoma				1 (100%)
Systems Examined With No Neoplasms Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Urinary System				
2-Year Study				
Alimentary System				
Intestine large, cecum	(50)			(50)
Leiomyosarcoma				1 (2%)
Intestine small, duodenum	(50)			(50)
Adenoma				1 (2%)
Intestine small, jejunum	(50)			(50)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Liver	(50)			(50)
Hemangioma				1 (2%)
Hemangiosarcoma	2 (4%)			
Hemangiosarcoma, multiple	1 (2%)			2 (4%)
Hemangiosarcoma, metastatic, skin	1 (2%)			
Hemangiosarcoma, metastatic, spleen				1 (2%)
Hepatoblastoma				1 (2%)
Hepatocellular carcinoma	9 (18%)			12 (24%)
Hepatocellular carcinoma, multiple	1 (2%)			2 (4%)
Hepatocellular adenoma	11 (22%)			12 (24%)
Hepatocellular adenoma, multiple	13 (26%)			13 (26%)
Histiocytic sarcoma				1 (2%)
Mesentery	(4)			(4)
Hemangioma	1 (25%)			1 (25%)
Histiocytic sarcoma				1 (25%)
Stomach, glandular	(50)			(50)
Carcinoid tumor malignant				1 (2%)
Cardiovascular System				
Heart	(50)			(50)
Hemangiosarcoma, metastatic, liver	1 (2%)			1 (2%)
Endocrine System				
Adrenal cortex	(50)			(50)
Histiocytic sarcoma				1 (2%)
Adrenal medulla	(50)			(50)
Pheochromocytoma benign	1 (2%)			
Islets, pancreatic	(49)			(50)
Adenoma	2 (4%)			1 (2%)
Pituitary gland	(48)			(48)
Histiocytic sarcoma				1 (2%)
Thyroid gland	(50)			(50)
Hemangiosarcoma, metastatic, spleen				1 (2%)
Follicular cell, adenoma				1 (2%)
Follicular cell, carcinoma	1 (2%)			1 (2%)
General Body System				
None				
Genital System				
Epididymis	(50)			(49)
Histiocytic sarcoma				1 (2%)
Testes	(50)			(50)
Interstitial cell, adenoma	1 (2%)			1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Hematopoietic System				
Bone marrow	(50)			(50)
Hemangiosarcoma, metastatic, liver	1 (2%)			
Hemangiosarcoma, metastatic, skin	1 (2%)			
Hemangiosarcoma, metastatic, spleen				1 (2%)
Histiocytic sarcoma				1 (2%)
Lymph node	(3)			(2)
Lymph node, mandibular	(49)			(48)
Lymph node, mesenteric	(48)			(47)
Spleen	(50)			(50)
Hemangiosarcoma				3 (6%)
Hemangiosarcoma, metastatic, liver	1 (2%)			
Histiocytic sarcoma				1 (2%)
Thymus	(31)			(39)
Integumentary System				
Skin, control	(50)	(50)	(50)	(50)
Melanoma benign				1 (2%)
Subcutaneous tissue, hemangioma	1 (2%)			
Subcutaneous tissue, hemangiosarcoma	1 (2%)			1 (2%)
Subcutaneous tissue, hemangiosarcoma, metastatic, spleen				1 (2%)
Skin, site of application-no mass	(50)	(50)	(50)	(50)
Musculoskeletal System				
None				
Nervous System				
Brain	(50)			(50)
Histiocytic sarcoma				1 (2%)
Respiratory System				
Lung	(50)			(50)
Alveolar/bronchiolar adenoma	11 (22%)			7 (14%)
Alveolar/bronchiolar adenoma, multiple	2 (4%)			1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)			
Alveolar/bronchiolar carcinoma, multiple				2 (4%)
Carcinoma, metastatic, harderian gland	1 (2%)			
Carcinoma, metastatic, thyroid gland				1 (2%)
Hepatocellular carcinoma, metastatic, liver	2 (4%)			5 (10%)
Histiocytic sarcoma				1 (2%)
Nose	(50)			(50)
Glands, carcinoma				1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Special Senses System				
Ear	(1)			(2)
Fibrosarcoma	1 (100%)			1 (50%)
Harderian gland	(43)			(37)
Adenoma	2 (5%)			2 (5%)
Carcinoma	1 (2%)			
Urinary System				
Kidney	(50)			(50)
Histiocytic sarcoma				1 (2%)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Lymphoma malignant lymphocytic	1 (2%)			
Lymphoma malignant mixed	1 (2%)			3 (6%)
Lymphoma malignant undifferentiated cell	1 (2%)			
Neoplasm Summary				
Total animals with primary neoplasms[c]				
15-Month interim evaluation	5			4
2-Year study	43			40
Total primary neoplasms				
15-Month interim evaluation	6			7
2-Year study	66			74
Total animals with benign neoplasms				
15-Month interim evaluation	4			4
2-Year study	35			30
Total benign neoplasms				
15-Month interim evaluation	4			4
2-Year study	45			42
Total animals with malignant neoplasms				
15-Month interim evaluation	2			2
2-Year study	16			22
Total malignant neoplasms				
15-Month interim evaluation	2			2
2-Year study	21			32
Total animals with metastatic neoplasms				
2-Year study	4			8
Total metastatic neoplasms				
2-Year study	8			11

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control

Number of Days on Study	5 7 7	6 0 3	6 1 3	6 3 7	6 5 5	7 1 7	7 1 9	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3
Carcass ID Number	0 5 7	0 2 3	0 1 9	0 3 9	0 0 9	0 1 2	0 4 5	0 0 1	0 0 2	0 0 3	0 0 4	0 0 5	0 0 6	0 0 7	0 0 8	0 1 1	0 1 3	0 1 4	0 1 6	0 1 7	0 2 1	0 2 2	0 2 4	0 2 5	0 2 6
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma	X					X																			
Hemangiosarcoma, multiple							X																		
Hemangiosarcoma, metastatic, skin																									
Hepatocellular carcinoma		X				X								X		X				X	X		X		
Hepatocellular carcinoma, multiple							X																		
Hepatocellular adenoma		X				X			X	X												X	X		X
Hepatocellular adenoma, multiple											X	X	X	X						X					
Mesentery														+										+	
Hemangioma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, liver	X																								
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, carcinoma																			X						
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 2 8	0 3 0	0 3 1	0 3 2	0 3 4	0 3 5	0 3 6	0 3 7	0 4 0	0 4 1	0 4 2	0 4 3	0 4 6	0 4 7	0 4 8	0 4 9	0 5 0	0 5 2	0 5 3	0 5 4	0 5 5	0 5 6	0 5 8	0 5 9	0 6 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										2
Hemangiosarcoma, multiple																										1
Hemangiosarcoma, metastatic, skin																								X		1
Hepatocellular carcinoma			X							X																9
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma						X	X				X			X												11
Hepatocellular adenoma, multiple					X							X	X		X		X	X	X		X					13
Mesentery													+		+											4
Hemangioma													X													1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth														+												1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, liver																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																	X									1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma								X											X							2
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, carcinoma																										1
General Body System																										
None																										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	5 7 7	6 0 3	6 1 3	6 3 7	6 5 5	7 1 7	7 1 9	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3
Carcass ID Number	0 5 7	0 2 3	0 1 9	0 3 9	0 0 9	0 1 2	0 4 5	0 0 1	0 0 2	0 0 3	0 0 4	0 0 5	0 0 6	0 0 7	0 0 8	0 1 1	0 1 3	0 1 4	0 1 6	0 1 7	0 2 1	0 2 2	0 2 4	0 2 5	0 2 6
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland		+			+	+			+					+					+			+			
Prostate	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma															X										
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, liver	X																								
Hemangiosarcoma, metastatic, skin																									
Lymph node									+							+									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, liver	X																								
Thymus	M	+	+	+	+	+	M	M	M	+	+	+	M	+	M	+	+	+	+	M	M	M	+	+	M
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	+	M	+	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangioma																									
Subcutaneous tissue, hemangiosarcoma																									
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X				X									X			X					
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, harderian gland																									
Hepatocellular carcinoma, metastatic, liver							X																		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 2 8	0 3 0	0 3 1	0 3 2	0 3 4	0 3 5	0 3 6	0 3 7	0 4 0	0 4 1	0 4 2	0 4 3	0 4 6	0 4 7	0 4 8	0 4 9	0 5 0	0 5 2	0 5 3	0 5 4	0 5 5	0 5 6	0 5 8	0 5 9	0 6 0	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland					+		+		+		+		+		+		+	+		+		+		+		18
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, liver																										1
Hemangiosarcoma, metastatic, skin																								X		1
Lymph node																							+			3
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, liver																										1
Thymus	M	+	M	M	M	+	+	+	+	+	+	M	M	M	+	+	+	+	+	+	+	+	+	M	M	31
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	+	M	M	+	M	M	M	M	M	M	M	+	M	M	M	M	M	M	6
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangioma																									X	1
Subcutaneous tissue, hemangiosarcoma																								X		1
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X		X		X										X	X						X		X	11
Alveolar/bronchiolar adenoma, multiple							X												X							2
Alveolar/bronchiolar carcinoma																		X								1
Carcinoma, metastatic, harderian gland																								X		1
Hepatocellular carcinoma, metastatic, liver			X																							2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	5	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	7	0	1	3	5	1	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	7	3	3	7	5	7	9	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	2	1	3	0	1	4	0	0	0	0	0	0	0	0	1	1	1	1	1	2	2	2	2	2
	7	3	9	9	9	2	5	1	2	3	4	5	6	7	8	1	3	4	6	7	1	2	4	5	6
Special Senses System																									
Ear																							+		
Fibrosarcoma																							X		
Eye					+																				
Harderian gland	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	M	M	M	+	M	+
Adenoma					X																				
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																X									
Lymphoma malignant mixed									X																
Lymphoma malignant undifferentiated cell type																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 2 8	0 3 0	0 3 1	0 3 2	0 3 4	0 3 5	0 3 6	0 3 7	0 4 0	0 4 1	0 4 2	0 4 3	0 4 6	0 4 7	0 4 8	0 4 9	0 5 0	0 5 2	0 5 3	0 5 4	0 5 5	0 5 6	0 5 8	0 5 9	0 6 0	Total Tissues/ Tumors
Special Senses System																										
Ear																										1
Fibrosarcoma																										1
Eye																										1
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Adenoma																		X								2
Carcinoma																								X		1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed																										1
Lymphoma malignant undifferentiated cell type																							X			1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: 0.15 mg/kg

Number of Days on Study	5	5	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	1	7	2	2	9	0	0	1	1	1	1	2	3	3	3	3	3	3	3	3	3	3	3	3
	3	1	1	4	5	1	3	2	2	7	9	1	1	1	1	1	1	1	1	1	1	1	1	1
Carcass ID Number	1	0	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	7	6	0	6	1	1	7	8	0	9	6	6	6	6	6	6	7	7	7	7	7	7	7
	0	3	2	3	3	3	4	8	8	7	3	5	1	4	7	8	9	0	1	2	4	5	6	7
Alimentary System																								
None																								
Cardiovascular System																								
None																								
Endocrine System																								
None																								
General Body System																								
None																								
Genital System																								
None																								
Hematopoietic System																								
None																								
Integumentary System																								
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																								
None																								
Nervous System																								
None																								
Respiratory System																								
None																								
Special Senses System																								
None																								
Urinary System																								
None																								
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: 0.15 mg/kg
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 7 9	0 8 0	0 8 1	0 8 2	0 8 3	0 8 4	0 8 5	0 9 0	0 9 1	0 9 2	0 9 4	0 9 5	0 9 6	0 9 8	0 9 9	1 0 0	1 0 1	1 0 6	1 0 8	1 0 9	1 1 2	1 1 5	1 1 6	1 1 7	1 1 9	1 2 0	Total Tissues/ Tumors
Alimentary System																											
None																											
Cardiovascular System																											
None																											
Endocrine System																											
None																											
General Body System																											
None																											
Genital System																											
None																											
Hematopoietic System																											
None																											
Integumentary System																											
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																											
None																											
Nervous System																											
None																											
Respiratory System																											
None																											
Special Senses System																											
None																											
Urinary System																											
None																											
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: 0.5 mg/kg

Number of Days on Study	5	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	6	3	3	6	6	8	1	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	0	5	8	0	3	4	3	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	8	3	2	4	4	6	7	5	2	2	2	2	2	2	2	3	3	3	3	3	3	4	4	4
	0	8	1	8	9	5	5	8	2	3	4	5	6	8	9	1	3	5	6	7	9	0	1	2
Alimentary System																								
None																								
Cardiovascular System																								
None																								
Endocrine System																								
None																								
General Body System																								
None																								
Genital System																								
None																								
Hematopoietic System																								
None																								
Integumentary System																								
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																								
None																								
Nervous System																								
None																								
Respiratory System																								
None																								
Special Senses System																								
None																								
Urinary System																								
None																								
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: 0.5 mg/kg
(continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	1 4 3	1 4 4	1 4 7	1 5 0	1 5 2	1 5 3	1 5 4	1 5 5	1 5 6	1 5 9	1 6 0	1 6 1	1 6 2	1 6 3	1 6 4	1 6 6	1 6 7	1 6 9	1 7 0	1 7 1	1 7 2	1 7 3	1 7 4	1 7 6	1 7 7	1 7 8	Total Tissues/ Tumors
Alimentary System																										
None																										
Cardiovascular System																										
None																										
Endocrine System																										
None																										
General Body System																										
None																										
Genital System																										
None																										
Hematopoietic System																										
None																										
Integumentary System																										
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
None																										
Nervous System																										
None																										
Respiratory System																										
None																										
Special Senses System																										
None																										
Urinary System																										
None																										
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg

Number of Days on Study	5 1 8	5 8 4	6 1 2	6 5 9	6 8 4	6 8 7	6 9 5	7 0 5	7 1 2	7 1 9	7 2 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	2 2 8	2 3 2	2 3 6	1 8 8	2 1 0	2 3 5	1 8 2	2 0 8	2 0 0	2 2 6	2 3 9	1 8 3	1 8 4	1 8 5	1 8 6	1 8 7	1 8 9	1 9 0	1 9 1	1 9 2	1 9 3	1 9 4	1 9 6	1 9 7	1 9 8
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																									
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X																					
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma					X																				
Hemangiosarcoma, multiple										X															
Hemangiosarcoma, metastatic, spleen						X																			
Hepatoblastoma												X													
Hepatocellular carcinoma								X	X		X	X	X			X							X		
Hepatocellular carcinoma, multiple					X																				
Hepatocellular adenoma		X		X		X		X	X							X				X					
Hepatocellular adenoma, multiple							X					X					X				X		X		X
Histiocytic sarcoma			X																						
Mesentery			+																						
Hemangioma																									
Histiocytic sarcoma			X																						
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoid tumor malignant														X											
Tooth							+																		
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, liver																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	M	+	+	M	+	+	
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, spleen						X																			
Follicular cell, adenoma																									
Follicular cell, carcinoma																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	
Carcass ID Number	19 9	20 1	20 3	20 5	20 6	20 9	21 1	21 3	21 4	21 6	21 8	21 9	22 0	22 1	22 2	22 4	22 5	22 7	22 9	23 0	23 3	23 4	23 7	23 8	24 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma															X											1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Hemangiosarcoma, multiple																								X		2
Hemangiosarcoma, metastatic, spleen																										1
Hepatoblastoma																										1
Hepatocellular carcinoma									X			X					X						X	X		12
Hepatocellular carcinoma, multiple																		X								2
Hepatocellular adenoma				X		X				X								X		X						12
Hepatocellular adenoma, multiple	X	X					X		X		X						X			X						13
Histiocytic sarcoma																										1
Mesentery		+			+						+															4
Hemangioma											X															1
Histiocytic sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoid tumor malignant																										1
Tooth									+																	2
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, liver																								X		1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma							X																			1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, spleen																										1
Follicular cell, adenoma																					X					1
Follicular cell, carcinoma																						X				1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	5 1 8	5 8 4	6 1 2	6 5 9	6 8 4	6 8 7	6 9 5	7 0 5	7 1 2	7 1 9	7 2 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	2 2 8	2 3 2	2 3 6	1 8 8	2 1 0	2 3 5	1 8 2	2 0 8	2 0 0	2 2 6	2 3 9	1 8 3	1 8 4	1 8 5	1 8 6	1 8 7	1 8 9	1 9 0	1 9 1	1 9 2	1 9 3	1 9 4	1 9 6	1 9 7	1 9 8
General Body System																									
Tissue nos														+											
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Preputial gland		+			+		+	+							+	+		+			M			+	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																					X				
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, spleen						X																			
Histiocytic sarcoma			X																						
Lymph node				+									+												
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma						X								X											
Histiocytic sarcoma			X																						
Thymus	M	+	+	M	M	+	+	+	+	M	M	+	M	+	+	+	+	+	+	+	M	+	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	+	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melanoma benign																									X
Subcutaneous tissue, hemangiosarcoma																									
Subcutaneous tissue, hemangiosarcoma, metastatic, spleen						X																			
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	
Carcass ID Number	19 99	20 01	20 03	20 05	20 06	20 09	21 01	21 13	21 14	21 16	21 18	21 19	22 20	22 21	22 22	22 24	22 25	22 27	22 29	23 00	23 03	23 04	23 07	23 08	24 00	Total Tissues/ Tumors
General Body System																										
Tissue nos																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Preputial gland		+			+		+		+		+			+		+						+		+		17
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, spleen																										1
Histiocytic sarcoma																										1
Lymph node																										2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																X										3
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	M	+	+	+	M	39
Integumentary System																										
Mammary gland	+	M	M	+	+	M	+	M	+	+	M	M	+	M	M	M	M	+	M	M	M	M	M	M	M	11
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Melanoma benign																										1
Subcutaneous tissue, hemangiosarcoma																							X			1
Subcutaneous tissue, hemangiosarcoma, metastatic, spleen																										1
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	5 1 8	5 8 4	6 1 2	6 5 9	6 8 4	6 8 7	6 9 5	7 0 5	7 1 2	7 1 9	7 2 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	2 2 8	2 3 2	2 3 6	1 8 8	2 1 0	2 3 5	1 8 2	2 0 8	2 0 0	2 2 6	2 3 9	1 8 3	1 8 4	1 8 5	1 8 6	1 8 7	1 9 9	1 9 0	1 9 1	1 9 2	1 9 3	1 9 4	1 9 6	1 9 7	1 9 8
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X					X											X			
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma, multiple																					X				
Carcinoma, metastatic, thyroid gland																									
Hepatocellular carcinoma, metastatic, liver					X				X															X	
Histiocytic sarcoma			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glands, carcinoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear			+										+												
Fibrosarcoma			X																						
Eye									+																
Harderian gland	+	+	+	+	M	M	M	+	+	M	+	+	M	+	+	M	+	+	+	+	+	M	+	M	M
Adenoma									X																
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Lymphoma malignant mixed				X					X				X												

