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				
Uterus	(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				
2-Year Study				
Alimentary System				
Intestine small, jejunum	(1)			
Liver	(51)			(53)
Pancreas	(51)			(53)
Salivary glands	(51)			(53)

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)				
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)
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%)

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 (continued)				
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	.5 mg/kg
2-Year Study (continued)				
Cardiovascular System				
None				
Endocrine System				
Adrenal cortex	(19)			(2)
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)
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%)

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	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
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	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	+																										2

TABLE B2
Individual Animal Tumor Pathology of Female Rats in th -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	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 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
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	
	6	3	6	9	0	8	1	2	2	2	2	3	8	9	7	0	4	6	0	7	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	2	6	0	2	0	4	0	3	4	3	2	1	4	4	5	0	0	5	0	0	0	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	
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 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	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 [illegible] [illegible]	3 [illegible] [illegible]	3 9 2	3 9 3	3 9 4	3 9 5	3 9 7	3 9 8	4 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 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
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		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 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 Tissue mors
Alimentary System																													
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Mesentery				+														+					+						3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, forestomach				+																									8
Tongue																													1
Cardiovascular System																													
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
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 1	[illegible]	[illegible]	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	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 B2
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 Neoplasm				
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, bone marrow, brain, clitoral gland, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, spleen, thyroid gland, and urinary bladder; 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%)

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%)

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, forestomach	(6)			(8)
Acanthosis				3 (38%)
Inflammation, chronic active				1 (13%)
Mineralization				1 (13%)
Ulcer	6 (100%)			5 (63%)
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%)

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)				
Genital System (continued)				
Uterus	(51)			(53)
Endometrium, hyperplasia, cystic, glandular	3 (6%)			2 (4%)
Vagina	(1)			
Lumen, hemorrhage	1 (100%)			
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%)

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)				
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%)
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%)

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

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				

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				
Alimentary System				
Intestine large, cecum	(50)			(50)
Leiomyosarcoma				1 (2%)
Intestine small, duodenum	(50)			(50)
Adenoma				1 (2%)
Intestine small, jejunum	(50)			(50)
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%)			

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
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																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland		+			+	+			+					+					+			+			
Prostate	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma															X										

+: 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																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland					+		+		+		+		+		+		+	+		+		+		+		18
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																										1

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
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																							+		
Fibrosarcoma																							X		
Eye					+																				
Harderian gland	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	M	M	M	+	M	+
Adenoma					X																				
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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 1 1	0 4	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
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
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

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
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
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
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	[illegible]	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	7	6	0	6	1	1	7		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	0 0 1	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	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	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	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	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 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	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 8 8	2 0 0	2 0 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	729	729	729	729	729	729	729	729	
	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	
Carcass ID Number	19	20	23	25	26	29	31	33	34	36	38	39	40	41	42	44	45	47	49	50	53	54	57	58	60	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	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	+	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	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
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	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
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																	
rinary 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 lysis 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 : Adenoma or Carcinoma				
Overall rate	3/50 (6%)	–[e]	–	2/50 (4%)
Adjusted ra	6.7%			4.9%
Terminal ra	2/43 (5%)			1/39 (3%)
First incidc s)	655			712
Life table te				P=0.531N
Logistic reg test[d]				P=0.495N
Fisher exact				P=0.500N
Liver: Her sarcoma				
Overall rate	3/50 (6%)	–	–	2/50 (4%)
Adjusted ra	6.4%			4.9%
Terminal rate	0/43 (0%)			1/39 (3%)
First incidenc s)	577			719
Life table te				P=0.531N
Logistic regre est				P=0.307N
Fisher exact t				P=0.500N
Liver: Hepa cellular 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 regres est				P=0.505
Fisher exact t				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 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: Hepatoblastoma or 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: Hepatoblastoma, Hepatocellular Adenoma, or 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
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: Malignant Lymphoma or Histiocytic Sarcoma				
Overall rate	3/50 (6%)	–	–	4/50 (8%)
Adjusted rate	7.0%			8.8%
Terminal rate	3/43 (7%)			1/39 (3%)
First incidence (days)	729 (T)			612
Life table test				P=0.468
Logistic regression test				P=0.520
Fisher exact test				P=0.500
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

