STATISTICAL METHODS

Calculation and Analysis of Lesion Incidences

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

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and vehicle control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to data to test for simultaneous equality of measurements across doses. The Fisher exact test was used to determine the significance of the functional observation battery data (Gart *et al.*, 1979).

QUALITY ASSURANCE METHODS

The 13-week studies of benzyltrimethylammonium chloride were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The quality assurance unit of Microbiological Associates, Inc., performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al.* (1988). Benzyltrimethylammonium chloride was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA1535, either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of benzyltrimethylammonium chloride. In the absence of toxicity, 10,000 μg/plate was selected as the high dose.

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

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 13-week toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 1,000 normochromatic erythrocytes (NCEs) in up to 10 animals per dose group.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dose group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the

micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dose group is less than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 13-week studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Evaluation Protocol

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

RESULTS

RATS

16-DAY STUDY

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

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

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

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

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

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

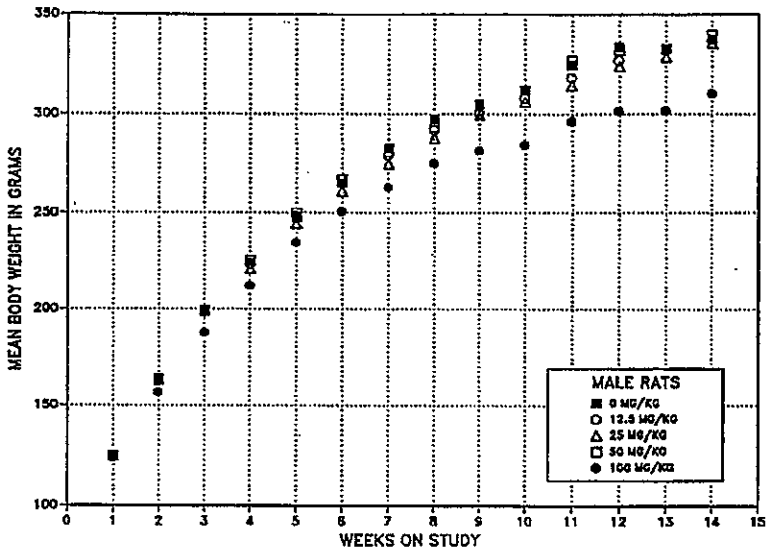
13-WEEK STUDY

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

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

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

** Significantly different ($P \leq 0.01$) from the vehicle control group by Dunnett's test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Week of death: 12 (gavage accident)
[d] Week of death: 10, 12



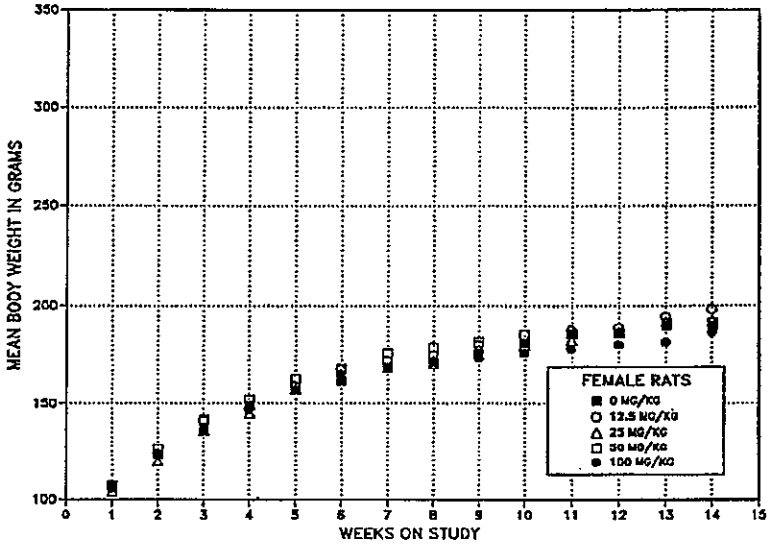


FIGURE 1
Body Weights of Rats Administered Benzyltrimethylammonium Chloride by Gavage for 13 Weeks

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

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

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

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

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

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

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

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

MICE

16-DAY STUDY

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

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

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

** Significantly different ($P \leq 0.01$) from the vehicle control group by Dunnett's test
[a] Number of animals surviving at 16 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. No data were calculated for groups with 100% mortality.
[c] Day of death: 1