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 9 9	2 0 1	2 0 3	2 0 5	2 0 6	2 0 9	2 1 1	2 1 3	2 1 4	2 1 6	2 1 8	2 1 9	2 2 0	2 2 1	2 2 2	2 2 4	2 2 5	2 2 7	2 2 9	2 3 0	2 3 3	2 3 4	2 3 7	2 3 8	2 4 0	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X							X									X	X						7
Alveolar/bronchiolar adenoma, multiple	X																									1
Alveolar/bronchiolar carcinoma, multiple															X											2
Carcinoma, metastatic, thyroid gland																						X				1
Hepatocellular carcinoma, metastatic, liver												X							X							5
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glands, carcinoma												X														1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										2
Fibrosarcoma																										1
Eye																										1
Harderian gland	+	M	+	M	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	37
Adenoma															X											2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant mixed																										3

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of Benzethonium Chloride

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Harderian Gland: Adenoma or Carcinoma				
Overall rate[a]	3/50 (6%)	–[e]	–	2/50 (4%)
Adjusted rate[b]	6.7%			4.9%
Terminal rate[c]	2/43 (5%)			1/39 (3%)
First incidence (days)	655			712
Life table test[d]				P=0.531N
Logistic regression test[d]				P=0.495N
Fisher exact test[d]				P=0.500N
Liver: Hemangiosarcoma				
Overall rate	3/50 (6%)	–	–	2/50 (4%)
Adjusted rate	6.4%			4.9%
Terminal rate	0/43 (0%)			1/39 (3%)
First incidence (days)	577			719
Life table test				P=0.531N
Logistic regression test				P=0.307N
Fisher exact test				P=0.500N
Liver: Hepatocellular Adenoma				
Overall rate	24/50 (48%)	–	–	25/50 (50%)
Adjusted rate	53.2%			55.2%
Terminal rate	22/43 (51%)			19/39 (49%)
First incidence (days)	603			584
Life table test				P=0.347
Logistic regression test				P=0.505
Fisher exact test				P=0.500
Liver: Hepatocellular Carcinoma				
Overall rate	10/50 (20%)	–	–	14/50 (28%)
Adjusted rate	21.6%			32.4%
Terminal rate	7/43 (16%)			10/39 (26%)
First incidence (days)	603			684
Life table test				P=0.189
Logistic regression test				P=0.363
Fisher exact test				P=0.241
Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma				
Overall rate	29/50 (58%)	–	–	33/50 (66%)
Adjusted rate	63.0%			70.1%
Terminal rate	26/43 (60%)			25/39 (64%)
First incidence (days)	603			584
Life table test				P=0.153
Logistic regression test				P=0.331
Fisher exact test				P=0.268

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	10/50 (20%)	–	–	14/50 (28%)
Adjusted rate	21.6%			32.4%
Terminal rate	7/43 (16%)			10/39 (26%)
First incidence (days)	603			684
Life table test				P=0.189
Logistic regression test				P=0.363
Fisher exact test				P=0.241
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	29/50 (58%)	–	–	33/50 (66%)
Adjusted rate	63.0%			70.1%
Terminal rate	26/43 (60%)			25/39 (64%)
First incidence (days)	603			584
Life table test				P=0.153
Logistic regression test				P=0.331
Fisher exact test				P=0.268
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	13/50 (26%)	–	–	8/50 (16%)
Adjusted rate	29.4%			19.3%
Terminal rate	12/43 (28%)			6/39 (15%)
First incidence (days)	637			687
Life table test				P=0.228N
Logistic regression test				P=0.181N
Fisher exact test				P=0.163N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	14/50 (28%)	–	–	10/50 (20%)
Adjusted rate	31.7%			24.2%
Terminal rate	13/43 (30%)			8/39 (21%)
First incidence (days)	637			687
Life table test				P=0.327N
Logistic regression test				P=0.274N
Fisher exact test				P=0.241N
Spleen: Hemangiosarcoma				
Overall rate	0/50 (0%)	–	–	3/50 (6%)
Adjusted rate	0.0%			7.2%
Terminal rate	0/43 (0%)			2/39 (5%)
First incidence (days)	–[f]			687
Life table test				P=0.112
Logistic regression test				P=0.162
Fisher exact test				P=0.121

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
All Organs: Hemangiosarcoma				
Overall rate	4/50 (8%)	–	–	6/50 (12%)
Adjusted rate	8.5%			14.4%
Terminal rate	1/43 (2%)			4/39 (10%)
First incidence (days)	577			687
Life table test				P=0.330
Logistic regression test				P=0.382
Fisher exact test				P=0.370
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	6/50 (12%)	–	–	8/50 (16%)
Adjusted rate	12.9%			18.6%
Terminal rate	3/43 (7%)			5/39 (13%)
First incidence (days)	577			684
Life table test				P=0.340
Logistic regression test				P=0.396
Fisher exact test				P=0.387
All Organs: Malignant Lymphoma (Lymphocytic, Mixed, or Undifferentiated Cell Type)				
Overall rate	3/50 (6%)	–	–	3/50 (6%)
Adjusted rate	7.0%			6.9%
Terminal rate	3/43 (7%)			1/39 (3%)
First incidence (days)	729 (T)			659
Life table test				P=0.628
Logistic regression test				P=0.662N
Fisher exact test				P=0.661N
All Organs: Benign Neoplasms				
Overall rate	37/50 (74%)	–	–	31/50 (62%)
Adjusted rate	77.1%			65.8%
Terminal rate	32/43 (74%)			23/39 (59%)
First incidence (days)	603			584
Life table test				P=0.349N
Logistic regression test				P=0.149N
Fisher exact test				P=0.142N
All Organs: Malignant Neoplasms				
Overall rate	18/50 (36%)	–	–	24/50 (48%)
Adjusted rate	38.1%			49.9%
Terminal rate	14/43 (33%)			15/39 (38%)
First incidence (days)	577			584
Life table test				P=0.121
Logistic regression test				P=0.164
Fisher exact test				P=0.156

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	44/50 (88%)	–	–	41/50 (82%)
Adjusted rate	89.8%			83.7%
Terminal rate	38/43 (88%)			31/39 (79%)
First incidence (days)	577			584
Life table test				P=0.533
Logistic regression test				P=0.300N
Fisher exact test				P=0.288N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and spleen; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Fisher exact test compares directly the overall incidence rates. For all tests, a lower incidence in a dose group is indicated by N.
[e] Organ was not examined at this dose level
[f] Not applicable; no neoplasms in animal group

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	9	9	10
Early deaths				
Moribund	3	8	4	9
Natural deaths	4	4	4	2
Survivors				
Terminal sacrifice	43	38	42	39
Missexed		1	1	
Animals examined microscopically	60	59	59	60
15-Month Interim Evaluation				
Alimentary System				
Liver	(10)			(10)
Clear cell focus	2 (20%)			1 (10%)
Fatty change, focal				1 (10%)
Mesentery	(1)			(1)
Fat, necrosis	1 (100%)			1 (100%)
Pancreas	(10)			(10)
Atrophy	1 (10%)			
Cytoplasmic alteration	1 (10%)			
Salivary glands	(10)			(10)
Atrophy	1 (10%)			
Endocrine System				
Adrenal cortex	(10)			(10)
Hyperplasia				1 (10%)
Islets, pancreatic	(10)			(10)
Hyperplasia	1 (10%)			1 (10%)
Pituitary gland	(10)			(10)
Pars intermedia, hyperplasia	1 (10%)			
Genital System				
Preputial gland	(1)			(1)
Duct, ectasia	1 (100%)			1 (100%)
Hematopoietic System				
Spleen	(10)			(10)
Hematopoietic cell proliferation	1 (10%)			2 (20%)
Hyperplasia, lymphoid	1 (10%)			
Thymus	(10)			(10)
Hyperplasia, lymphoid	1 (10%)			

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

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
15-Month Interim Evaluation (continued)				
Integumentary System				
Skin, control	(10)	(9)	(9)	(10)
Skin, site of application-no mass	(10)	(9)	(9)	(10)
Epithelial hyperplasia			2 (22%)	10 (100%)
Respiratory System				
Lung	(10)			(10)
Alveolar epithelium, hyperplasia	1 (10%)			
Urinary System				
Kidney	(10)			(10)
Nephropathy	10 (100%)			9 (90%)
Systems Examined With No Lesions Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Special Senses System				
2-Year Study				
Alimentary System				
Intestine small, duodenum	(50)			(50)
Erosion	1 (2%)			
Intestine small, jejunum	(50)			(50)
Hyperplasia, lymphoid	1 (2%)			1 (2%)
Inflammation, chronic active				1 (2%)
Liver	(50)			(50)
Basophilic focus	2 (4%)			5 (10%)
Clear cell focus	11 (22%)			11 (22%)
Eosinophilic focus	14 (28%)			16 (32%)
Hematopoietic cell proliferation	2 (4%)			
Infarct	1 (2%)			
Mixed cell focus	5 (10%)			4 (8%)
Necrosis	1 (2%)			2 (4%)
Mesentery	(4)			(4)
Inflammation, chronic active				1 (25%)
Fat, necrosis	3 (75%)			
Vein, thrombosis				1 (25%)
Pancreas	(50)			(50)
Atrophy	2 (4%)			4 (8%)
Atypia cellular	4 (8%)			2 (4%)
Concretion				1 (2%)
Necrosis				1 (2%)
Duct, cyst				1 (2%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Stomach, forestomach	(50)			(50)
Erosion				2 (4%)
Hyperplasia	1 (2%)			4 (8%)
Stomach, glandular	(50)			(50)
Erosion	1 (2%)			
Tooth	(1)			(2)
Inflammation, chronic active				1 (50%)
Inflammation, suppurative	1 (100%)			1 (50%)
Cardiovascular System				
Heart	(50)			(50)
Inflammation, chronic active	2 (4%)			2 (4%)
Artery, inflammation, chronic active	1 (2%)			
Valve, inflammation, chronic active				1 (2%)
Endocrine System				
Adrenal cortex	(50)			(50)
Accessory adrenal cortical nodule				1 (2%)
Hyperplasia	40 (80%)			32 (64%)
Capsule, hyperplasia, adenomatous	5 (10%)			9 (18%)
Islets, pancreatic	(49)			(50)
Hyperplasia	21 (43%)			18 (36%)
Parathyroid gland	(47)			(45)
Cyst	1 (2%)			
Pituitary gland	(48)			(48)
Cyst	2 (4%)			2 (4%)
Pars distalis, hyperplasia				2 (4%)
Pars intermedia, hyperplasia	2 (4%)			1 (2%)
Thyroid gland	(50)			(50)
Inflammation				1 (2%)
Ultimobranchial cyst	1 (2%)			
Follicle, cyst	3 (6%)			1 (2%)
Follicular cell, hyperplasia	10 (20%)			7 (14%)
General Body System				
Tissue NOS				(1)
Hemorrhage				1 (100%)
Genital System				
Epididymis	(50)			(49)
Inflammation	3 (6%)			5 (10%)
Preputial gland	(18)			(17)
Inflammation, chronic active	6 (33%)			3 (18%)
Duct, ectasia	18 (100%)			16 (94%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Genital System (continued)				
Prostate	(49)			(50)
Inflammation, suppurative	1 (2%)			
Artery, inflammation, chronic active				1 (2%)
Seminal vesicle	(50)			(50)
Inflammation	1 (2%)			
Hematopoietic System				
Bone marrow	(50)			(50)
Erythroid cell, hyperplasia	4 (8%)			12 (24%)
Myeloid cell, hyperplasia	3 (6%)			4 (8%)
Lymph node, mandibular	(49)			(48)
Hyperplasia, lymphoid				1 (2%)
Lymph node, mesenteric	(48)			(47)
Hyperplasia, lymphoid				1 (2%)
Inflammation, granulomatous				1 (2%)
Spleen	(50)			(50)
Depletion lymphoid	3 (6%)			
Hematopoietic cell proliferation	11 (22%)			20 (40%)
Hyperplasia, lymphoid				1 (2%)
Inflammation, granulomatous				1 (2%)
Pigmentation, hemosiderin	1 (2%)			
Thymus	(31)			(39)
Depletion lymphoid	4 (13%)			3 (8%)
Hyperplasia, lymphoid				2 (5%)
Integumentary System				
Skin, control	(50)	(50)	(50)	(50)
Skin, site of application-no mass	(50)	(50)	(50)	(50)
Epithelial hyperplasia	2 (4%)	7 (14%)	16 (32%)	23 (46%)
Inflammation, chronic				2 (4%)
Ulcer	1 (2%)	1 (2%)	4 (8%)	2 (4%)
Sebaceous gland, hyperplasia			1 (2%)	
Musculoskeletal System				
None				
Nervous System				
Brain	(50)			(50)
Neuron, necrosis	2 (4%)			

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Respiratory System				
Lung	(50)			(50)
Thrombosis				2 (4%)
Alveolar epithelium, hyperplasia	5 (10%)			3 (6%)
Bronchiole, hyperplasia				1 (2%)
Special Senses System				
Ear	(1)			(2)
Necrosis				1 (50%)
Eye	(1)			(1)
Cornea, inflammation	1 (100%)			1 (100%)
Harderian gland	(43)			(37)
Hyperplasia	2 (5%)			
Urinary System				
Kidney	(50)			(50)
Cyst				1 (2%)
Hydronephrosis	2 (4%)			1 (2%)
Nephropathy	48 (96%)			49 (98%)

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR DERMAL STUDY OF BENZETHONIUM CHLORIDE

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	8	7	10	6
Early deaths				
Accidental death	1			
Moribund	10	4	8	13
Natural deaths	3	15	9	7
Survivors				
Terminal sacrifice	38	34	31	34
Missexed			2	
Animals examined microscopically	60	60	58	60
15-Month Interim Evaluation				
Alimentary System				
Liver	(8)			(6)
Hepatocellular adenoma	1 (13%)			1 (17%)
Integumentary System				
Skin, control	(8)	(7)	(10)	(6)
Skin, site of application	(8)	(7)	(10)	(6)
Systems Examined With No Neoplasms Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
2-Year Study				
Alimentary System				
Gallbladder	(52)			(53)
Histiocytic sarcoma	1 (2%)			
Intestine large, rectum	(52)			(53)
Liposarcoma, metastatic, skeletal muscle				1 (2%)
Intestine large, cecum	(52)			(53)
Intestine small, jejunum	(52)			(52)
Carcinoma	1 (2%)			
Hemangiosarcoma	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Liver	(52)			(54)
Hemangioma				1 (2%)
Hemangiosarcoma	1 (2%)			
Hemangiosarcoma, metastatic, spleen	1 (2%)			
Hepatocellular carcinoma	7 (13%)			10 (19%)
Hepatocellular carcinoma, multiple	5 (10%)			1 (2%)
Hepatocellular adenoma	9 (17%)			12 (22%)
Hepatocellular adenoma, multiple	11 (21%)			6 (11%)
Hepatocholangiocarcinoma, multiple				1 (2%)
Histiocytic sárcoma	3 (6%)			
Plasma cell tumor malignant, metastatic, spleen	1 (2%)			
Mesentery	(10)			(9)
Hemangioma	1 (10%)			
Hepatocholangiocarcinoma, metastatic, liver				1 (11%)
Histiocytic sarcoma	1 (10%)			
Myxosarcoma, metastatic, skin	1 (10%)			
Pancreas	(52)			(53)
Histiocytic sarcoma	1 (2%)			
Leiomyosarcoma, metastatic, uterus				1 (2%)
Salivary glands	(52)			(54)
Stomach, forestomach	(51)			(53)
Cardiovascular System				
Heart	(52)			(54)
Plasma cell tumor malignant, metastatic, spleen	1 (2%)			
Endocrine System				
Adrenal cortex	(52)			(54)
Histiocytic sarcoma	1 (2%)			
Pituitary gland	(52)			(50)
Histiocytic sarcoma	1 (2%)			
Pars distalis, adenoma	6 (12%)			5 (10%)
Thyroid gland	(52)			(54)
Follicular cell, adenoma	3 (6%)			1 (2%)
Follicular cell, carcinoma	1 (2%)			
General Body System				
None				
Genital System				
Ovary	(52)			(52)
Cystadenoma	3 (6%)			2 (4%)
Hemangioma				1 (2%)
Histiocytic sarcoma	2 (4%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Genital System (continued)				
Uterus	(52)			(53)
Carcinoma				1 (2%)
Histiocytic sarcoma	3 (6%)			1 (2%)
Leiomyosarcoma				1 (2%)
Polyp stromal	1 (2%)			1 (2%)
Hematopoietic System				
Blood				(1)
Bone marrow	(52)			(53)
Hemangiosarcoma, metastatic, spleen				3 (6%)
Histiocytic sarcoma	2 (4%)			
Plasma cell tumor malignant, metastatic, spleen	1 (2%)			
Lymph node	(7)			(2)
Lumbar, histiocytic sarcoma	1 (14%)			
Mediastinal, histiocytic sarcoma	1 (14%)			
Pancreatic, hepatocholangiocarcinoma, metastatic, liver				1 (50%)
Lymph node, mandibular	(52)			(54)
Hemangioma	1 (2%)			
Histiocytic sarcoma	3 (6%)			
Plasma cell tumor malignant, metastatic, spleen	1 (2%)			
Lymph node, mesenteric	(50)			(49)
Histiocytic sarcoma	3 (6%)			
Plasma cell tumor malignant, metastatic, spleen	1 (2%)			
Spleen	(52)			(53)
Hemangiosarcoma	1 (2%)			3 (6%)
Histiocytic sarcoma	1 (2%)			
Plasma cell tumor malignant	1 (2%)			
Thymus	(41)			(45)
Plasma cell tumor malignant, metastatic, spleen	1 (2%)			
Integumentary System				
Skin, control	(52)	(52)	(48)	(53)
Subcutaneous tissue, fibrosarcoma	2 (4%)			
Subcutaneous tissue, hemangiosarcoma			1 (2%)	
Subcutaneous tissue, sarcoma	1 (2%)			
Skin, site of application-no mass	(52)	(52)	(48)	(53)
Subcutaneous tissue, hemangioma	1 (2%)			
Skin, site of application-mass	(52)			(53)
Subcutaneous tissue, sarcoma	1 (2%)			1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Musculoskeletal System				
Bone	(52)			(53)
Liposarcoma, metastatic, skeletal muscle				1 (2%)
Skeletal muscle				(4)
Hemangiosarcoma, metastatic, spleen				1 (25%)
Leiomyosarcoma, metastatic, uterus				1 (25%)
Liposarcoma				1 (25%)
Nervous System				
Spinal cord				(2)
Hemangiosarcoma, metastatic, spleen				1 (50%)
Respiratory System				
Lung	(52)			(54)
Alveolar/bronchiolar adenoma	1 (2%)			2 (4%)
Alveolar/bronchiolar carcinoma	1 (2%)			1 (2%)
Carcinoma, metastatic, harderian gland	1 (2%)			
Hepatocellular carcinoma, metastatic, liver	5 (10%)			3 (6%)
Hepatocholangiocarcinoma, metastatic, liver				1 (2%)
Histiocytic sarcoma	2 (4%)			
Liposarcoma, metastatic, skeletal muscle				1 (2%)
Plasma cell tumor malignant, metastatic, spleen	1 (2%)			
Special Senses System				
Harderian gland	(44)			(35)
Adenoma	2 (5%)			2 (6%)
Carcinoma	2 (5%)			1 (3%)
Zymbal's gland				(1)
Carcinoma				1 (100%)
Urinary System				
Kidney	(52)			(54)
Histiocytic sarcoma	3 (6%)			
Plasma cell tumor malignant, metastatic, spleen	1 (2%)			
Urinary bladder	(50)			(53)
Systemic Lesions				
Multiple organs[b]	(52)	(53)	(48)	(54)
Histiocytic sarcoma	3 (6%)			1 (2%)
Leukemia lymphocytic				1 (2%)
Lymphoma malignant lymphocytic	1 (2%)	1 (2%)		2 (4%)
Lymphoma malignant mixed	5 (10%)			3 (6%)
Lymphoma malignant undifferentiated cell	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]				
15-Month interim evaluation	1			1
2-Year study	41	1	1	42
Total primary neoplasms				
15-Month interim evaluation	1			1
2-Year study	74	1	1	62
Total animals with benign neoplasms				
15-Month interim evaluation	1			1
2-Year study	28			28
Total benign neoplasms				
15-Month interim evaluation	1			1
2-Year study	39			33
Total animals with malignant neoplasms				
2-Year study	27	1	1	27
Total malignant neoplasms				
2-Year study	35	1	1	29
Total animals with metastatic neoplasms				
2-Year study	9			8
Total metastatic neoplasms				
2-Year study	16			16