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: 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
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 adrenal gland, bone marrow, brain, epididymis, gallbladder, heart, kidney, larynx, liver, lung, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; 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%)			
Integumentary System				
Skin, control	(10)	(9)	(9)	(10)
Skin, site of application-no mass	(10)	(9)	(9)	(10)
Epithelial hyperplasia			2 (22%)	10 (100%)

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)				
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%)
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%)

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)				
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%)
Prostate	(49)			(50)
Inflammation, suppurative	1 (2%)			
Artery, inflammation, chronic active				1 (2%)
Seminal vesicle	(50)			(50)
Inflammation	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)				
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%)			
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%)			

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)				
Urinary System				
Kidney	(50)			(50)
Cyst				1 (2%)
Hydronephrosis	2 (4%)			1 (2%)
Nephropathy	48 (96%)			49 (98%)

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

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 sarcoma	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				

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%)
Hepatocellular carcinoma, multiple				1 (2%)
Histiocytic sarcoma	3 (6%)			
Plasma cell tumor, malignant, metastatic, spleen	1 (2%)			
Mesentery	(10)			(9)
Hemangioma	1 (10%)			
Hepatocellular carcinoma, 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				

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				
Ovary	(52)			(52)
Cystadenoma	3 (6%)			2 (4%)
Hemangioma				1 (2%)
Histiocytic sarcoma	2 (4%)			
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	62	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	30			33
Total animals with malignant neoplasms				
2-Year study	27	1	1	27
Total malignant neoplasms				
2-Year study	32	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 2
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	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			4
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 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
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	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
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	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: .15 mg/kg

Number of Days on Study	2 6 8	3 0 8	3 7 2	4 5 9	4 7 1	5 1 2	5 1 8	5 4 3	5 6 5	5 8 3	5 9 6	5 9 7	5 1 3	6 2 6	6 3 5	6 5 6	6 9 9	7 0 1	7 1 5	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 5 2	3 3 8	3 2 0	3 6 0	3 5 6	3 2 3	3 3 0	3 4 1	3 1 4	3 2 9	3 0 9	3 3 6	3 5 5	3 5 8	3 1 0	3 0 7	3 2 1	3 3 4	3 2 7	3 0 1	3 0 2	3 0 3	3 0 4	3 0 5	3 0 6	3 0 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: .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 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	
Carcass ID Number	3 1 1	3 1 2	3 1 3	3 1 6	3 1 7	3 1 9	3 2 2	3 2 4	3 2 5	3 2 8	3 3 1	3 3 2	3 3 3	3 3 9	3 4 0	4 4 2	4 4 4	4 4 5	4 4 6	4 4 7	4 4 8	4 4 9	5 5 0	5 5 3	5 5 4	5 5 7	5 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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	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 2 6	5 6 8	5 9 1	5 9 1	6 0 9	6 2 1	6 4 8	6 6 4	6 7 4	6 7 7	6 9 0	6 9 7	6 9 8	7 0 4	7 1 2	7 1 7	7 1 8	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 7 6	3 6 3	3 8 1	4 0 4	3 8 3	4 1 8	4 0 8	3 8 9	4 2 0	3 9 2	3 9 9	3 6 2	3 7 1	4 0 1	4 1 9	4 0 3	3 9 7	3 6 1	3 6 4	3 6 5	3 6 6	3 6 8	3 7 2	3 7 5	3 7 7
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	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	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	
Carcass ID Number	3	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4		Total
	7	7	8	8	8	8	8	9	9	9	9	0	0	0	0	1	1	1	1	1	1	1		Tissues
	8	9	0	2	4	5	8	3	4	5	6	0	5	6	7	0	1	2	3	4	5	6	7	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 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
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
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							
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	43340	43346	43347	43349	43440	43442	43443	43444	43446	43447	43451	43452	43453	43454	43455	43456	43457	43459	43465	43466	43467	43471	43473	43474	43475	43478	43480	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 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
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)

