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

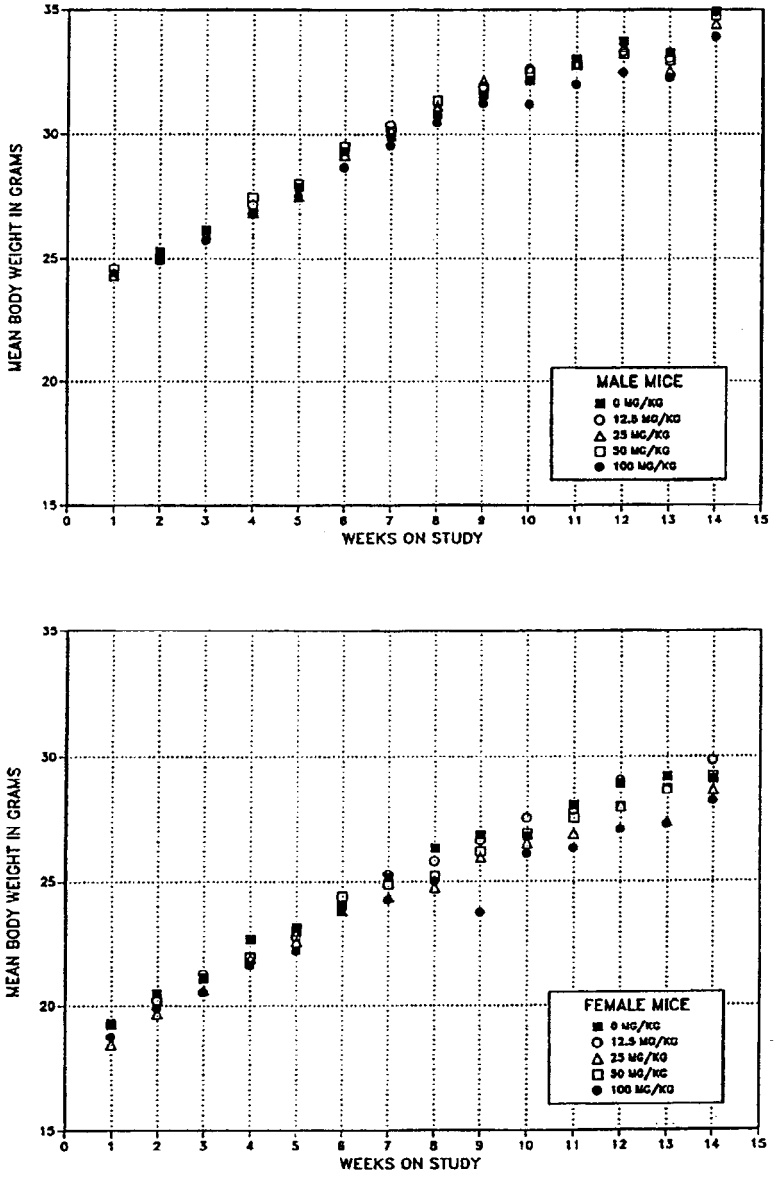

FIGURE 2
Body Weights of Mice Administered Benzyltrimethylammonium Chloride by Gavage for 13 Weeks

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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APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS IN RATS AND MICE

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

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

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

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

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

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

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

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

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Integumentary System					
None					
Musculoskeletal System					
None					
Nervous System					
None					
Respiratory System					
Lung	(10)		(1)	(10)	(10)
Congestion					1 (10%)
Inflammation, focal	3 (30%)		1 (100%)	3 (30%)	6 (60%)
Nose	(10)				(10)
Inflammation, chronic active	1 (10%)				
Special Senses System					
None					
Urinary System					
None					

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

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

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

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

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

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

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

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

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

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

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