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control

Number of Days on Study	3 1 7	4 2 8	5 6 8	6 3 2	6 3 9	6 6 0	6 7 4	6 7 7	6 8 7	6 8 9	6 9 1	6 9 8	7 1 9	7 2 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3
Carcass ID Number	2 6 1	2 8 2	2 9 5	2 8 6	2 5 2	2 6 9	2 8 3	2 8 1	2 5 3	2 5 1	2 4 3	2 9 1	3 0 0	2 6 7	2 4 1	2 4 2	2 4 4	2 4 5	2 4 6	2 4 7	2 5 0	2 5 4	2 5 5	2 5 6	2 5 7
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																						X			
Hemangiosarcoma						X																			
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma							X																		
Hemangiosarcoma, metastatic, spleen										X															
Hepatocellular carcinoma								X			X							X					X	
Hepatocellular carcinoma, multiple														X								X			
Hepatocellular adenoma																X				X					
Hepatocellular adenoma, multiple							X			X					X									X	X
Histiocytic sarcoma			X										X												
Plasma cell tumor malignant, metastatic, spleen																									
Mesentery	+	+		+												+		+							
Hemangioma																X									
Histiocytic sarcoma																									
Myxosarcoma, metastatic, skin																		X							
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Plasma cell tumor malignant, metastatic, spleen																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Pars distalis, adenoma																									X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma						X																			
Follicular cell, carcinoma																							X		

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	2 5 8	2 6 0	2 6 2	2 6 3	2 6 4	2 6 8	2 7 0	2 7 1	2 7 2	2 7 3	2 7 4	2 7 5	2 7 6	2 7 7	2 7 8	2 7 9	2 8 0	2 8 4	2 8 5	2 8 7	2 8 9	2 9 0	2 9 3	2 9 4	2 9 6	2 9 7	2 9 9	Total Tissues/ Tumors
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Histiocytic sarcoma																	X										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, duodenum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Carcinoma																											1
Hemangiosarcoma																											1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Hemangiosarcoma																											1
Hemangiosarcoma, metastatic, spleen																											1
Hepatocellular carcinoma					X												X								X		7
Hepatocellular carcinoma, multiple			X	X										X													5
Hepatocellular adenoma	X		X		X		X									X			X			X					9
Hepatocellular adenoma, multiple														X	X		X	X			X				X		11
Histiocytic sarcoma																	X										3
Plasma cell tumor malignant, metastatic, spleen															X												1
Mesentery												+			+		+	+			+						10
Hemangioma																											1
Histiocytic sarcoma																	X										1
Myxosarcoma, metastatic, skin																											1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Histiocytic sarcoma																	X										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Stomach, forestomach	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Stomach, glandular	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Cardiovascular System																											
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Plasma cell tumor malignant, metastatic, spleen															X												1
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Histiocytic sarcoma																											1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	M	+	+	+	43
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Histiocytic sarcoma																											1
Pars distalis, adenoma															X		X					X			X	X	6
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Follicular cell, adenoma								X										X									3
Follicular cell, carcinoma																											1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	3 1 7	4 2 8	5 6 8	6 3 2	6 3 9	6 6 0	6 7 4	6 7 7	6 8 7	6 8 9	6 9 1	6 9 1	7 1 8	7 2 9	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3
Carcass ID Number	2 6 1	2 8 2	2 9 5	2 8 6	2 5 2	2 6 9	2 8 3	2 8 1	2 5 3	2 5 1	2 4 3	2 9 1	3 0 0	2 6 7	2 4 1	2 4 2	2 4 4	2 4 5	2 4 6	2 4 7	2 5 0	2 5 4	2 5 5	2 5 6	2 5 7
General Body System																									
None																									
Genital System																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																X					X				
Histiocytic sarcoma			X																						
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X										X												
Polyp stromal																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Plasma cell tumor malignant, metastatic, spleen																									
Lymph node		+				+																			
Lumbar, histiocytic sarcoma																									
Mediastinal, histiocytic sarcoma																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Histiocytic sarcoma			X										X												
Plasma cell tumor malignant, metastatic, spleen																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X										X												
Plasma cell tumor malignant, metastatic, spleen																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma										X															
Histiocytic sarcoma			X																						
Plasma cell tumor malignant																									
Thymus	M	+	+	+	+	M	+	M	M	+	M	+	M	+	M	+	+	M	+	+	+	+	+	+	+
Plasma cell tumor malignant, metastatic, spleen																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma												X							X						
Subcutaneous tissue, sarcoma																		X							
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangioma																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	2 5 8	2 6 0	2 6 2	2 6 3	2 6 4	2 6 8	2 7 0	2 7 1	2 7 2	2 7 3	2 7 4	2 7 5	2 7 6	2 7 7	2 7 8	2 7 9	2 8 0	2 8 4	2 8 5	2 8 7	2 8 9	2 9 0	2 9 3	2 9 4	2 9 6	2 9 7	2 9 9	Total Tissues/ Tumors
General Body System																												
None																												
Genital System																												
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Cystadenoma									X																			3
Histiocytic sarcoma																	X											2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Histiocytic sarcoma																	X											3
Polyp stromal																							X					1
Hematopoietic System																												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Histiocytic sarcoma																	X											2
Plasma cell tumor malignant, metastatic, spleen															X													1
Lymph node			+													+	+						+				+	7
Lumbar, histiocytic sarcoma																	X											1
Mediastinal, histiocytic sarcoma																	X											1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Hemangioma																					X							1
Histiocytic sarcoma																	X											3
Plasma cell tumor malignant, metastatic, spleen															X													1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																	X											3
Plasma cell tumor malignant, metastatic, spleen															X													1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Hemangiosarcoma																												1
Histiocytic sarcoma																												1
Plasma cell tumor malignant															X													1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	M	41
Plasma cell tumor malignant, metastatic, spleen															X													1
Integumentary System																												
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Subcutaneous tissue, fibrosarcoma																												2
Subcutaneous tissue, sarcoma																												1
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Subcutaneous tissue, hemangioma																								X				1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	3 1 7	4 2 8	5 6 8	6 3 2	6 3 9	6 6 0	6 7 4	6 7 7	6 8 7	6 8 9	6 9 1	6 9 8	7 1 9	7 2 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3
Carcass ID Number	2 6 1	2 8 2	2 9 5	2 8 6	2 5 2	2 6 9	2 8 3	2 8 1	2 5 3	2 5 1	2 4 3	2 9 1	2 3 0	2 6 7	2 2 1	2 4 2	2 4 4	2 4 5	2 4 6	2 4 7	2 5 0	2 5 4	2 5 5	2 5 6	2 5 7
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma															X										
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, harderian gland																								X	
Hepatocellular carcinoma, metastatic, liver								X		X			X												
Histiocytic sarcoma												X													
Plasma cell tumor malignant, metastatic, spleen																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																+								+	
Harderian gland	+	+	M	+	M	+	M	M	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+
Adenoma																X									
Carcinoma												X												X	
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X										X												
Plasma cell tumor malignant, metastatic, spleen																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X										X												
Lymphoma malignant lymphocytic						X																			
Lymphoma malignant mixed									X																
Lymphoma malignant undifferentiated cell type																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: Vehicle Control (continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	2 5 8	2 6 0	2 6 2	2 6 3	2 6 4	2 6 8	2 7 0	2 7 1	2 7 2	2 7 3	2 7 4	2 7 5	2 7 6	2 7 7	2 7 8	2 7 9	2 8 0	2 8 4	2 8 5	2 8 7	2 8 9	2 9 0	2 9 3	2 9 4	2 9 6	2 9 7	2 9 9	Total Tissues/ Tumors
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Alveolar/bronchiolar adenoma																											1
Alveolar/bronchiolar carcinoma												X															1
Carcinoma, metastatic, harderian gland																											1
Hepatocellular carcinoma, metastatic, liver				X										X													5
Histiocytic sarcoma																	X										2
Plasma cell tumor malignant, metastatic, spleen															X												1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Special Senses System																											
Eye						+	+																				4
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	44
Adenoma							X																				2
Carcinoma																											2
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Histiocytic sarcoma																	X										3
Plasma cell tumor malignant, metastatic, spleen															X												1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Histiocytic sarcoma																	X										3
Lymphoma malignant lymphocytic																											1
Lymphoma malignant mixed	X		X												X											X	5
Lymphoma malignant undifferentiated cell type																						X					1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: 0.15 mg/kg

Number of Days on Study	2	3	3	4	4	5	5	5	5	5	5	5	6	6	6	6	6	7	7	7	7	7	7	7	7	7
	6	0	7	5	7	1	1	4	6	8	9	9	1	2	3	5	9	0	1	3	3	3	3	3	3	3
	8	8	2	9	1	2	8	3	5	3	6	7	3	6	5	6	9	1	5	1	1	1	1	1	1	1
Carcass ID Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	5	3	2	6	5	2	3	4	1	2	0	3	5	5	1	0	2	3	2	0	0	0	0	0	0	0
	2	8	0	0	6	3	0	1	4	9	9	6	5	8	0	7	1	4	7	1	2	3	4	5	6	8
Alimentary System																										
None																										
Cardiovascular System																										
None																										
Endocrine System																										
None																										
General Body System																										
None																										
Genital System																										
None																										
Hematopoietic System																										
None																										
Integumentary System																										
Skin, control	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																										
None																										
Nervous System																										
None																										
Respiratory System																										
None																										
Special Senses System																										
None																										
Urinary System																										
None																										
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic		X																								

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: 0.15 mg/kg
(continued)

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
Carcass ID Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Total
	1	1	1	1	1	1	2	2	2	2	3	3	3	3	4	4	4	4	4	4	4	4	5	5	5	5	5	Tissues/
	1	2	3	6	7	9	2	4	5	8	1	2	3	9	0	2	4	5	6	7	8	9	0	3	4	7	9	Tumors
Alimentary System																												
None																												
Cardiovascular System																												
None																												
Endocrine System																												
None																												
General Body System																												
None																												
Genital System																												
None																												
Hematopoietic System																												
None																												
Integumentary System																												
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Musculoskeletal System																												
None																												
Nervous System																												
None																												
Respiratory System																												
None																												
Special Senses System																												
None																												
Urinary System																												
None																												
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Lymphoma malignant lymphocytic																												1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: 0.5 mg/kg

Number of Days on Study	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7
	2	6	9	9	0	2	4	6	7	7	9	9	9	0	1	1	1	3	3	3	3	3	3	3
	6	8	1	1	9	1	8	4	4	7	0	7	8	4	2	7	8	0	0	0	0	0	0	0
Carcass ID Number	3	3	3	4	3	4	4	3	4	3	3	3	3	4	4	4	3	3	3	3	3	3	3	3
	7	6	8	0	8	1	0	8	2	9	9	6	7	0	1	0	9	6	6	6	6	6	7	7
	6	3	1	4	3	8	8	9	0	2	9	2	1	1	9	3	7	1	4	5	6	8	2	5
Alimentary System																								
None																								
Cardiovascular System																								
None																								
Endocrine System																								
None																								
General Body System																								
None																								
Genital System																								
None																								
Hematopoietic System																								
None																								
Integumentary System																								
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma				X																				
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																								
None																								
Nervous System																								
None																								
Respiratory System																								
None																								
Special Senses System																								
None																								
Urinary System																								
None																								
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: 0.5 mg/kg
(continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	3 7 8	3 7 9	3 8 0	3 8 2	3 8 4	3 8 5	3 8 8	3 9 3	3 9 4	3 9 5	3 9 6	4 0 0	4 0 5	4 0 6	4 0 7	4 1 0	4 1 1	4 1 2	4 1 3	4 1 4	4 1 5	4 1 6	4 1 7	Total Tissues/ Tumors
Alimentary System																								
None																								
Cardiovascular System																								
None																								
Endocrine System																								
None																								
General Body System																								
None																								
Genital System																								
None																								
Hematopoietic System																								
None																								
Integumentary System																								
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma																								1
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Musculoskeletal System																								
None																								
Nervous System																								
None																								
Respiratory System																								
None																								
Special Senses System																								
None																								
Urinary System																								
None																								
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg

Number of Days on Study	1 2 3 4 4 4 5 5 5 6 6 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 2 4 1 2 7 8 2 5 8 1 4 8 8 9 9 9 9 9 9 2 2 2 2 2 2 2 2 0 9 6 8 9 5 0 1 9 2 7 2 9 0 1 5 8 8 8 0 9 9 9 9 9 9 9
Carcass ID Number	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 2 7 2 6 4 3 4 7 5 2 6 7 3 3 4 6 3 6 7 5 2 2 2 2 3 3 7 7 9 0 9 1 1 6 0 3 1 2 8 3 5 9 5 2 9 8 1 2 5 6 8 0 2
Alimentary System	
Esophagus	+ +
Gallbladder	+ + A +
Intestine large, colon	+ + M +
Intestine large, rectum	+ + M +
Liposarcoma, metastatic, skeletal muscle	X
Intestine large, cecum	+ + M +
Intestine small, duodenum	+ + M +
Intestine small, jejunum	+ M M +
Intestine small, ileum	+ M M +
Liver	+ +
Hemangioma	X
Hepatocellular carcinoma	X X X X X X
Hepatocellular carcinoma, multiple	X
Hepatocellular adenoma	X X X
Hepatocellular adenoma, multiple	X
Hepatocholangiocarcinoma, multiple	X
Mesentery	+ + + + + + + + +
Hepatocholangiocarcinoma, metastatic, liver	X
Pancreas	+ + M +
Leiomyosarcoma, metastatic, uterus	X
Salivary glands	+ +
Stomach, forestomach	+ + M +
Stomach, glandular	+ + M +
Cardiovascular System	
Heart	+ +
Endocrine System	
Adrenal cortex	+ +
Adrenal medulla	+ +
Islets, pancreatic	+ + M + + + + + + + + + + + + M + + + + + + + + + +
Parathyroid gland	+ + M + + + + + + + + + + + + + M M + + + + + + + M +
Pituitary gland	+ + M + + + + M + + + + + + M + + M + + + + + + + +
Pars distalis, adenoma	X X X
Thyroid gland	+ +
Follicular cell, adenoma	
General Body System	
None	
Genital System	
Clitoral gland	
Ovary	+ + M + + + + + + + + + + + + + + + M + + + + + + + +
Cystadenoma	
Hemangioma	X