	1	2	3	4	4	4	5	5	5	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7
Number of Days on Study	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
	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	4
Carcass ID Number	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
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	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	4	4 8 8	4 8 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: Malignant Lymphoma or Histiocytic Sarcoma				
Overall rate	10/52 (19%)	-	-	6/54 (11%)
Adjusted rate	23.0%			14.5%
Terminal rate	6/38 (16%)			3/34 (9%)
First incidence (days)	568			249
Life table test				P=0.280N
Logistic regression test				P=0.155N
Fisher exact test				P=0.185N
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

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

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, gallbladder, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, spleen, thyroid gland, and urinary bladder; 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%)
Respiratory System				
Lung	(8)			(6)
Alveolar epithelium, hyperplasia	1 (13%)			1 (17%)

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)				
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, chronic				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%)
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%)

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)				
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				
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%)

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)				
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%)		
Skin, site of application-no mass	(52)	(52)	(48)	(53)
Epithelial hyperplasia	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%)

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)				
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%)

[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
2-Year Study (continued)				
Alimentary System (continued)				
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				
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%)

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, 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 positive trials were repeated under the conditions that elicited the positive response. If no positive responses were seen, 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 Chinese hamster ovary cells, benzethonium chloride did not induce SCEs (Table E2) or Abs (Table E3), with or without S9. Although an increase in chromosomal aberrations 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 Abs test or the SCE 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	0.00	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				
	0.10	19 ± 2.1	7 ± 0.3				
	0.30	18 ± 0.9	10 ± 1.8				
	1.00	22 ± 1.5	10 ± 4.2	15 ± 1.9	28 ± 4.1	21 ± 2.0	17 ± 1.9
	3.30			16 ± 2.9	20 ± 0.7	24 ± 3.2	20 ± 2.4
	10.00			16 ± 1.7	20 ± 1.3	24 ± 1.5	16 ± 1.8
	33.00			10 ± 2.1	19 ± 2.9	16 ± 0.7	24 ± 1.2
	100.00			7 ± 1.2	21 ± 0.9	18 ± 0.7	18 ± 3.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		180 ± 63.8	123 ± 9.0	1,152 ± 40.1	719 ± 45.8	872 ± 70.4	1,150 ± 21.3
TA100	0.00	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				
	0.10	82 ± 5.2	103 ± 5.3				
	0.30	94 ± 4.7	99 ± 4.3				
	1.00	92 ± 5.0	66 ± 1.7	105 ± 1.5	130 ± 5.9	109 ± 7.4	122 ± 8.0
	3.30			100 ± 3.2	133 ± 16.3	121 ± 6.1	132 ± 19.1
	10.00			94 ± 2.3	140 ± 14.2	118 ± 13.0	144 ± 6.9
	33.00			84 ± 4.1	144 ± 8.8	123 ± 8.7	141 ± 1.3
	100.00			34 ± 3.8	143 ± 15.2	128 ± 6.0	toxic
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		936 ± 11.1	1,024 ± 18.1	1,636 ± 125.5	1,021 ± 63.2	1,264 ± 205.4	2,105 ± 62.1
TA1535	0.00	15 ± 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				
	0.10	14 ± 3.2	5 ± 0.7				
	0.30	8 ± 1.5	2 ± 1.2				
	1.00	8 ± 0.6	2 ± 0.6	18 ± 3.3	4 ± 1.8	16 ± 0.9	5 ± 1.9
	3.30			26 ± 1.3	5 ± 1.5	20 ± 2.0	6 ± 2.5
	10.00			15 ± 4.2	3 ± 0.9	17 ± 1.5	5 ± 1.7
	33.00			9 ± 0.9	4 ± 0.7	8 ± 1.5	4 ± 0.7
	100.00			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	0.00	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				
	0.10	14 ± 1.0	7 ± 0.9				
	0.30	16 ± 0.7	7 ± 2.3				
	1.00	11 ± 3.8	7 ± 1.2	22 ± 2.4	13 ± 1.0	23 ± 3.0	12 ± 1.5
	3.30			26 ± 4.3	10 ± 2.1	20 ± 3.4	15 ± 1.7
	10.00			14 ± 0.6	9 ± 2.4	13 ± 2.0	7 ± 2.1
	33.00			13 ± 1.2	15 ± 2.6	13 ± 2.5	12 ± 1.7
	100.00			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] 2-Aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-o-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