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

13-WEEK STUDY

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

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

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

[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. Differences from the vehicle control groups were not significant by Dunnett's test.
[c] Week of death: 9
[d] Week of death: 6

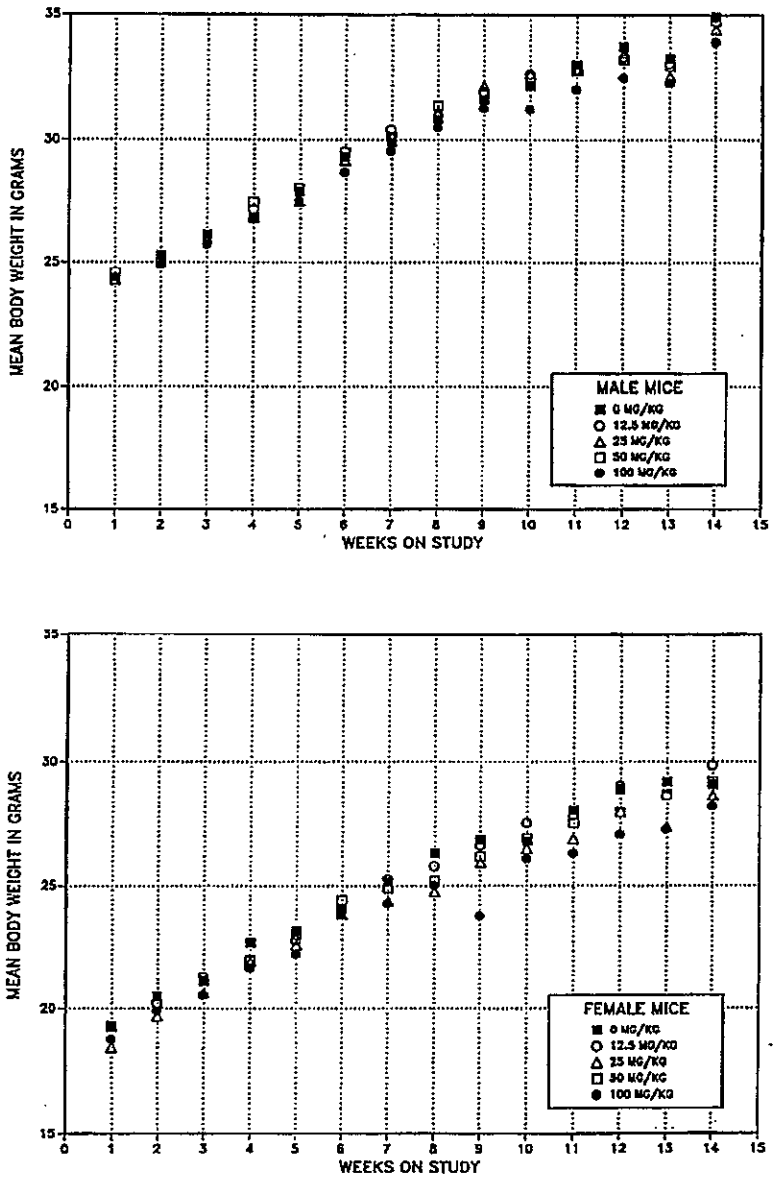


FIGURE 2
Body Weights of Mice Administered Benzyltrimethylammonium Chloride by Gavage for 13 Weeks

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

GENETIC TOXICOLOGY

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

DISCUSSION

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

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

The NTP also conducted 14-day dermal studies (unpublished) of benzyltrimethylammonium chloride. Male and female F344/N rats were administered 0, 11.9, 39.6, or 118.8 mg per day (equivalent to 0, 170, 340, or 680 mg/kg per day for males and 0, 260, 520, or 860 mg/kg per day for females), and $B6C3F_1$ mice were administered 0, 3.96, 11.9, or 39.6 mg per day (equivalent to 0, 385, 790, or 1,580 mg/kg per day for males and 0, 450, 900, or 1,800 mg/kg per day for females). Results of these dermal studies were similar to those of the 16-day gavage studies except that the animals were more sensitive to toxic effects following gavage administration. Deaths, ataxia, and tremors occurred in rats administered 118.8 mg and mice administered 39.6 mg; these doses were 1.5 to 3.5 orders of magnitude greater than the doses used in the gavage studies.

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

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

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

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

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

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

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