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	
Carcass ID Number	43 34	43 36	43 37	43 39	44 40	44 42	44 43	44 44	44 46	44 47	45 51	45 52	45 53	45 54	45 55	45 56	45 57	45 59	46 55	46 56	46 57	47 71	47 73	47 74	47 75	48 80	Total Tissues/ Tumors
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liposarcoma, metastatic, skeletal muscle																											1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Hemangioma																											1
Hepatocellular carcinoma							X	X								X							X	X			10
Hepatocellular carcinoma, multiple																											1
Hepatocellular adenoma	X				X				X				X			X					X	X	X			X	12
Hepatocellular adenoma, multiple							X	X		X									X						X		6
Hepatocholangiocarcinoma, multiple																											1
Mesentery								+											+								9
Hepatocholangiocarcinoma, metastatic, liver																											1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Leiomyosarcoma, metastatic, uterus																											1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Cardiovascular System																											
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Parathyroid gland	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+		47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma							X		X																		5
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Follicular cell, adenoma				X																							1
General Body System																											
None																											
Genital System																											
Clitoral gland																											1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Cystadenoma											X														X		2
Hemangioma																											1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	1 2 3 4 4 4 5 5 5 6 6 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 2 4 1 2 7 8 2 5 8 1 4 8 8 9 9 9 9 9 9 2 2 2 2 2 2 2 2 0 9 6 8 9 5 0 1 9 2 7 2 9 0 1 5 8 8 8 0 9 9 9 9 9 9 9 9
Carcass ID Number	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 2 7 2 6 4 3 4 7 5 2 6 7 3 3 4 6 3 6 7 5 2 2 2 2 2 3 3 7 7 9 0 9 1 1 6 0 3 1 2 8 3 5 9 5 2 9 8 1 2 5 6 8 0 2
Genital System (continued)	
Uterus	+ + M +
Carcinoma	
Histiocytic sarcoma	
Leiomyosarcoma	X
Polyp stromal	X
Hematopoietic System	
Blood	+
Bone marrow	+ + M +
Hemangiosarcoma, metastatic, spleen	X X
Lymph node	+
Pancreatic, hepatocholangiocarcinoma, metastatic, liver	X
Lymph node, mandibular	+ +
Lymph node, mesenteric	+ + M + M + + M
Spleen	+ + M +
Hemangiosarcoma	X X
Thymus	+ + M + + + + + + + + M M + + + + + + + + + + M + + +
Integumentary System	
Mammary gland	+ + M + + + M +
Skin, control	+ + A +
Skin, site of application-no mass	+ + A +
Skin, site of application-mass	+
Subcutaneous tissue, sarcoma	X
Musculoskeletal System	
Bone	+ + M +
Liposarcoma, metastatic, skeletal muscle	X
Skeletal muscle	+ + + +
Hemangiosarcoma, metastatic, spleen	X
Leiomyosarcoma, metastatic, uterus	X
Liposarcoma	X
Nervous System	
Brain	+ + M +
Peripheral nerve	+ +
Spinal cord	+ +
Hemangiosarcoma, metastatic, spleen	X

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	4 3 4	4 3 6	4 3 7	4 3 9	4 4 0	4 4 2	4 4 3	4 4 4	4 4 6	4 4 7	4 5 1	4 5 2	4 5 3	4 5 4	4 5 5	4 5 6	4 5 7	4 5 9	4 6 5	4 6 6	4 6 7	4 7 1	4 7 3	4 7 4	4 7 5	4 7 8	4 8 0	Total Tissues/ Tumors
Genital System (continued)																												
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma			X																									1
Histiocytic sarcoma																						X						1
Leiomyosarcoma																												1
Polyp stromal																												1
Hematopoietic System																												
Blood																												1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Hemangiosarcoma, metastatic, spleen																												3
Lymph node																	+											2
Pancreatic, hepatocholangiocarcinoma, metastatic, liver																												1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Hemangiosarcoma																											X	3
Thymus	M	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	M	+	M	+	+	+	+	+	+	+	45
Integumentary System																												
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Skin, control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Skin, site of application-no mass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Skin, site of application-mass																												1
Subcutaneous tissue, sarcoma																												1
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liposarcoma, metastatic, skeletal muscle																												1
Skeletal muscle																												4
Hemangiosarcoma, metastatic, spleen																												1
Leiomyosarcoma, metastatic, uterus																												1
Liposarcoma																												1
Nervous System																												
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Peripheral nerve																												2
Spinal cord																												2
Hemangiosarcoma, metastatic, spleen																												1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	1 2 0	2 4 9	3 1 6	4 2 8	4 7 9	4 8 5	5 2 0	5 5 1	5 8 9	6 1 2	6 4 7	6 8 2	6 8 9	6 9 0	6 9 1	6 9 5	6 9 8	6 9 8	6 9 8	7 2 0	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	4 2 7	4 7 7	4 2 9	4 6 0	4 4 9	4 3 1	4 4 1	4 7 6	4 5 0	4 2 3	4 6 1	4 7 2	4 3 8	4 3 3	4 4 5	4 6 9	4 3 5	4 6 2	4 7 9	4 5 8	4 2 1	4 2 2	4 2 5	4 2 6	4 2 8	4 3 0	4 3 2
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X																						
Alveolar/bronchiolar carcinoma																											
Hepatocellular carcinoma, metastatic, liver								X					X						X								
Hepatocholangiocarcinoma, metastatic, liver																						X					
Liposarcoma, metastatic, skeletal muscle														X													
Nose	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																											
Eye																											
Harderian gland	+	M	M	+	+	+	+	+	+	+	M	M	+	+	+	M	M	M	+	+	+	+	+	M	M	+	+
Adenoma																										X	
Carcinoma																											
Zymbal's gland																											
Carcinoma																											
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																											
Leukemia lymphocytic																		X									
Lymphoma malignant lymphocytic											X																
Lymphoma malignant mixed		X								X																	

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Dermal Study of Benzethonium Chloride: 1.5 mg/kg
(continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	
Carcass ID Number	43 4	43 6	43 7	43 9	44 0	44 2	44 3	44 4	44 6	44 7	45 1	45 2	45 3	45 4	45 5	45 6	45 7	45 9	46 5	46 6	46 7	47 1	47 3	47 4	47 5	47 8	48 0	Total Tissues/ Tumors
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Alveolar/bronchiolar adenoma							X																					2
Alveolar/bronchiolar carcinoma										X																		1
Hepatocellular carcinoma, metastatic, liver																												3
Hepatocholangiocarcinoma, metastatic, liver																												1
Liposarcoma, metastatic, skeletal muscle																												1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Special Senses System																												
Eye															+					+								2
Harderian gland	M	+	+	+	M	+	+	+	M	M	M	+	M	+	+	M	+	+	M	+	+	+	M	M	+	+	+	35
Adenoma															X													2
Carcinoma																				X								1
Zymbal's gland	+																											1
Carcinoma	X																											1
Urinary System																												
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Histiocytic sarcoma																							X					1
Leukemia lymphocytic																												1
Lymphoma malignant lymphocytic																									X			2
Lymphoma malignant mixed																	X											3

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Harderian Gland: Adenoma or Carcinoma				
Overall rate[a]	4/52 (8%)	–[e]	–	3/54 (6%)
Adjusted rate[b]	10.1%			8.8%
Terminal rate[c]	3/38 (8%)			3/34 (9%)
First incidence (days)	698			729 (T)
Life table test[d]				P=0.558N
Logistic regression test[d]				P=0.550N
Fisher exact test[d]				P=0.479N
Liver: Hepatocellular Adenoma				
Overall rate	20/52 (38%)	–	–	18/54 (33%)
Adjusted rate	49.7%			51.1%
Terminal rate	18/38 (47%)			17/34 (50%)
First incidence (days)	674			589
Life table test				P=0.584
Logistic regression test				P=0.573N
Fisher exact test				P=0.364N
Liver: Hepatocellular Carcinoma				
Overall rate	12/52 (23%)	–	–	11/54 (20%)
Adjusted rate	29.0%			26.2%
Terminal rate	9/38 (24%)			5/34 (15%)
First incidence (days)	677			485
Life table test				P=0.583
Logistic regression test				P=0.482N
Fisher exact test				P=0.459N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	27/52 (52%)	–	–	25/54 (46%)
Adjusted rate	62.6%			60.2%
Terminal rate	22/38 (58%)			18/34 (53%)
First incidence (days)	674			485
Life table test				P=0.531
Logistic regression test				P=0.512N
Fisher exact test				P=0.350N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma				
Overall rate	2/52 (4%)	–	–	3/54 (6%)
Adjusted rate	5.3%			7.8%
Terminal rate	2/38 (5%)			2/34 (6%)
First incidence (days)	729 (T)			479
Life table test				P=0.460
Logistic regression test				P=0.571
Fisher exact test				P=0.518

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Ovary: Cystadenoma				
Overall rate	3/52 (6%)	–	–	2/52 (4%)
Adjusted rate	7.9%			5.9%
Terminal rate	3/38 (8%)			2/34 (6%)
First incidence (days)	729 (T)			729 (T)
Life table test				P=0.551N
Logistic regression test				P=0.551N
Fisher exact test				P=0.500N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	6/52 (12%)	–	–	5/50 (10%)
Adjusted rate	15.8%			14.3%
Terminal rate	6/38 (16%)			4/34 (12%)
First incidence (days)	729 (T)			720
Life table test				P=0.578N
Logistic regression test				P=0.578N
Fisher exact test				P=0.528N
Skin, Control (Subcutaneous Tissue): Fibrosarcoma or Sarcoma				
Overall rate	3/52 (6%)	0/53 (0%)	0/48 (0%)	1/54 (2%)
Adjusted rate	7.6%	0.0%	0.0%	2.5%
Terminal rate	2/38 (5%)	0/34 (0%)	0/31 (0%)	0/34 (0%)
First incidence (days)	698	–[f]	–	691
Life table test	P=0.461N	P=0.143N	P=0.156N	P=0.339N
Logistic regression test	P=0.452N	P=0.145N	P=0.141N	P=0.321N
Cochran-Armitage test	P=0.438N			
Fisher exact test		P=0.118N	P=0.137N	P=0.295N
Spleen: Hemangiosarcoma				
Overall rate	1/52 (2%)	–	–	3/53 (6%)
Adjusted rate	2.3%			7.5%
Terminal rate	0/38 (0%)			1/34 (3%)
First incidence (days)	689			589
Life table test				P=0.285
Logistic regression test				P=0.315
Fisher exact test				P=0.316
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	3/52 (6%)	–	–	1/54 (2%)
Adjusted rate	7.3%			2.9%
Terminal rate	2/38 (5%)			1/34 (3%)
First incidence (days)	660			729 (T)
Life table test				P=0.345N
Logistic regression test				P=0.327N
Fisher exact test				P=0.295N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	4/52 (8%)	–	–	1/54 (2%)
Adjusted rate	9.9%			2.9%
Terminal rate	3/38 (8%)			1/34 (3%)
First incidence (days)	660			729 (T)
Life table test				P=0.215N
Logistic regression test				P=0.202N
Fisher exact test				P=0.170N
All Organs: Hemangioma				
Overall rate	3/52 (6%)	–	–	2/54 (4%)
Adjusted rate	7.9%			4.4%
Terminal rate	3/38 (8%)			0/34 (0%)
First incidence (days)	729 (T)			316
Life table test				P=0.534N
Logistic regression test				P=0.419N
Fisher exact test				P=0.482N
All Organs: Hemangiosarcoma				
Overall rate	3/52 (6%)	–	–	3/54 (6%)
Adjusted rate	6.5%			7.5%
Terminal rate	0/38 (0%)			1/34 (3%)
First incidence (days)	660			589
Life table test				P=0.626
Logistic regression test				P=0.632N
Fisher exact test				P=0.643N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	6/52 (12%)	–	–	5/54 (9%)
Adjusted rate	13.9%			11.6%
Terminal rate	3/38 (8%)			1/34 (3%)
First incidence (days)	660			316
Life table test				P=0.549N
Logistic regression test				P=0.416N
Fisher exact test				P=0.473N
All Organs: Malignant Lymphoma (Lymphocytic, Mixed, or Undifferentiated Cell Type)				
Overall rate	7/52 (13%)	–	–	5/54 (9%)
Adjusted rate	16.9%			11.8%
Terminal rate	5/38 (13%)			2/34 (6%)
First incidence (days)	660			249
Life table test				P=0.444N
Logistic regression test				P=0.307N
Fisher exact test				P=0.354N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride
(continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
All Organs: Histiocytic Sarcoma				
Overall rate	3/52 (6%)	–	–	1/54 (2%)
Adjusted rate	7.0%			2.9%
Terminal rate	1/38 (3%)			1/34 (3%)
First incidence (days)	568			729 (T)
Life table test				P=0.347N
Logistic regression test				P=0.291N
Fisher exact test				P=0.295N
All Organs: Benign Neoplasms				
Overall rate	28/52 (54%)	–	–	29/54 (54%)
Adjusted rate	68.0%			71.9%
Terminal rate	25/38 (66%)			23/34 (68%)
First incidence (days)	660			316
Life table test				P=0.282
Logistic regression test				P=0.403
Fisher exact test				P=0.571N
All Organs: Malignant Neoplasms				
Overall rate	27/52 (52%)	–	–	27/54 (50%)
Adjusted rate	56.2%			55.9%
Terminal rate	17/38 (45%)			13/34 (38%)
First incidence (days)	568			249
Life table test				P=0.413
Logistic regression test				P=0.545N
Fisher exact test				P=0.499N
All Organs: Benign or Malignant Neoplasms				
Overall rate	41/52 (79%)	–	–	43/54 (80%)
Adjusted rate	85.4%			84.3%
Terminal rate	31/38 (82%)			26/34 (76%)
First incidence (days)	568			249
Life table test				P=0.214
Logistic regression test				P=0.406
Fisher exact test				P=0.555

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, pituitary gland, skin, spleen, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Organ was not examined at this dose level.
[f] Not applicable; no neoplasm in animal group

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	8	7	10	6
Early deaths				
Accidental death	1			
Moribund	10	4	8	13
Natural deaths	3	15	9	7
Survivors				
Terminal sacrifice	38	34	31	34
Missexed			2	
Animals examined microscopically	60	60	58	60
15-Month Interim Evaluation				
Alimentary System				
Liver	(8)			(6)
Basophilic focus				1 (17%)
Endocrine System				
Pituitary gland	(8)			(5)
Pars distalis, hyperplasia	2 (25%)			1 (20%)
Genital System				
Ovary	(8)			(6)
Cyst	2 (25%)			1 (17%)
Uterus	(8)			(6)
Hyperplasia, cystic	7 (88%)			4 (67%)
Hematopoietic System				
Lymph node				(1)
Pancreatic, hyperplasia				1 (100%)
Lymph node, mandibular	(8)			(6)
Hyperplasia				1 (17%)
Spleen	(8)			(6)
Hematopoietic cell proliferation				1 (17%)
Hyperplasia, lymphoid				1 (17%)
Thymus	(8)			(6)
Depletion lymphoid				1 (17%)
Integumentary System				
Skin, control	(8)	(7)	(10)	(6)
Skin, site of application-no mass	(8)	(7)	(10)	(6)
Epithelial hyperplasia			3 (30%)	4 (67%)

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

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
15-Month Interim Evaluation (continued)				
Respiratory System				
Lung	(8)			(6)
Alveolar epithelium, hyperplasia	1 (13%)			1 (17%)
Urinary System				
Kidney	(8)			(6)
Nephropathy	2 (25%)			1 (17%)
Systems Examined With No Lesions Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Special Senses System				
2-Year Study				
Alimentary System				
Gallbladder	(52)			(53)
Ulcer				1 (2%)
Intestine large, rectum	(52)			(53)
Artery, inflammation, chronic active				1 (2%)
Intestine small, duodenum	(51)			(53)
Erosion	2 (4%)			
Intestine small, ileum	(52)			(52)
Inflammation, acute				1 (2%)
Liver	(52)			(54)
Angiectasis	2 (4%)			1 (2%)
Basophilic focus	1 (2%)			2 (4%)
Clear cell focus	1 (2%)			1 (2%)
Developmental malformation	1 (2%)			
Eosinophilic focus	12 (23%)			17 (31%)
Fibrosis	1 (2%)			
Hematopoietic cell proliferation	2 (4%)			1 (2%)
Hemorrhage				1 (2%)
Inflammation, chronic active	3 (6%)			
Mixed cell focus	2 (4%)			3 (6%)
Necrosis	5 (10%)			
Bile duct, cyst				1 (2%)
Centrilobular, fatty change	1 (2%)			
Hepatocyte, hyperplasia	1 (2%)			1 (2%)
Mesentery	(10)			(9)
Inflammation, chronic active				1 (11%)
Inflammation, suppurative	2 (20%)			1 (11%)
Fat, necrosis	6 (60%)			5 (56%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Pancreas	(52)			(53)
Atrophy	2 (4%)			5 (9%)
Atypia cellular	1 (2%)			2 (4%)
Inflammation, chronic active				1 (2%)
Duct, cyst				3 (6%)
Stomach, forestomach	(51)			(53)
Erosion	1 (2%)			
Hyperplasia	1 (2%)			2 (4%)
Stomach, glandular	(51)			(53)
Erosion	1 (2%)			
Inflammation, acute				1 (2%)
Mineralization				1 (2%)
Cardiovascular System				
Heart	(52)			(54)
Degeneration				1 (2%)
Inflammation, chronic active	3 (6%)			
Mineralization	1 (2%)			
Artery, inflammation, chronic active	1 (2%)			
Endocrine System				
Adrenal cortex	(52)			(54)
Accessory adrenal cortical nodule	4 (8%)			3 (6%)
Hyperplasia	3 (6%)			1 (2%)
Capsule, hyperplasia, adenomatous	1 (2%)			
Adrenal medulla	(51)			(54)
Hyperplasia	4 (8%)			2 (4%)
Islets, pancreatic	(52)			(52)
Hyperplasia	2 (4%)			3 (6%)
Pituitary gland	(52)			(50)
Angiectasis	2 (4%)			
Pars distalis, hyperplasia	25 (48%)			29 (58%)
Thyroid gland	(52)			(54)
Follicle, cyst	3 (6%)			
Follicular cell, hyperplasia	20 (38%)			21 (39%)
General Body System				
None				