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	0.960	50	1,049	458	0.43	9.2	26.0	5.33
	3.000	50	1,050	457	0.43	9.1	26.0	5.00
	9.600	50	1,048	473	0.45	9.5	26.0	8.68
								P=0.115[c]
+S9								
Summary: Negative								
Distilled water		50	1,043	369	0.35	7.4	26.0	
Cyclophosphamide	1.00	50	1,045	790	0.75	15.8	26.0	113.69
Benzethonium chloride	3.00	50	1,048	347	0.33	6.9	26.0	−6.41
	9.60	50	1,050	359	0.34	7.2	26.0	−3.36
	30.00	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.* (1985). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine.
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells × 100
[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	Percent Cells w/Abs	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells w/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	15.00	50	16	0.32	28.0
Benzethonium chloride					Benzethonium chloride				
0.96	100	11	0.11	10.0	3.00	100	5	0.05	5.0
3.00	100	10	0.10	10.0	9.60	100	6	0.06	5.0
9.60	100	8	0.08	8.0	30.00	100	6	0.06	6.0
				P=0.162					P=0.172[b]

[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	10.580 ± 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 ± 0.05	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 ± 0.15	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 ($P \le 0.05$) from the control group by Williams' or Dunnett's test
** $P \le 0.01$
[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	1.563 mg/kg	3.125 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg
	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**
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 ($P \leq 0.05$) 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	1.563 mg/kg	3.125 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	1.736 ± 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 ($P \le 0.05$) from the control group by Williams' or Dunnett's test
** $P \le 0.01$
[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 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 ± 0.055*
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 ± 0.66
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 ($P \le 0.05$) 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	1.563 mg/kg	3.125 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 ± 0.90**
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 ± 0.003
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	0.220 ± 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 ($P \le 0.05$) from the control group by Williams' or Dunnett's test
** $P \le 0.01$
[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	9
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 ($P \le 0.05$) from the control group by Williams' or Dunnett's test
** $P \le 0.01$
[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 (NIEHS).

The chemical, a white powdered solid, 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. High performance liquid chromatography 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, 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(s)% 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. 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 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 high performance liquid chromatography 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 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), with octanophenone added 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% (169/173) 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).