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Genital System				
Clitoral gland				(1)
Duct, ectasia				1 (100%)
Ovary	(52)			(52)
Angiectasis				1 (2%)
Cyst	25 (48%)			16 (31%)
Cyst dermoid				1 (2%)
Inflammation, suppurative	2 (4%)			1 (2%)
Interstitium, hyperplasia	1 (2%)			2 (4%)
Uterus	(52)			(53)
Angiectasis	1 (2%)			1 (2%)
Cyst	1 (2%)			
Hyperplasia, cystic	32 (62%)			39 (74%)
Infiltration cellular, histiocyte	1 (2%)			
Inflammation, chronic active	1 (2%)			1 (2%)
Hematopoietic System				
Bone marrow	(52)			(53)
Myelofibrosis	13 (25%)			13 (25%)
Erythroid cell, hyperplasia	9 (17%)			3 (6%)
Myeloid cell, hyperplasia	6 (12%)			4 (8%)
Lymph node	(7)			(2)
Mediastinal, necrosis	1 (14%)			
Lymph node, mandibular	(52)			(54)
Atrophy	1 (2%)			
Hematopoietic cell proliferation				1 (2%)
Hyperplasia, lymphoid	2 (4%)			1 (2%)
Lymph node, mesenteric	(50)			(49)
Atrophy	1 (2%)			
Hematopoietic cell proliferation	1 (2%)			2 (4%)
Hyperplasia, lymphoid	2 (4%)			
Spleen	(52)			(53)
Angiectasis	1 (2%)			
Depletion lymphoid	2 (4%)			
Hematopoietic cell proliferation	16 (31%)			23 (43%)
Hyperplasia, lymphoid	1 (2%)			
Hyperplasia, macrophage				1 (2%)
Thymus	(41)			(45)
Depletion lymphoid	5 (12%)			6 (13%)
Hyperplasia, lymphoid	3 (7%)			4 (9%)
Integumentary System				
Mammary gland	(52)			(52)
Hyperplasia	3 (6%)			2 (4%)
Skin, control	(52)	(52)	(48)	(53)
Epithelial hyperplasia			1 (2%)	
Inflammation, chronic		1 (2%)		

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Benzethonium Chloride (continued)

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
2-Year Study (continued)				
Integumentary System (continued)				
Skin, site of application-no mass	(52)	(52)	(48)	(53)
Epithelial hyperlasia	3 (6%)	7 (13%)	6 (13%)	22 (42%)
Inflammation, chronic	1 (2%)	2 (4%)		
Ulcer			2 (4%)	
Sebaceous gland, hyperplasia			1 (2%)	
Musculoskeletal System				
Bone	(52)			(53)
Osteopetrosis	1 (2%)			
Nervous System				
Brain	(52)			(53)
Hemorrhage	1 (2%)			
Hydrocephalus				1 (2%)
Neuron, necrosis	1 (2%)			1 (2%)
Peripheral nerve				(2)
Degeneration				2 (100%)
Respiratory System				
Lung	(52)			(54)
Hemorrhage				1 (2%)
Alveolar epithelium, hyperplasia	2 (4%)			2 (4%)
Alveolus, pigmentation, hemosiderin	2 (4%)			2 (4%)
Pleura, infiltration cellular, lymphocyte	1 (2%)			
Pleura, inflammation	1 (2%)			
Special Senses System				
Eye	(4)			(2)
Degeneration	3 (75%)			
Cornea, inflammation	1 (25%)			1 (50%)
Harderian gland	(44)			(35)
Hyperplasia	1 (2%)			2 (6%)
Urinary System				
Kidney	(52)			(54)
Glomerulosclerosis	1 (2%)			1 (2%)
Nephropathy	31 (60%)			38 (70%)

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1987). Benzethonium chloride was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) 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 at least five doses of benzethonium chloride. The high dose was limited by toxicity. All negative trials were repeated.

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, not reproducible, or is of insufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1987). Benzethonium chloride was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of benzethonium chloride; the high dose was limited by toxicity. A single flask per dose was used.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with benzethonium chloride in McCoy's 5A medium supplemented with fetal bovine serum, *l*-glutamine, and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing benzethonium chloride was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with benzethonium chloride, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no benzethonium chloride and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.005$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with benzethonium chloride for 12 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with benzethonium chloride and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 12 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. One hundred first-division metaphase cells were scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Chromosomal aberration data are presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P\leq0.05$) difference for one dose point and a significant trend ($P\leq0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend test in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.*, 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

RESULTS

Benzethonium chloride (0.010 to 100 μg/plate) was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with a preincubation protocol with or without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Zeiger *et al.*, 1987; Table E1). In cytogenetic tests with cultured CHO cells, benzethonium chloride did not induce SCEs (Table E2) or Abs (Table E3) with or without S9. Although an increase in Abs was observed in each of the two trials conducted, these increases were not statistically significant or dose related. No cell cycle delay was noted in either the SCE test or the Abs test.

TABLE E1
Mutagenicity of Benzethonium Chloride in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA98	0	20 ± 1.8	14 ± 4.1	19 ± 2.5	28 ± 3.2	22 ± 3.0	21 ± 0.9
	0.01	21 ± 0.7	7 ± 1.2				
	0.03	18 ± 1.8	6 ± 0.7				
	0.1	19 ± 2.1	7 ± 0.3				
	0.3	18 ± 0.9	10 ± 1.8				
	1	22 ± 1.5	10 ± 4.2	15 ± 1.9	28 ± 4.1	21 ± 2.0	17 ± 1.9
	3.3			16 ± 2.9	20 ± 0.7	24 ± 3.2	20 ± 2.4
	10			16 ± 1.7	20 ± 1.3	24 ± 1.5	16 ± 1.8
	33			10 ± 2.1	19 ± 2.9	16 ± 0.7	24 ± 1.2
	100			7 ± 1.2	21 ± 0.9	18 ± 0.7	18 ± 3.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		180 ± 63.8	123 ± 9.0	1,152 ± 40.1	719 ± 45.8	872 ± 70.4	1,150 ± 21.3
TA100	0	90 ± 1.8	101 ± 9.0	109 ± 7.1	152 ± 5.2	128 ± 9.3	142 ± 4.7
	0.01	87 ± 0.3	92 ± 3.8				
	0.03	85 ± 4.2	107 ± 0.3				
	0.1	82 ± 5.2	103 ± 5.3				
	0.3	94 ± 4.7	99 ± 4.3				
	1	92 ± 5.0	66 ± 1.7	105 ± 1.5	130 ± 5.9	109 ± 7.4	122 ± 8.0
	3.3			100 ± 3.2	133 ± 16.3	121 ± 6.1	132 ± 10.1
	10			94 ± 2.3	140 ± 14.2	118 ± 13.0	144 ± 6.9
	33			84 ± 4.1	144 ± 8.8	123 ± 8.7	141 ± 1.3
	100			34 ± 3.8	143 ± 15.2	128 ± 6.0	toxic
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		936 ± 11.1	1,024 ± 18.1	1,636 ± 125.5	1,021 ± 63.2	1,264 ± 205.4	2,105 ± 62.1
TA1535	0	18 ± 1.2	4 ± 1.5	17 ± 1.5	6 ± 0.0	25 ± 1.5	7 ± 1.7
	0.01	14 ± 1.9	4 ± 0.7				
	0.03	16 ± 3.7	4 ± 1.2				
	0.1	14 ± 3.2	5 ± 0.7				
	0.3	8 ± 1.5	2 ± 1.2				
	1	8 ± 0.6	2 ± 0.6	18 ± 3.3	4 ± 1.8	16 ± 0.9	5 ± 1.9
	3.3			26 ± 1.3	5 ± 1.5	20 ± 2.0	6 ± 2.5
	10			15 ± 4.2	3 ± 0.9	17 ± 1.5	5 ± 1.7
	33			9 ± 0.9	4 ± 0.7	8 ± 1.5	4 ± 0.7
	100			toxic	toxic	toxic	toxic
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		942 ± 36.6	446 ± 18.0	125 ± 10.7	97 ± 15.5	184 ± 15.0	60 ± 8.4

TABLE E1
Mutagenicity of Benzethonium Chloride in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA1537	0	12 ± 1.5	10 ± 1.2	24 ± 2.4	15 ± 1.5	19 ± 0.3	12 ± 1.5
	0.01	9 ± 0.6	13 ± 0.7				
	0.03	10 ± 2.8	9 ± 1.5				
	0.1	14 ± 1.0	7 ± 0.9				
	0.3	16 ± 0.7	7 ± 2.3				
	1	11 ± 3.8	7 ± 1.2	22 ± 2.4	13 ± 1.0	23 ± 3.0	12 ± 1.5
	3.3			26 ± 4.3	10 ± 2.1	20 ± 3.4	15 ± 1.7
	10			14 ± 0.6	9 ± 2.4	13 ± 2.0	7 ± 2.1
	33			13 ± 1.2	15 ± 2.6	13 ± 2.5	12 ± 1.7
	100			1 ± 0.7	13 ± 2.5	toxic	toxic
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		207 ± 94.2	177 ± 104.5	356 ± 21.3	197 ± 15.3	432 ± 29.1	122 ± 43.3

[a] The study was performed at Case Western Reserve University. The detailed protocol and these data are presented in Zeiger *et al.* (1987). The high dose was limited by toxicity; 0 μg/plate dose is the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Benzethonium Chloride[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Summary: Negative								
Distilled water		50	1,047	434	0.41	8.7	26.0	
Mitomycin-C	0.005	25	523	639	1.22	25.6	26.0	194.76
Benzethonium chloride	0.96	50	1,049	458	0.43	9.2	26.0	5.33
	3	50	1,050	457	0.43	9.1	26.0	5.00
	9.6	50	1,048	473	0.45	9.5	26.0	8.88
					P=0.115[c]			
+S9								
Summary: Negative								
Distilled water		50	1,043	369	0.35	7.4	26.0	
Cyclophosphamide	1	50	1,045	790	0.75	15.8	26.0	113.69
Benzethonium chloride	3	50	1,048	347	0.33	6.9	26.0	−6.41
	9.6	50	1,050	359	0.34	7.2	26.0	−3.36
	30	50	1,047	367	0.35	7.3	26.0	−0.92
					P=0.495			

[a] Study performed at Columbia University. A detailed description of the protocol and these data are presented in Galloway *et al.* (1987). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine.
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells.
[c] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose

TABLE E3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Benzethonium Chloride[a]

-S9					+S9				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)
Harvest time: 14.0 hours Summary: Negative					Harvest time: 14.0 hours Summary: Negative				
Distilled water					Distilled water				
	100	4	0.04	4.0		100	3	0.03	3.0
Mitomycin-C					Cyclophosphamide				
0.15	50	34	0.68	42.0	15	50	16	0.32	28.0
Benzethonium chloride					Benzethonium chloride				
0.96	100	11	0.11	10.0	3	100	5	0.05	5.0
3	100	10	0.10	10.0	9.6	100	6	0.06	5.0
9.6	100	8	0.08	8.0	30	100	6	0.06	6.0
				P=0.162[b]					P=0.172

[a] Study performed at Columbia University. The detailed protocol and these data are presented in Galloway *et al.* (1987). Abs=aberrations.
[b] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose

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

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 16-Day Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	6.3 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	167 ± 7	165 ± 5	162 ± 7	154 ± 4	140 ± 6**	127 ± 6**
Brain						
Absolute	1.710 ± 0.008	1.685 ± 0.028	1.701 ± 0.017	1.671 ± 0.023	1.663 ± 0.012	1.647 ± 0.010*
Relative	10.30 ± 0.44	10.25 ± 0.18	10.57 ± 0.37	10.90 ± 0.32	11.93 ± 0.46*	13.11 ± 0.70**
Heart						
Absolute	0.686 ± 0.022	0.647 ± 0.016	0.637 ± 0.014	0.651 ± 0.016	0.631 ± 0.028	0.592 ± 0.014**
Relative	4.12 ± 0.14	3.93 ± 0.06	3.95 ± 0.11	4.24 ± 0.13	4.50 ± 0.11	4.72 ± 0.28*
R. Kidney						
Absolute	0.935 ± 0.034	0.916 ± 0.028	0.882 ± 0.030	0.817 ± 0.020**	0.806 ± 0.024**	0.780 ± 0.025**
Relative	5.60 ± 0.11	5.56 ± 0.06	5.46 ± 0.13	5.32 ± 0.10	5.77 ± 0.21	6.18 ± 0.25
Liver						
Absolute	10.880 ± 0.433	9.896 ± 0.421	9.485 ± 0.390*	9.093 ± 0.379**	8.794 ± 0.434**	7.881 ± 0.190**
Relative	65.14 ± 1.59	60.06 ± 1.18	58.67 ± 1.33	59.11 ± 1.38	62.65 ± 1.12	62.62 ± 3.04
Lungs						
Absolute	1.516 ± 0.131	1.301 ± 0.111	1.181 ± 0.026	1.518 ± 0.145	1.108 ± 0.064**	1.065 ± 0.028**
Relative	9.26 ± 1.20	7.85 ± 0.45	7.33 ± 0.24	9.98 ± 1.14	7.90 ± 0.35	8.45 ± 0.34
R. Testis						
Absolute	1.022 ± 0.023	0.956 ± 0.022	0.949 ± 0.018	0.907 ± 0.020	0.868 ± 0.075*	0.887 ± 0.066*
Relative	6.13 ± 0.16	5.82 ± 0.10	5.89 ± 0.20	5.92 ± 0.20	6.15 ± 0.37	6.97 ± 0.32*
Thymus						
Absolute	0.457 ± 0.018	0.410 ± 0.006	0.391 ± 0.032*	0.378 ± 0.024*	0.373 ± 0.015**	0.267 ± 0.017**
Relative	2.74 ± 0.08	2.50 ± 0.08	2.44 ± 0.24	2.48 ± 0.19	2.67 ± 0.13	2.12 ± 0.16*
Female						
Necropsy body wt	123 ± 3	120 ± 3	124 ± 2	116 ± 3	109 ± 5*	108 ± 6*
Brain						
Absolute	1.617 ± 0.029	1.611 ± 0.008	1.582 ± 0.029	1.570 ± 0.025	1.564 ± 0.033	1.543 ± 0.025
Relative	13.22 ± 0.17	13.45 ± 0.30	12.80 ± 0.18	13.61 ± 0.23	14.37 ± 0.42*	14.42 ± 0.56*
Heart						
Absolute	0.578 ± 0.009	0.567 ± 0.015	0.549 ± 0.013	0.549 ± 0.012	0.516 ± 0.018**	0.521 ± 0.007**
Relative	4.73 ± 0.12	4.74 ± 0.18	4.44 ± 0.13	4.76 ± 0.10	4.73 ± 0.06	4.88 ± 0.21
R. Kidney						
Absolute	0.677 ± 0.014	0.705 ± 0.013	0.690 ± 0.014	0.694 ± 0.027	0.691 ± 0.022	0.672 ± 0.026
Relative	5.53 ± 0.09	5.88 ± 0.09	5.58 ± 0.12	6.01 ± 0.09**	6.34 ± 0.15**	6.25 ± 0.13**
Liver						
Absolute	6.251 ± 0.234	6.532 ± 0.170	6.388 ± 0.195	6.463 ± 0.264	6.112 ± 0.212	5.964 ± 0.245
Relative	51.06 ± 1.47	54.42 ± 0.89	51.64 ± 1.28	55.99 ± 1.87*	56.06 ± 1.49*	55.45 ± 1.06*
Lungs						
Absolute	1.023 ± 0.035	1.062 ± 0.038	0.941 ± 0.038	0.958 ± 0.054	0.863 ± 0.077*	0.863 ± 0.058*
Relative	8.35 ± 0.18	8.87 ± 0.40	7.62 ± 0.32	8.28 ± 0.32	7.88 ± 0.57	7.99 ± 0.32
Thymus						
Absolute	0.358 ± 0.012	0.366 ± 0.019	0.374 ± 0.018	0.308 ± 0.016	0.273 ± 0.017**	0.249 ± 0.017**
Relative	2.93 ± 0.07	3.05 ± 0.13	3.02 ± 0.14	2.66 ± 0.10	2.49 ± 0.04**	2.30 ± 0.07**

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	1.56 mg/kg	3.13 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	327 ± 6	326 ± 6	333 ± 6	323 ± 6	319 ± 5	287 ± 7**
Brain						
Absolute	1.900 ± 0.019	1.902 ± 0.021	1.952 ± 0.022	1.928 ± 0.017	1.933 ± 0.024	1.947 ± 0.019
Relative	5.82 ± 0.07	5.84 ± 0.06	5.87 ± 0.10	5.98 ± 0.09	6.07 ± 0.05	6.81 ± 0.15**
Heart						
Absolute	1.009 ± 0.022	1.014 ± 0.023	1.054 ± 0.023	1.029 ± 0.025	1.045 ± 0.019	1.048 ± 0.029
Relative	3.09 ± 0.03	3.11 ± 0.05	3.17 ± 0.06	3.18 ± 0.07	3.28 ± 0.07	3.67 ± 0.15**
R. Kidney						
Absolute	1.339 ± 0.033	1.318 ± 0.033	1.355 ± 0.026	1.309 ± 0.038	1.341 ± 0.023	1.353 ± 0.035
Relative	4.10 ± 0.05	4.04 ± 0.07	4.07 ± 0.06	4.05 ± 0.07	4.21 ± 0.09	4.72 ± 0.12**
Liver						
Absolute	16.102 ± 0.503	15.648 ± 0.660	16.669 ± 0.362	15.628 ± 0.424	15.629 ± 0.250	14.451 ± 0.400*
Relative	49.22 ± 1.02	47.86 ± 1.53	50.07 ± 0.80	48.29 ± 0.64	49.10 ± 0.98	50.39 ± 1.21
Lungs						
Absolute	2.030 ± 0.075	1.952 ± 0.069	2.058 ± 0.096	1.988 ± 0.055	1.914 ± 0.080	1.876 ± 0.034
Relative	6.20 ± 0.17	5.98 ± 0.17	6.17 ± 0.22	6.16 ± 0.17	6.00 ± 0.24	6.55 ± 0.15
R. Testis						
Absolute	1.452 ± 0.038	1.408 ± 0.022[b]	1.486 ± 0.029	1.455 ± 0.016	1.485 ± 0.015	1.440 ± 0.032
Relative	4.45 ± 0.12	4.35 ± 0.09[b]	4.47 ± 0.08	4.51 ± 0.06	4.66 ± 0.07	5.04 ± 0.16**
Thymus						
Absolute	0.301 ± 0.010	0.298 ± 0.013	0.301 ± 0.013	0.287 ± 0.016	0.275 ± 0.013	0.230 ± 0.008**[b]
Relative	0.92 ± 0.03	0.91 ± 0.03	0.91 ± 0.04	0.89 ± 0.04	0.86 ± 0.04	0.79 ± 0.03*[b]
Female						
Necropsy body wt	185 ± 3	186 ± 3	182 ± 2	183 ± 3	183 ± 2	181 ± 3
Brain						
Absolute	1.754 ± 0.019	1.771 ± 0.012	1.750 ± 0.018	1.736 ± 0.023	1.765 ± 0.021	1.733 ± 0.019
Relative	9.51 ± 0.18	9.55 ± 0.16	9.62 ± 0.11	9.51 ± 0.15	9.68 ± 0.16	9.59 ± 0.14
Heart						
Absolute	0.680 ± 0.010	0.722 ± 0.025	0.676 ± 0.007	0.674 ± 0.013	0.702 ± 0.009[b]	0.687 ± 0.016
Relative	3.68 ± 0.05	3.87 ± 0.08	3.72 ± 0.03	3.69 ± 0.07	3.84 ± 0.07[b]	3.80 ± 0.07
R. Kidney						
Absolute	0.770 ± 0.016	0.780 ± 0.018	0.783 ± 0.012	0.781 ± 0.015	0.785 ± 0.016	0.818 ± 0.013*
Relative	4.17 ± 0.05	4.19 ± 0.06	4.31 ± 0.06	4.27 ± 0.05	4.30 ± 0.07	4.52 ± 0.06**
Liver						
Absolute	7.212 ± 0.150	8.001 ± 0.217*	7.767 ± 0.218	7.434 ± 0.179	7.859 ± 0.219	7.660 ± 0.254
Relative	39.03 ± 0.67	43.00 ± 0.80*	42.75 ± 1.33	40.73 ± 1.04	43.03 ± 1.10*	42.33 ± 1.23
Lungs						
Absolute	1.346 ± 0.035	1.490 ± 0.072	1.346 ± 0.041	1.386 ± 0.052	1.401 ± 0.109	1.306 ± 0.039
Relative	7.29 ± 0.21	8.02 ± 0.39	7.39 ± 0.18	7.59 ± 0.28	7.70 ± 0.65	7.21 ± 0.16
Thymus						
Absolute	0.242 ± 0.007	0.242 ± 0.006	0.222 ± 0.011	0.228 ± 0.007	0.240 ± 0.007	0.232 ± 0.011
Relative	1.31 ± 0.04	1.30 ± 0.04	1.22 ± 0.05	1.25 ± 0.05	1.31 ± 0.04	1.29 ± 0.07

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

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 15-Month Interim Evaluation in the 2-Year Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Male				
n	8	8	5	4
Necropsy body wt	498 ± 10	496 ± 18	520 ± 30	486 ± 12
L. Kidney				
Absolute	1.833 ± 0.032	1.929 ± 0.063	2.042 ± 0.139	1.835 ± 0.069
Relative	3.69 ± 0.09	3.90 ± 0.06	3.93 ± 0.13	3.78 ± 0.15
R. Kidney				
Absolute	1.823 ± 0.031	1.946 ± 0.061	2.017 ± 0.091	1.811 ± 0.070
Relative	3.67 ± 0.09	3.93 ± 0.07	3.90 ± 0.14	3.73 ± 0.11
Liver				
Absolute	19.236 ± 0.723	20.325 ± 1.167	21.970 ± 1.425	19.519 ± 0.510
Relative	38.73 ± 1.57	40.84 ± 1.28	42.31 ± 1.87	40.21 ± 0.73
Female				
n	9	7	9	7
Necropsy body wt	289 ± 8	289 ± 6	295 ± 9	285 ± 9
L. Kidney				
Absolute	1.158 ± 0.039	1.129 ± 0.033	1.215 ± 0.026	1.168 ± 0.045
Relative	4.01 ± 0.13	3.91 ± 0.11	4.14 ± 0.10	4.10 ± 0.15
R. Kidney				
Absolute	1.137 ± 0.034	1.142 ± 0.039	1.229 ± 0.034	1.139 ± 0.045
Relative	3.95 ± 0.13	3.95 ± 0.13	4.18 ± 0.06	4.01 ± 0.16
Liver				
Absolute	10.579 ± 0.324	10.849 ± 0.304	11.651 ± 0.581	10.434 ± 0.300
Relative	36.74 ± 1.32	37.54 ± 0.83	39.49 ± 1.27	36.64 ± 0.72

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

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 16-Day Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	6.3 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male						
n	5	5	5	5	5	4
Necropsy body wt	25.1 ± 0.4	25.3 ± 0.6	25.9 ± 0.4	26.5 ± 0.5	26.4 ± 0.7	26.5 ± 0.5
Brain						
Absolute	0.472 ± 0.012	0.470 ± 0.004	0.475 ± 0.007	0.446 ± 0.014	0.455 ± 0.012	0.454 ± 0.007
Relative	18.83 ± 0.68	18.63 ± 0.45	18.37 ± 0.48	16.85 ± 0.55*	17.29 ± 0.74*	17.17 ± 0.23
Heart						
Absolute	0.154 ± 0.009	0.155 ± 0.010	0.154 ± 0.009	0.167 ± 0.006	0.155 ± 0.005	0.188 ± 0.017*
Relative	6.14 ± 0.39	6.16 ± 0.46	5.97 ± 0.46	6.31 ± 0.17	5.89 ± 0.23	7.06 ± 0.53
R. Kidney						
Absolute	0.285 ± 0.008	0.283 ± 0.005	0.273 ± 0.014	0.273 ± 0.008	0.262 ± 0.015	0.303 ± 0.003
Relative	11.34 ± 0.28	11.20 ± 0.23	10.55 ± 0.44	10.32 ± 0.32	9.94 ± 0.63	11.45 ± 0.25
Liver						
Absolute	1.663 ± 0.017	1.615 ± 0.047	1.689 ± 0.017	1.737 ± 0.062	1.687 ± 0.074	1.857 ± 0.035*
Relative	66.28 ± 1.32	63.99 ± 1.98	65.30 ± 0.60	65.57 ± 1.17	63.75 ± 1.46	70.21 ± 2.02
Lungs						
Absolute	0.241 ± 0.014[b]	0.207 ± 0.006	0.230 ± 0.016	0.206 ± 0.003	0.222 ± 0.009	0.226 ± 0.019
Relative	9.64 ± 0.49[b]	8.21 ± 0.27	8.88 ± 0.63	7.81 ± 0.18*	8.39 ± 0.24	8.50 ± 0.60
R. Testis						
Absolute	0.112 ± 0.005	0.115 ± 0.003	0.109 ± 0.005	0.106 ± 0.004	0.108 ± 0.005	0.112 ± 0.005
Relative	4.44 ± 0.17	4.55 ± 0.21	4.20 ± 0.15	4.00 ± 0.11	4.08 ± 0.13	4.21 ± 0.12
Thymus						
Absolute	0.052 ± 0.004	0.052 ± 0.003	0.051 ± 0.003	0.052 ± 0.004	0.041 ± 0.004	0.046 ± 0.001
Relative	2.07 ± 0.16	2.05 ± 0.14	1.96 ± 0.13	1.97 ± 0.17	1.54 ± 0.16*	1.72 ± 0.07
Female						
n	5	5	5	5	5	5
Necropsy body wt	21.2 ± 0.5	21.6 ± 0.4	21.0 ± 0.4	21.3 ± 0.3	21.1 ± 0.3	20.9 ± 0.5
Brain						
Absolute	0.460 ± 0.012	0.472 ± 0.007	0.455 ± 0.009	0.457 ± 0.004	0.440 ± 0.006	0.450 ± 0.005
Relative	21.71 ± 0.45	21.95 ± 0.62	21.69 ± 0.66	21.46 ± 0.18	20.87 ± 0.29	21.60 ± 0.44
Heart						
Absolute	0.119 ± 0.007	0.126 ± 0.004	0.123 ± 0.004	0.124 ± 0.002	0.125 ± 0.005	0.136 ± 0.006*
Relative	5.60 ± 0.23	5.85 ± 0.19	5.87 ± 0.25	5.84 ± 0.13	5.91 ± 0.22	6.54 ± 0.38*
R. Kidney						
Absolute	0.200 ± 0.008	0.190 ± 0.004	0.193 ± 0.003	0.193 ± 0.006	0.191 ± 0.006	0.206 ± 0.008
Relative	9.44 ± 0.25	8.83 ± 0.23	9.18 ± 0.22	9.06 ± 0.25	9.06 ± 0.22	9.87 ± 0.22
Liver						
Absolute	1.325 ± 0.016	1.334 ± 0.044	1.393 ± 0.048	1.347 ± 0.016	1.392 ± 0.028	1.401 ± 0.067
Relative	62.67 ± 1.30	61.81 ± 0.98	66.15 ± 1.49	63.36 ± 1.56	66.00 ± 0.73	66.97 ± 1.74*
Lungs						
Absolute	0.184 ± 0.007	0.213 ± 0.012	0.189 ± 0.006	0.196 ± 0.013	0.184 ± 0.004	0.186 ± 0.008
Relative	8.70 ± 0.21	9.88 ± 0.56	8.97 ± 0.21	9.23 ± 0.61	8.75 ± 0.27	8.87 ± 0.17
Thymus						
Absolute	0.069 ± 0.005	0.072 ± 0.003	0.066 ± 0.003	0.061 ± 0.005	0.061 ± 0.004	0.054 ± 0.006*
Relative	3.23 ± 0.19	3.32 ± 0.09	3.11 ± 0.10	2.88 ± 0.23	2.90 ± 0.18	2.59 ± 0.24*

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=4

TABLE F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	1.56 mg/kg	3.13 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	33.5 ± 1.0	32.6 ± 0.7	32.5 ± 1.0	32.4 ± 0.5	32.2 ± 0.8	31.4 ± 0.7
Brain						
Absolute	0.466 ± 0.007	0.454 ± 0.007	0.453 ± 0.009	0.472 ± 0.005	0.486 ± 0.007	0.465 ± 0.005
Relative	14.01 ± 0.46	13.98 ± 0.32	14.05 ± 0.41	14.61 ± 0.21	15.18 ± 0.31*	14.86 ± 0.23*
Heart						
Absolute	0.176 ± 0.005	0.176 ± 0.006	0.173 ± 0.007[b]	0.183 ± 0.004	0.183 ± 0.007	0.180 ± 0.006
Relative	5.26 ± 0.18	5.40 ± 0.15	5.26 ± 0.13[b]	5.68 ± 0.19	5.68 ± 0.13	5.77 ± 0.20*
R. Kidney						
Absolute	0.329 ± 0.011	0.323 ± 0.008	0.338 ± 0.012	0.339 ± 0.013	0.349 ± 0.007	0.346 ± 0.008
Relative	9.82 ± 0.24	9.95 ± 0.30	10.42 ± 0.28	10.46 ± 0.33	10.89 ± 0.21**	11.03 ± 0.17**
Liver						
Absolute	1.753 ± 0.054	1.684 ± 0.032	1.754 ± 0.058	1.764 ± 0.036	1.821 ± 0.052	1.786 ± 0.032
Relative	52.38 ± 1.23	51.86 ± 1.11	54.04 ± 1.00	54.50 ± 0.82	56.62 ± 0.61**	57.07 ± 0.99**
Lungs						
Absolute	0.283 ± 0.011	0.289 ± 0.017	0.281 ± 0.013	0.295 ± 0.010	0.273 ± 0.018	0.260 ± 0.016
Relative	8.53 ± 0.44	8.85 ± 0.40	8.62 ± 0.26	9.15 ± 0.40	8.43 ± 0.39	8.23 ± 0.41
R. Testis						
Absolute	0.117 ± 0.003	0.118 ± 0.003	0.116 ± 0.003	0.118 ± 0.002	0.114 ± 0.003	0.117 ± 0.002
Relative	3.48 ± 0.08	3.64 ± 0.08	3.56 ± 0.07	3.65 ± 0.09	3.55 ± 0.06	3.75 ± 0.03*
Thymus						
Absolute	0.042 ± 0.003	0.036 ± 0.002	0.038 ± 0.002	0.037 ± 0.002	0.038 ± 0.002	0.042 ± 0.001
Relative	1.27 ± 0.09	1.12 ± 0.06	1.16 ± 0.06	1.15 ± 0.06	1.17 ± 0.04	1.33 ± 0.05
Female						
Necropsy body wt	27.5 ± 0.8	27.5 ± 0.6	27.9 ± 0.6	27.8 ± 0.7	27.3 ± 0.7	27.8 ± 1.0
Brain						
Absolute	0.482 ± 0.007	0.464 ± 0.008	0.465 ± 0.009	0.473 ± 0.005	0.471 ± 0.008	0.484 ± 0.007
Relative	17.61 ± 0.42	16.96 ± 0.46	16.67 ± 0.32	17.08 ± 0.35	17.32 ± 0.48	17.57 ± 0.52
Heart						
Absolute	0.149 ± 0.007	0.144 ± 0.004	0.159 ± 0.008	0.169 ± 0.008	0.150 ± 0.006	0.155 ± 0.006
Relative	5.46 ± 0.28	5.25 ± 0.17	5.72 ± 0.31	6.08 ± 0.25	5.52 ± 0.21	5.62 ± 0.26
R. Kidney						
Absolute	0.217 ± 0.008	0.230 ± 0.006	0.227 ± 0.008	0.229 ± 0.005	0.219 ± 0.006	0.233 ± 0.006
Relative	7.92 ± 0.29	8.39 ± 0.26	8.13 ± 0.25	8.25 ± 0.19	8.03 ± 0.13	8.42 ± 0.14
Liver						
Absolute	1.491 ± 0.064	1.460 ± 0.048	1.510 ± 0.050	1.527 ± 0.039	1.522 ± 0.051	1.635 ± 0.050
Relative	54.32 ± 2.10	53.25 ± 1.82	54.00 ± 1.08	55.05 ± 1.30	55.75 ± 1.44	59.16 ± 1.84
Lungs						
Absolute	0.256 ± 0.012	0.250 ± 0.010	0.273 ± 0.016	0.261 ± 0.013	0.255 ± 0.009	0.250 ± 0.009
Relative	9.36 ± 0.46	9.10 ± 0.36	9.82 ± 0.64	9.37 ± 0.42	9.36 ± 0.33	9.07 ± 0.39
Thymus						
Absolute	0.051 ± 0.002	0.049 ± 0.002	0.048 ± 0.003	0.049 ± 0.003	0.050 ± 0.002	0.050 ± 0.003
Relative	1.85 ± 0.05	1.77 ± 0.07	1.72 ± 0.08	1.77 ± 0.07	1.81 ± 0.06	1.81 ± 0.07

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

TABLE F6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 15-Month Interim Evaluation in the 2-Year Dermal Study of Benzethonium Chloride[a]

	Vehicle Control	0.15 mg/kg	0.5 mg/kg	1.5 mg/kg
Male				
n	10	9	9	10
Necropsy body wt	51.2 ± 1.2	52.0 ± 1.4	49.6 ± 1.7	50.2 ± 0.8
L. Kidney				
Absolute	0.466 ± 0.014	0.436 ± 0.013	0.433 ± 0.010	0.453 ± 0.010
Relative	9.12 ± 0.31	8.40 ± 0.21	8.78 ± 0.23	9.05 ± 0.26
R. Kidney				
Absolute	0.485 ± 0.015	0.462 ± 0.011	0.450 ± 0.008	0.489 ± 0.010
Relative	9.48 ± 0.29	8.92 ± 0.20	9.11 ± 0.22	9.78 ± 0.26
Liver				
Absolute	3.296 ± 0.362	2.591 ± 0.142	2.978 ± 0.338	3.082 ± 0.358
Relative	65.51 ± 8.61	49.95 ± 2.65	61.70 ± 9.23	61.60 ± 7.37
Female				
n	8	7	10	6
Necropsy body wt	50.5 ± 1.5	52.3 ± 0.9	51.5 ± 2.5	51.1 ± 3.0
L. Kidney				
Absolute	0.280 ± 0.007	0.303 ± 0.016	0.296 ± 0.008	0.307 ± 0.013
Relative	5.55 ± 0.06	5.77 ± 0.24	5.82 ± 0.19	6.04 ± 0.17
R. Kidney				
Absolute	0.297 ± 0.009	0.320 ± 0.012	0.308 ± 0.009	0.322 ± 0.015
Relative	5.89 ± 0.10	6.12 ± 0.18	6.06 ± 0.25	6.35 ± 0.23
Liver				
Absolute	1.959 ± 0.074	2.061 ± 0.068[b]	2.224 ± 0.090*[c]	2.184 ± 0.102
Relative	38.82 ± 1.01	39.82 ± 0.85[b]	42.09 ± 0.75*[c]	42.95 ± 0.89**

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=6
[c] n=9

APPENDIX G
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF BENZETHONIUM CHLORIDE

United States Pharmacopeia (USP) grade benzethonium chloride was obtained from Rohm and Haas (Philadelphia, PA) in one lot (W0061), which was used throughout the 16-day, 13-week, and 2-year dermal studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). Reports on analyses performed in support of the benzethonium chloride studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a white powder, was identified as benzethonium chloride by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with the literature spectra (*Sadtler Standard Spectra, Aldrich Library*) of benzethonium chloride (Figures G1 and G2).

The purity was determined by elemental analyses, Karl Fischer water analysis, functional group titration, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). For functional group titration, samples were dissolved in 20 mL glacial acetic acid and 10 mL 2% mercury (II) acetate. The sample solutions were then titrated with 0.1 N perchloric acid and monitored potentiometrically using a combination pH/mV electrode filled with 3 M aqueous potassium chloride. TLC was performed on Silica Gel 60 F-254 plates using two solvent systems: A) *n*-butanol:water:glacial acetic acid (66:17:17), and B) acetone:concentrated ammonium hydroxide (90:10). Nicotinamide was used as a reference standard. Plates were examined under shortwave (254 nm) ultraviolet light and a spray of iodoplatinate reagent. HPLC was performed using a Waters μBondapak C_{18} column using ultraviolet detection (280 nm) and a solvent system of 0.1 M methanesulfonic acid in water adjusted to pH 2.0 with 10 N sodium hydroxide: 0.1 M methanesulfonic acid, or 0.1 M methanesulfonic acid in methanol adjusted to pH 2.0 with 10 N sodium hydroxide (20:80). The flow rate was 1.0 mL/minute.