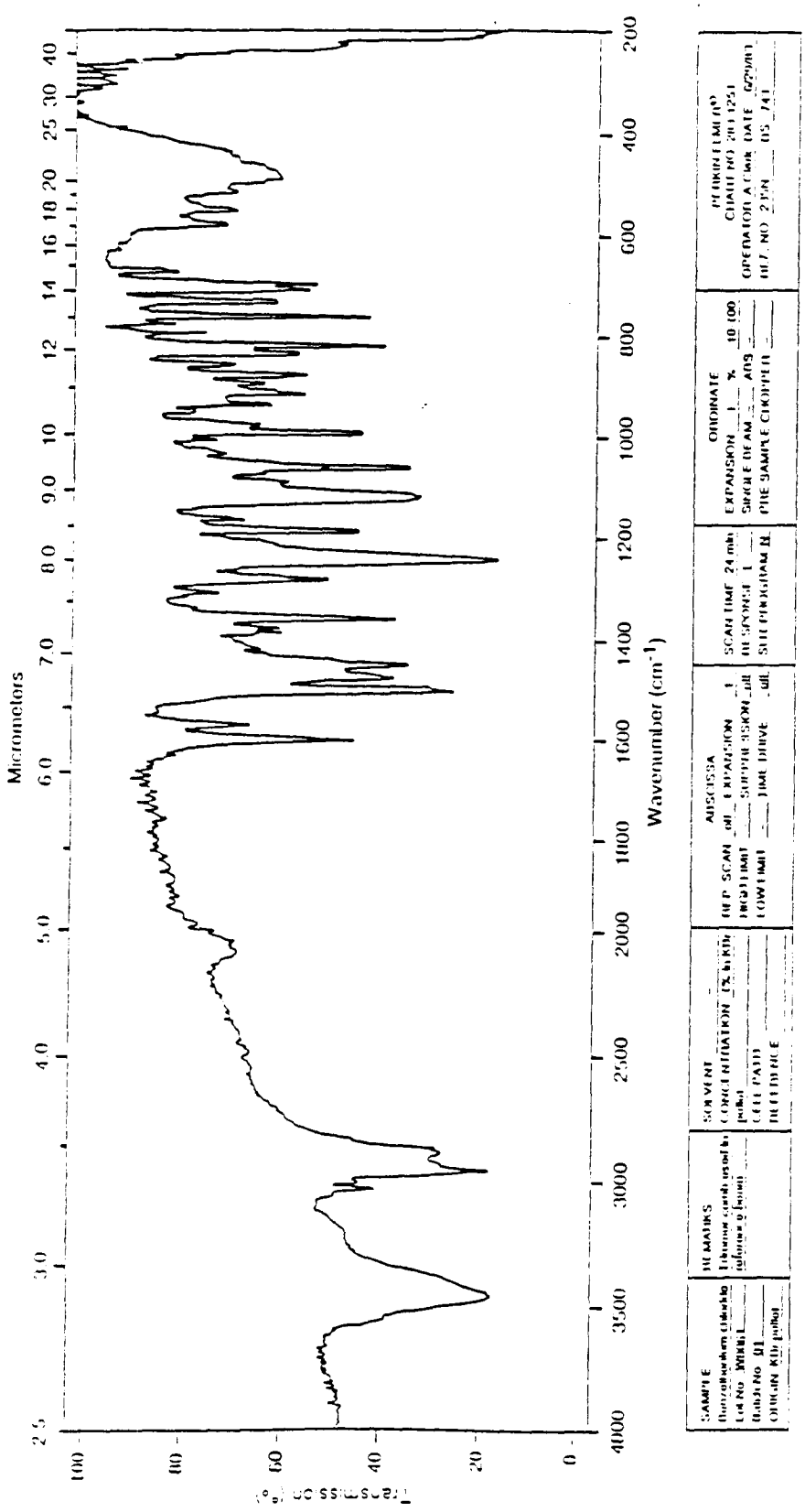

FIGURE G1
Infrared Absorption Spectrum of Benzethonium Chloride

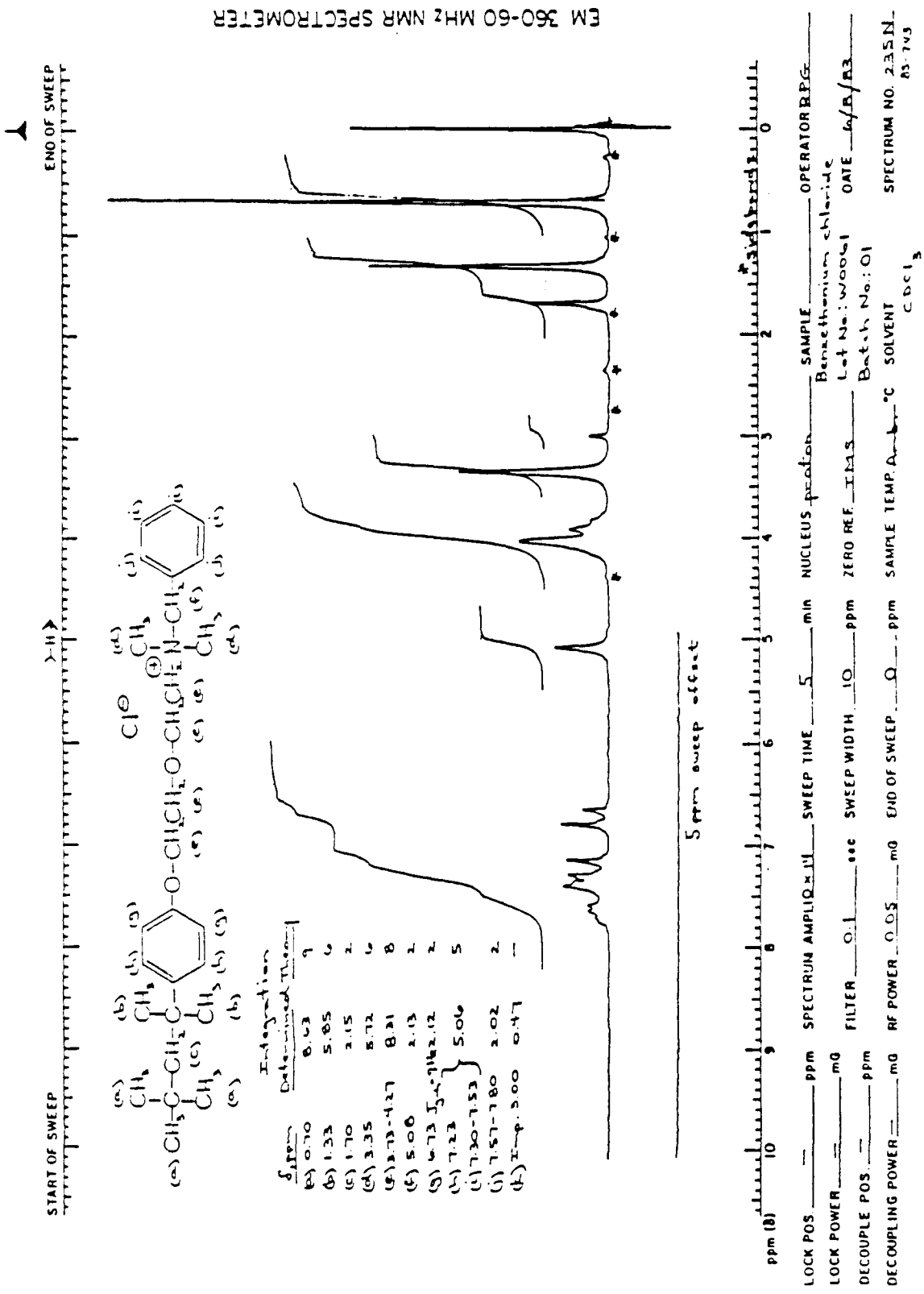

FIGURE G2
Nuclear Magnetic Resonance Spectrum of Benzethonium Chloride

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	Same as 16-day studies	Same as 16-day studies
Maximum Storage Time		
3 weeks	Same as 16-day studies	Same as 16-day studies
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[b] (mg/mL)	% Difference from Target
Rats				
Males				
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
Females				
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
		96.0	97.7	+2

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

Date Prepared	Date Analyzed	Target Concentration (mg/mL)[a]	Determined Concentration[b] (mg/mL)	% Difference from Target
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 = 0.250 mL (rats) or 0.100 mL (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 mg/mL = 50 mg/kg; 96 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 mg/mL = 50 mg/kg; 64 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 mg/mL = 50 mg/kg; 24 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[a]