Elemental analyses for carbon, hydrogen, nitrogen, and chlorine were in agreement with the theoretical values for benzethonium chloride. Karl Fischer water analysis indicated 0.6% ± 0.3% water. Functional group titration indicated a purity of 98.5% ± 0.5%. Thin-layer chromatography by system A indicated a major spot and a trace impurity near the origin; system B indicated a major spot and a minor impurity near the origin. HPLC detected a major peak and no impurities greater than or equal to 0.1% of the major peak area. The overall purity was determined to be greater than 98%.

The analytical chemistry laboratory analyzed the chemical to determine if it met USP purity requirements. The complete battery of USP analyses was performed as a supplement to the chemical characterization of benzethonium chloride. The USP tests included a test for chloride, reaction with nitric acid and mercuric chloride, as well as reaction with sodium nitrite. Further tests included determination of melting point range, weight loss on drying, residue on ignition, and containment of ammonium compounds. The assay was a titration with sodium tetraphenylboron and a bromophenol blue indicator. The melting point range was 161.2° to 161.4° C, and the test for weight loss on drying yielded a value of 0.4% ± 0.3% water. These values conform to the USP requirements for both analyses. The sample met USP specifications for the residue on ignition (0.007% ± 0.002%) and ammonium compounds (no perceptible ammonium odor) tests. The titrimetric assay indicated that the sample contained 99.7% ± 0.05% benzethonium chloride, which met USP requirements for purity.

Stability studies were performed by the analytical chemistry laboratory using the HPLC system previously described. These studies indicated that benzethonium chloride was stable as a bulk chemical for at least 2 weeks when stored protected from light at temperatures up to 60° C. The bulk chemical was stored at room temperature protected from light. The stability of the chemical was monitored periodically using HPLC methods similar to those previously described. No degradation of the bulk chemical was observed.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Dose formulation solutions were prepared by mixing benzethonium chloride and 95% ethanol (USP grade) to give the required concentrations (Table G1). The dose formulations were prepared once for the 16-day studies and every 2 weeks for the 13-week and 2-year studies and were stored protected from light in sealed glass vials at room temperature. Dose formulations were discarded 3 weeks after the date of preparation.

Dose formulation stability studies were performed by the analytical chemistry laboratory. Aliquots of the 0.03 mg/mL formulation of benzethonium chloride were evaporated under nitrogen and redissolved in 5 mL of internal standard solution (octanophenone, 0.1 mg/mL in acetonitrile). HPLC was performed using a Chromanetics Licrosorb RP-2 column with a flow rate of 2.0 mL/minute, a mobile phase of water:acetonitrile:glacial acetic acid (30:69:1), octanophenone as an internal standard, and ultraviolet detection at 280 nm. The stability of the benzethonium chloride dose formulation was confirmed for at least 3 weeks at room temperature when stored protected from light, and for 3 hours when exposed to light and air.

Periodic analyses of the dose formulations of benzethonium chloride were conducted by the study laboratory and the analytical chemistry laboratory using ethanol dilutions and subsequent determination of absorbance at 227 nm. The study laboratory analyzed the dose formulations once during the 16-day studies (Table G2), three times during the 13-week studies (Table G3), and approximately every 2 months during the 2-year studies (Table G4). All of the dose formulations from the 16-day and 13-week studies were found to be within 10% of the target concentrations. In the 2-year study, 98% (159/163) of the dose formulations analyzed were within 10% of the target concentrations. Results of the periodic referee analyses performed by the analytical chemistry laboratory were in agreement with the results obtained by the study laboratory (Table G5).

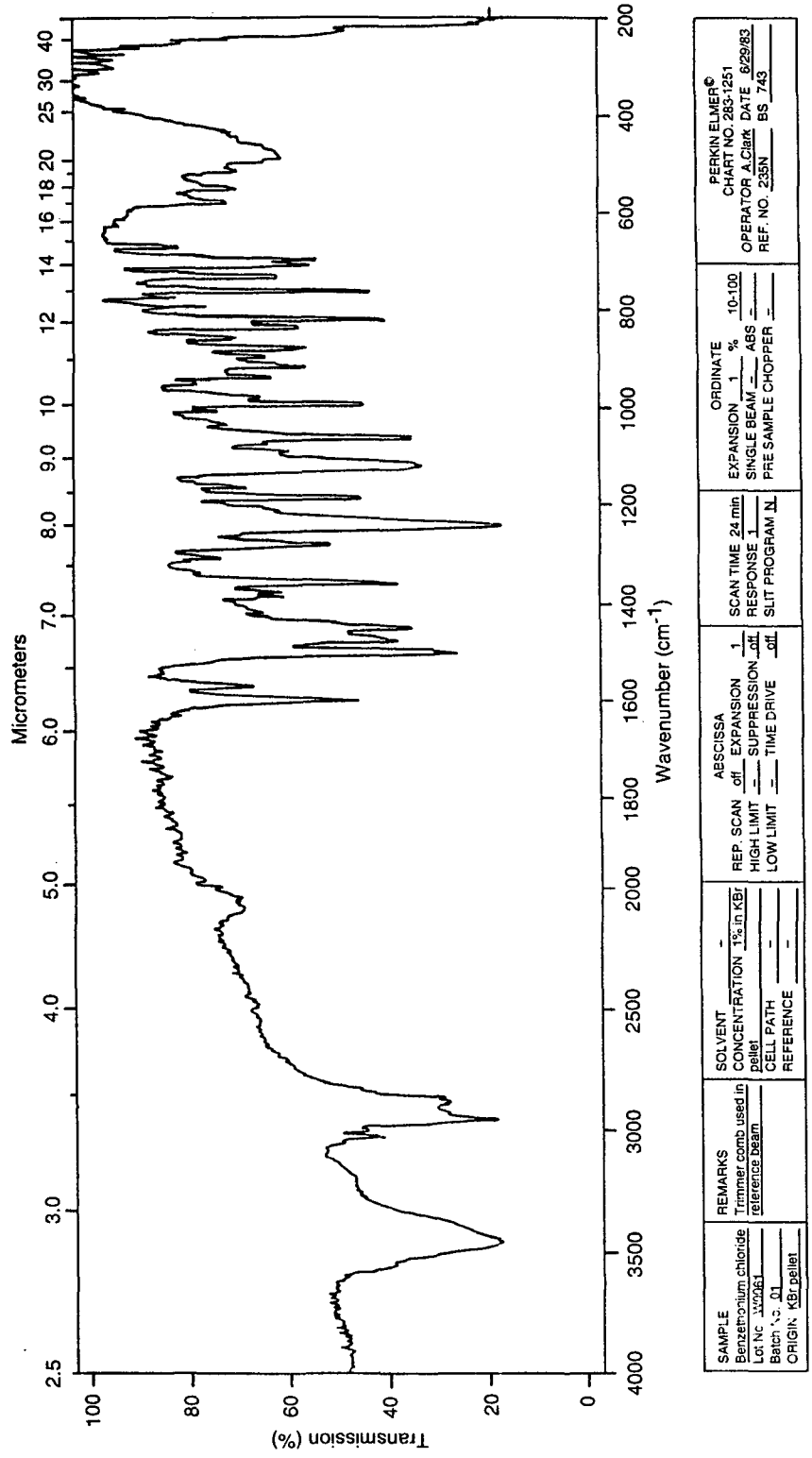

FIGURE G1
Infrared Absorption Spectrum of Benzethonium Chloride

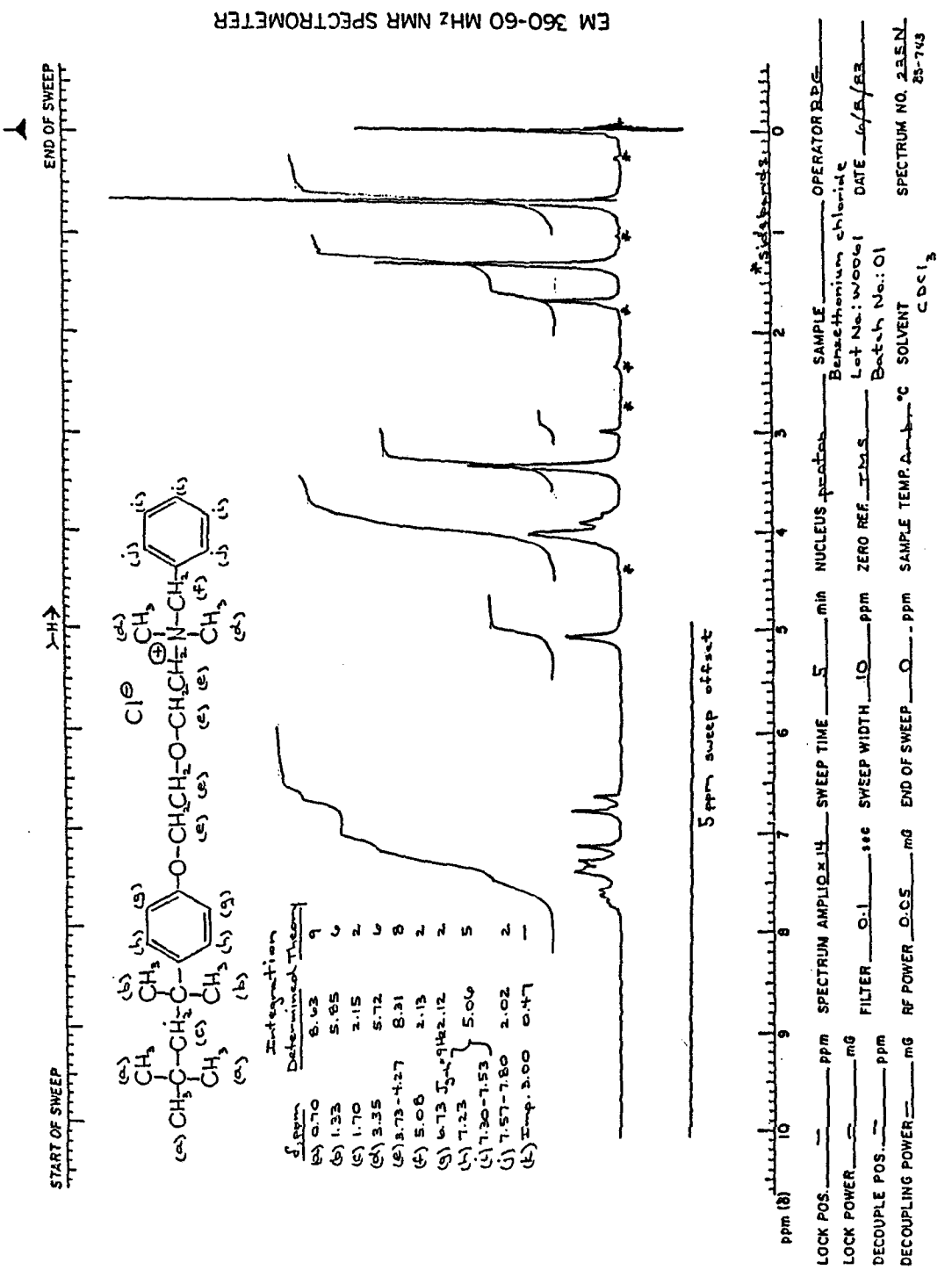

FIGURE G2
Nuclear Magnetic Resonance Spectrum of Benzethonium Chloride

TABLE G1
Preparation and Storage of Dose Formulations in the Dermal Studies of Benzethonium Chloride

16-Day Studies	13-Week Studies	2-Year Studies
Preparation		
Benzethonium chloride was weighed and transferred to a graduated cylinder, and 95% ethanol was added to obtain the desired volume.	Same as 16-day studies	Same as 16-day studies
Chemical Lot Number		
W0061	W0061	W0061
Maximum Storage Time		
3 weeks	3 weeks	3 weeks
Storage Conditions		
Stored at room temperature in sealed vials, protected from light	Same as 16-day studies	Same as 16-day studies
Study Laboratory		
Battelle Columbus Laboratories (Columbus, OH)	Same as 16-day studies	Same as 16-day studies
Referee Laboratory		
Midwest Research Institute (Kansas City, MO)	Same as 16-day studies	Same as 16-day studies

TABLE G2
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 16-Day Dermal Studies of Benzethonium Chloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)[a]	Determined Concentration (mg/mL)[b]	% Difference from Target
Rats				
Male				
12 December 1984	13 December 1984	6.0	5.93	–1
		12.0	11.9	–1
		24.0	23.6	–2
		48.0	47.0	–2
		96.0	94.8	–1
	27 December 1984[c]	6.0	6.44	+7
		12.0	12.7	+6
		24.0	25.0	+4
		48.0	49.6	+3
		96.0	97.7	+2
Female				
12 December 1984	13 December 1984	4.0	4.07	+2
		8.0	8.07	+1
		16.0	15.9	–1
		32.0	30.6	–4
		64.0	63.1	–1
	27 December 1984[c]	4.0	4.37	+9
		8.0	8.77	+10
		16.0	16.9	+6
		32.0	33.7	+5
		64.0	66.4	+4
Mice				
12 December 1984	13 December 1984	1.5	1.55	+3
		3.0	3.05	+2
		6.0	6.06	+1
		12.0	12.1	+1
		24.0	23.6	–2
	27 December 1984[c]	1.5	1.57	+5
		3.0	3.20	+7
		6.0	6.57	+10
		12.0	12.7	+6
		24.0	25.0	+4

[a] Dosing volume = 250 µL (rats) or 100 µL (mice). For male rats, 6.0 mg/mL=6.3 mg/kg; 12.0 mg/mL=12.5 mg/kg; 24.0 mg/mL=25 mg/kg; 48.0 mg/mL=50 mg/kg; 96.0 mg/mL=100 mg/kg. For female rats, 4.0 mg/mL=6.3 mg/kg; 8.0 mg/mL=12.5 mg/kg; 16.0 mg/mL=25 mg/kg; 32.0 mg/mL=50 mg/kg; 64.0 mg/mL=100 mg/kg. For mice, 1.5 mg/mL=6.3 mg/kg; 3.0 mg/mL=12.5 mg/kg; 6.0 mg/mL=25 mg/kg; 12.0 mg/mL=50 mg/kg; 24.0 mg/mL=100 mg/kg.
[b] Results of duplicate analyses
[c] Animal room samples

TABLE G3
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 13-Week Dermal Studies of Benzethonium Chloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)[a]	Determined Concentration (mg/mL)[b]	% Difference from Target
Rats				
25 April 1985	29 April 1985	1.56	1.58	+1
		3.18	3.04	–4
		6.25	6.41	+3
		12.5	12.7	+2
		25.0	26.0	+4
	14 May 1985[c]	1.56	1.60	+2
		3.18	3.13	–2
		6.25	6.39	+2
		12.5	12.9	+3
		25.0	25.6	+2
6 June 1985	7 June 1985	1.56	1.59	+2
		3.13	3.10	–1
		6.25	6.38	+2
		12.5	13.0	+4
		25.0	25.9	+4
	26 June 1985[c]	1.56	1.60	+2
		3.13	3.24	+4
		6.25	6.47	+4
		12.5	13.1	+5
		25.0	26.6	+6
18 July 1985	19 July 1985	1.56	1.55	–1
		3.13	3.03	–3
		6.25	6.12	–2
		12.5	12.2	–2
		25.0	24.5	–2
		50.0	50.4	+1
	5 August 1985[c]	1.56	1.60	+2
		3.18	3.24	+2
		6.25	6.27	0
		12.5	12.6	+1
		25.0	25.7	+3

TABLE G3
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 13-Week Dermal Studies of Benzethonium Chloride (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	% Difference from Target
Mice				
25 April 1985	29 April 1985	0.5	0.52	+4
		1.0	0.96	–4
		2.0	2.02	+1
		4.0	4.00	0
		8.0	8.05	+1
	14 May 1985[c]	0.5	0.52	+4
		1.0	1.00	0
		2.0	1.99	–1
		4.0	4.03	+1
		8.0	8.05	+1
6 June 1985	7 June 1985	4.0	4.33	+8
	10 June 1985	8.0	8.35	+4
	26 June 1985[c]	4.0	4.38[d]	+10
		8.0	8.28	+4
10 June 1985[e]	10 June 1985	0.5	0.49	–3
		1.0	1.03	+3
		2.0	1.94	–3
	26 June 1985	0.5	0.50	0
		1.0	1.04	+4
		2.0	2.06	+3
18 July 1985	19 July 1985	0.5	0.48	–4
		1.0	0.97	–3
		2.0	2.00	0
		4.0	3.93	–2
		8.0	7.93	–1
		16.0	15.9	–1
	5 August 1985[c]	0.5	0.49	–3
		1.0	1.01	+1
		2.0	2.00	0
		4.0	4.22	+6
		8.0	8.16	+2
		16.0	16.1	+1

[a] The dosing volume was adjusted weekly following mean body weight measurements, but did not exceed 300 µL for rats or 100 µL for mice. For rats and mice, the doses were 1.56 mg/kg, 3.13 mg/kg, 6.25 mg/kg, 12.5 mg/kg, or 25 mg/kg.
[b] Results of duplicate analyses
[c] Animal room samples
[d] Result is average of analysis of three samples
[e] Because of an interfering static charge on the preparation balance, samples originally mixed on 6 June 1985 were remixed and reanalyzed on 10 June 1985.

TABLE G4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies of Benzethonium Chloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)[a]	Determined Concentration (mg/mL)[b]	% Difference from Target
Rats				
9 June 1987	11 June 1987	0.15	0.149[c]	–1
		0.25	0.249[c]	0
		0.5	0.497	–1
		0.83	0.829	0
		1.5	1.52	+1
		2.5	2.51	0
	23 June 1987[d]	0.15	0.170[c]	+13
		0.25	0.256[c]	+2
		0.5	0.510	+2
		0.83	0.842	+1
		1.5	1.53	+2
		2.5	2.58	+3
	24 June 1987[d]	0.15	0.154[c]	+3
5 August 1987	6 August 1987	0.15	0.149[c]	–1
		0.25	0.248[c]	–1
		0.5	0.507	+1
		0.83	0.834	0
		1.5	1.55	+3
		2.5	2.54	+2
30 September 1987	1 October 1987	0.5	0.489	–2
		0.83	0.819	–1
		1.5	1.52	+1
		2.5	2.50	0
	7 October 1987	0.15	0.141	–6
		0.25	0.238	–5
9 December 1987	10 December 1987	0.15	0.145	–3
		0.25	0.246	–2
		0.5	0.497	–1
		0.83	0.817	–2
		1.5	1.49	–1
		2.5	2.49	0
	4 January 1988[d]	0.15	0.166	+11
		0.25	0.277	+11
		0.5	0.537	+7
		0.83	0.870	+5
		1.5	1.59	+6
		2.5	2.66	+6

TABLE G4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies of Benzethonium Chloride (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	% Difference from Target
Rats (continued)				
20 January 1988	21 January 1988	0.15	0.146	–3
		0.25	0.246	–2
		0.5	0.502	0
		0.83	0.827	0
		1.5	1.52	+1
		2.5	2.51	0
16 March 1988	17 March 1988	0.15	0.146	–3
		0.25	0.250	0
		0.5	0.489	–2
		0.83	0.837	+1
		1.5	1.53	+2
		2.5	2.53	+1
11 May 1988	12 May 1988	0.15	0.150	0
		0.25	0.249	0
		0.5	0.499	0
		0.83	0.837	+1
		1.5	1.54	+3
		2.5	2.60	+4
	26 May 1988[d]	0.15	0.155	+3
		0.25	0.255	+2
		0.5	0.502	0
		0.83	0.857	+3
		1.5	1.52	+1
		2.5	2.54	+2
5 July 1988	6 July 1988	0.15	0.146	–3
		0.25	0.243	–3
		0.5	0.487	–3
		0.83	0.807	–3
		1.5	1.47	–2
		2.5	2.44	–2
30 August 1988	31 August 1988	0.15	0.148	–1
		0.25	0.246	–2
		0.5	0.490	–2
		0.83	0.815	–2
		1.5	1.49	–1
		2.5	2.47	–1

TABLE G4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies of Benzethonium Chloride (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	% Difference from Target
Rats (continued)				
26 October 1988	28 October 1988	0.15	0.149	–1
		0.25	0.251	0
		0.5	0.500	0
		0.83	0.825	–1
		1.5	1.54	+3
		2.5	2.52	+1
	14 November 1988[d]	0.15	0.161	+7
		0.25	0.261	+4
		0.5	0.509	+2
		0.83	0.850	+2
		1.5	1.55	+3
		2.5	2.54	+2
27 December 1988	29 December 1988	0.15	0.148	–1
		0.25	0.246	–2
		0.5	0.504	+1
		0.83	0.823	–1
		1.5	1.50	0
		2.5	2.48	–1
22 February 1989	23 February 1989	0.15	0.157	+5
		0.25	0.267	+7
		0.5	0.525	+5
		0.83	0.877	+6
		1.5	1.52	+1
		2.5	2.71	+8
19 April 1989	20 April 1989	0.15	0.149	–1
		0.25	0.246	–2
		0.5	0.493	–1
		0.83	0.820	–1
		1.5	1.49	–1
		2.5	2.47	–1
	2 May 1989[d]	0.15	0.157	+5
		0.25	0.249	0
		0.5	0.505	+1
		0.83	0.837	+1
		1.5	1.51	+1
		2.5	2.49	0

TABLE G4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies of Benzethonium Chloride (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	% Difference from Target
Mice				
15 June 1987	15 June 1987	0.06	0.060[c]	0
		0.2	0.196[c]	–2
		0.6	0.595	–1
	29 June 1987[d]	0.06	0.066	+10
		0.2	0.204	+2
		0.6	0.613	+2
5 August 1987	6 August 1987	0.06	0.057[c]	–4
		0.2	0.198[c]	–1
		0.6	0.604	+1
30 September 1987	1 October 1987	0.06	0.056	–6
		0.2	0.195	–2
		0.6	0.603	+1
9 December 1987	10 December 1987	0.06	0.055	–8
		0.2	0.198	–1
		0.6	0.589	–2
	4 January 1988[d]	0.06	0.056	–7
		0.2	0.211	+6
		0.6	0.627	+5
20 January 1988	21 January 1988	0.06	0.058	–3
		0.2	0.198	–1
		0.6	0.594	–1
16 March 1988	17 March 1988	0.06	0.058	–3
		0.2	0.193	–3
		0.6	0.582	+3
11 May 1988	12 May 1988	0.06	0.059	–2
		0.2	0.201	+1
		0.6	0.599	0
	26 May 1988[d]	0.06	0.059	–2
		0.2	0.204	+2
		0.6	0.616	+3
5 July 1988	6 July 1988	0.06	0.058	–3
		0.2	0.194	–3
		0.6	0.585	–2

TABLE G4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies of Benzethonium Chloride (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	% Difference from Target
Mice (continued)				
30 August 1988	31 August 1988	0.06	0.061	+2
		0.2	0.197	–1
		0.6	0.585	–2
26 October 1988	28 October 1988	0.06	0.063	+5
		0.2	0.201	+1
		0.6	0.597	0
	14 November 1988[d]	0.06	0.063	+5
		0.2	0.203	+2
		0.6	0.605	+1
27 December 1988	29 December 1988	0.06	0.059	–2
		0.2	0.196	–2
		0.6	0.590	–2
22 February 1989	23 February 1989	0.06	0.062	+3
		0.2	0.210	+5
		0.6	0.623	+4
19 April 1989	20 April 1989	0.06	0.058	–3
		0.2	0.198	–1
		0.6	0.585	–2
	2 May 1989[d]	0.06	0.073	+22
		0.2	0.206	+3
		0.6	0.619	+3

[a] The dosing volume was based on mean body weight measurements and ranged from 63 to 296 μL for male rats, from 95 to 317 μL for female rats, and from 50 to 131 μL for male and female mice. For male rats, 0.25 mg/mL=0.15 mg/kg, 0.83 mg/mL=0.5 mg/kg, 2.5 mg/mL=1.5 mg/kg. For female rats, 0.15 mg/mL=0.15 mg/kg, 0.5 mg/mL=0.5 mg/kg, 1.5 mg/mL=1.5 mg/kg. For mice, 0.06 mg/mL=0.15 mg/kg, 0.2 mg/mL=0.5 mg/kg, 0.6 mg/mL=1.5 mg/kg.
[b] Results of duplicate analyses
[c] Results of single analysis
[d] Animal room sample

TABLE G5
Results of Referee Analysis of Dose Formulations Administered to Rats and Mice in the 13-Week and 2-Year Dermal Studies of Benzethonium Chloride

Date Mixed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	
		Study Laboratory[a]	Referee Laboratory[b]
13-Week Studies			
Mice			
25 April 1985	1.0	0.96	1.04 ± 0.01
18 July 1985	8.0	7.93	7.99 ± 0.01
2-Year Studies			
Rats			
9 June 1987	0.25	0.249	1.12 ± 0.0[c]
	0.83	0.829	0.830 ± 0.002
11 May 1988	2.5	2.60	2.51 ± 0.01
19 April 1989	0.83	0.820	0.922 ± 0.003
Mice			
9 December 1987	0.2	0.198	0.193 ± 0.008
26 October 1988	0.06	0.063	0.0583 ± 0.0002

[a] Results of duplicate analyses
[b] Results of triplicate analyses
[c] No explanation for this discrepancy was identified.

APPENDIX H
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-07 RAT AND MOUSE RATION

TABLE H1
Ingredients of NIH-07 Rat and Mouse Ration[a]

Ingredients[b]	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

[a] NCI, 1976; NIH, 1978
[b] Ingredients were ground to pass through a U.S. Standard Screen No. 16 before being mixed.

TABLE H2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 μg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product

TABLE H3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.76 ± 0.79	21.70 – 24.20	25
Crude fat (% by weight)	5.39 ± 0.37	4.60 – 5.90	25
Crude fiber (% by weight)	3.52 ± 0.31	2.80 – 4.20	25
Ash (% by weight)	6.81 ± 0.25	6.26 – 7.30	25
Amino Acids (% of total diet)			
Arginine	1.287 ± 0.084	1.100 – 1.390	10
Cystine	0.306 ± 0.075	0.181 – 0.400	10
Glycine	1.160 ± 0.050	1.060 – 1.220	10
Histidine	0.580 ± 0.024	0.531 – 0.608	10
Isoleucine	0.917 ± 0.034	0.867 – 0.965	10
Leucine	1.972 ± 0.052	1.850 – 2.040	10
Lysine	1.273 ± 0.051	1.200 – 1.370	10
Methionine	0.437 ± 0.115	0.306 – 0.699	10
Phenylalanine	0.994 ± 0.125	0.665 – 1.110	10
Threonine	0.896 ± 0.055	0.824 – 0.985	10
Tryptophan	0.223 ± 0.160	0.107 – 0.671	10
Tyrosine	0.677 ± 0.105	0.564 – 0.794	10
Valine	1.089 ± 0.057	0.962 – 1.170	10
Essential Fatty Acids (% of total diet)			
Linoleic	2.389 ± 0.233	1.830 – 2.570	9
Linolenic	0.277 ± 0.036	0.210 – 0.320	9
Vitamins			
Vitamin A (IU/kg)	6,750 ± 1,439	4,430 – 10,860	25
Vitamin D (IU/kg)	4,450 ± 1,382	3,000 – 6,300	4
α-Tocopherol (ppm)	36.92 ± 9.32	22.5 – 48.9	9
Thiamine (ppm)	18.64 ± 2.12	14.0 – 23.0	25
Riboflavin (ppm)	7.92 ± 0.93	6.10 – 9.00	10
Niacin (ppm)	100.95 ± 25.92	65.0 – 150.0	9
Pantothenic acid (ppm)	30.30 ± 3.60	23.0 – 34.6	10
Pyridoxine (ppm)	9.25 ± 2.62	5.60 – 14.0	10
Folic acid (ppm)	2.51 ± 0.64	1.80 – 3.70	10
Biotin (ppm)	0.267 ± 0.049	0.19 – 0.35	10
Vitamin B_{12} (ppb)	40.14 ± 20.04	10.6 – 65.0	10
Choline (ppm)	3,068 ± 314	2,400 – 3,430	9
Minerals			
Calcium (%)	1.29 ± 0.12	1.00 – 1.54	25
Phosphorus (%)	0.95 ± 0.04	0.86 – 1.00	25
Potassium (%)	0.887 ± 0.067	0.772 – 0.971	8
Chloride (%)	0.526 ± 0.092	0.380 – 0.635	8
Sodium (%)	0.315 ± 0.344	0.258 – 0.370	10
Magnesium (%)	0.168 ± 0.008	0.151 – 0.180	10
Sulfur (%)	0.274 ± 0.063	0.208 – 0.420	10
Iron (ppm)	356.2 ± 90.0	255.0 – 523.0	10
Manganese (ppm)	92.24 ± 5.35	81.70 – 99.40	10
Zinc (ppm)	58.14 ± 9.91	46.10 – 81.60	10
Copper (ppm)	11.50 ± 2.40	8.090 – 15.39	10
Iodine (ppm)	3.70 ± 1.14	1.52 – 5.83	10
Chromium (ppm)	1.71 ± 0.45	0.85 – 2.09	9
Cobalt (ppm)	0.797 ± 0.23	0.490 – 1.150	6

TABLE H4
Contaminant Levels in NIH-07 Rat and Mouse Ration

	Mean ± Standard Deviation[a]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.18 ± 0.12	0.05 – 0.55	25
Cadmium (ppm)	0.10 ± 0.02	<0.10 – 0.20	25
Lead (ppm)	0.32 ± 0.26	0.05 – 1.00	25
Mercury (ppm)	0.05 ± 0.01	0.05 – 0.11	25
Selenium (ppm)[b]	0.39 ± 0.20	0.16 – 1.21	25
Aflatoxins (ppb)	<5.0		25
Nitrate nitrogen (ppm)[c]	20.04 ± 7.55	9.90 – 39.0	25
Nitrite nitrogen (ppm)[c]	0.19 ± 0.14	<0.10 – 0.60	25
BHA (ppm)[d]	1.88 ± 0.51	<0.10 – 3.00	25
BHT (ppm)[d]	1.12 ± 0.52	<0.10 – 3.00	25
Aerobic plate count (CFU/g)[e,f]	132,320 ± 192,307	13,000 – 940,000	25
Coliform (MPN/g)[g]	55.60 ± 219.57	3.00 – 11.0	25
E. coli (MPN/g)	3.04 ± 0.20	3.00 – 4.00	25
Salmonella (MPN/g)	Negative		25
Total nitrosoamines (ppb)[h]	10.65 ± 4.99	3.60 – 20.00	25
N-Nitrosodimethylamine (ppb)	8.30 ± 4.62	2.60 – 19.00	25
N-Nitrosopyrrolidine (ppb)	2.36 ± 1.43	0.90 – 5.40	25
Pesticides (ppm)			
α-BHC[i]	<0.01		25
ß-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.1		25
Estimated PCBs	<0.2		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.1		25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.19 ± 0.17	<0.05 – 0.60	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

TABLE H4
Contaminant Levels in NIH-07 Rat and Mouse Ration (continued)

[a] For values less than the limit of detection, the detection limit is given as the mean.
[b] One lot milled on 2 March 1989 contained more than 0.65 ppm. All other lots measured less than or equal to the detection limit.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] CFU = colony forming unit
[f] One lot milled on 5 November 1987 contained more than 600,000 CFU/g.
[g] MPN = most probable number
[h] All values were corrected for percent recovery.
[i] BHC is hexachlorocyclohexane or benzene hexachloride

APPENDIX I
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Rats

At the end of the 13-week study, samples for viral screening were collected from five male and five female vehicle control rats. These samples were processed appropriately and submitted to Microbiological Associates, Inc. (Bethesda, MD), for viral titer screening. The following tests were performed on the sera:

Method of Analysis	Time of Analysis
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	Study termination
Sendai	Study termination
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	Study termination
KRV (Kilham rat virus)	Study termination

Prior to the beginning of the 2-year study, blood was collected once (during one quarantine screening) from five male and five female rats. Serum samples were also collected from as many as five male and five female rats at 6, 12, and 18 months into the study and from as many as five male and five female high-dose rats at the end of the study (24 months). Blood from each collection was processed appropriately, shipped to Microbiological Associates, Inc., and screened for the following:

Method of Analysis	Time of Analysis
ELISA	
M. arthritidis	24 months
M. pulmonis	24 months
PVM	Quarantine, 6, 12, 18, and 24 months
RCV/SDA	Quarantine, 6, 12, 18, and 24 months
Sendai	Quarantine, 6, 12, 18, and 24 months
Hemagglutination Inhibition	
H-1	Quarantine, 6, 12, 18, and 24 months
KRV	Quarantine, 6, 12, 18, and 24 months

Mice

At the end of the 13-week study, samples for viral screening were collected from five male and five female vehicle control mice. These samples were processed appropriately and submitted to Microbiological Associates, Inc., for viral titer screening. The following tests were performed on the sera:

Method of Analysis	Time of Analysis
Complement Fixation	
LCM (lymphocytic choriomeningitis virus)	Study termination
ELISA	
Ectromelia virus	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
Mouse adenoma virus	Study termination
MHV (mouse hepatitis virus)	Study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	Study termination
Reovirus 3	Study termination
Sendai	Study termination
Hemagglutination Inhibition	
K (papovavirus)	Study termination
MVM (minute virus of mice)	Study termination
Polyoma virus	Study termination
Immunofluorescence Assay	
EDIM (epizootic diarrhea of infant mice)	Study termination

Prior to the beginning of the 2-year study, blood was collected once (during one quarantine screening) from five male and five female mice. Serum samples were also collected from as many as five males and five females at 6, 12, and 18 months into the study and from five male and five female high-dose mice at the end of the study (24 months). In addition, an unscheduled screening was conducted on five male and five female vehicle control mice at about 22 months into the study. Blood from each collection was processed appropriately, shipped to Microbiological Associates, Inc., and screened for the following:

Method of Analysis	Time of Analysis
ELISA	
Ectromelia virus	Quarantine, 6, 12, 18, 22, and 24 months
GDVII	Quarantine, 6, 12, 18, 22, and 24 months
LCM	Quarantine, 6, and 12 months
MVM	Quarantine, 6, 12, 18, 22, and 24 months
Mouse adenoma virus	Quarantine, 6, 12, 18, 22, and 24 months
MHV	Quarantine, 6, 12, 18, 22, and 24 months
M. arthritidis	24 months
M. pulmonis	24 months
PVM	Quarantine, 6, 12, 18, 22, and 24 months
Reovirus 3	Quarantine, 6, 18, 22, and 24 months
Sendai	Quarantine, 6, 12, 18, 22, and 24 months
Hemagglutination Inhibition	
K	Quarantine, 6, 12, 18, 22, and 24 months
Polyoma virus	Quarantine, 6, 12, 18, 22, and 24 months

Mice (continued)

Method of Analysis	Time of Analysis
Immunofluorescence Assay	
EDIM	Quarantine, 6, 12, 18, 22, and 24 months
LCM	18, 22, and 24 months
Reovirus 3	12 months

Serology results are presented in Table I1.

TABLE I1
Murine Virus Antibody Determinations for Rats and Mice in the 13-Week and 2-Year Dermal Studies of Benzethonium Chloride

Interval	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
13-Week Studies		
Rats		
Study termination	1/10	*M. arthritidis*[a]
Mice		
Study termination	0/10	None positive
2-Year Studies		
Rats		
Quarantine screening	0/10	None positive
6 months	0/9	None positive
12 months	0/10	None positive
18 months	0/8	None positive
24 months	2/10	*M. arthritidis*[a]
Mice		
Quarantine screening	0/10	None positive
6 months	0/10	None positive
12 months	0/10	None positive
18 months	0/8	None positive
22 months	7/10	Mouse hepatitis virus
24 months	8/10	Mouse hepatitis virus

[a] Further evaluation of samples positive for *M. arthritidis* by immunoblot and Western blot procedures indicated that the positive titers may be due to cross reaction with antibodies of nonpathogenic *Mycoplasma* or other agents. Only sporadic samples were positive, and there were no clinical signs or histopathologic changes of *M. arthritidis* infection in rats with positive titers. Accordingly, *M. arthritidis*-positive titers were considered to be false positives.

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