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[b]	% Difference from Target
Rats				
25 April 1985	29 April 1985	1.563	1.58	+1
		3.125	3.04	−4
		6.25	6.41	+3
		12.5	12.7	+2
		25.0	26.0	+4
	14 May 1985[c]	1.563	1.60	+2
		3.125	3.13	+1
		6.25	6.39	+2
		12.5	12.9	+3
		25.0	25.6	+2
6 June 1985	7 June 1985	1.563	1.59	+2
		3.125	3.10	−1
		6.25	6.38	+2
		12.5	13.0	+4
		25.0	25.9	+4
	26 June 1985[c]	1.563	1.60	+2
		3.125	3.24	+4
		6.25	6.47	+4
		12.5	13.1	+5
		25.0	26.6	+6

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
Rats (continued)				
18 July 1985	19 July 1985	1.563	1.55	−1
		3.125	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]	1.563	1.60	+2
		3.175	3.24	+2
		6.25	6.27	0
		12.5	12.6	+1
		25.0	25.7	+3
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	0.519	+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

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 (continued)				
6 June 1985	26 June 1985[d]	4.0	4.38	+10
		8.0	8.28	+4
10 June 1985[e]	10 June 1985	0.5	0.486	-3
		1.0	1.03	+3
		2.0	1.94	-3
10 June 1985	26 June 1985	0.5	0.50	0
		1.0	1.04	+4
		2.0	2.06	+3
15 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	0.5	0.485	-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 2.5 mL for rats or 100 µL for mice. For rats, 1.563 mg/mL = 1.563 mg/kg; 3.125 mg/mL = 3.125 mg/kg; 6.25 mg/mL = 6.25 mg/kg; 12.5 mg/mL = 12.5 mg/kg; 25 mg/mL = 25 mg/kg. For mice, 0.5 mg/mL = 1.563 mg/kg; 1 mg/mL = 3.125 mg/kg; 2 mg/mL = 6.25 mg/kg; 4 mg/mL = 12.5 mg/kg; 8 mg/mL = 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 prep 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[a] (mg/mL)	Determined Concentration[b] (mg/mL)	% 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
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

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 (continued)				
6 June 1985	26 June 1985[d]	4.0	4.38	+10
		8.0	8.28	+4
10 June 1985[e]	10 June 1985	0.5	0.486	−3
		1.0	1.03	+3
		2.0	1.94	−3
19 July 1985	26 June 1985	0.5	0.50	0
		1.0	1.04	+4
		2.0	2.06	+3
29 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	0.5	0.485	−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, 1.563 mg/mL = 1.563 mg/kg; 3.125 mg/mL = 3.125 mg/kg; 6.25 mg/mL = 6.25 mg/kg; 12.5 mg/mL = 12.5 mg/kg; 25 mg/mL = 25 mg/kg. For mice, 0.5 mg/mL = 1.563 mg/kg; 1 mg/mL = 3.125 mg/kg; 2 mg/mL = 6.25 mg/kg; 4 mg/mL = 12.5 mg/kg; 8 mg/mL = 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 prep balance, samples originally mixed on 6 June 1985 were remixed and reanalyzed on 10 June 1985.

TABLE G-
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 (cont.)				
16 March	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 19	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	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
26 September 1988	28 September 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

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)				
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
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	0.0658	+10
		0.2	0.204	+2
		0.6	0.613	+2
5 August 1987	6 August 1987	0.06	0.0574[c]	−4
		0.2	0.198[c]	−1
		0.6	0.604	+1
30 September 1987	1 October 1987	0.06	0.0561	−6
		0.2	0.195	−2
		0.6	0.603	+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
Mice (continued)				
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
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 1988	23 February 1988	0.06	0.062	+3
		0.2	0.210	+5
		0.6	0.623	+4

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)				
19 April 1988	20 April 1988	0.06	0.058	−3
		0.2	0.198	−1
		0.6	0.585	−2
	2 May 1989	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 63-296 µL for male rats, 95-317 µL for female rats and 50-131 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 2-Year Dermal Studies of Benzethonium Chloride

Date Mixed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	
		Study Laboratory[a]	Referee Laboratory[b]
13 Weeks			
25 April 1985	1.0	0.96	1.04 ± 0.01
18 July 1985	8.0	7.93	7.99 ± 0.01
2 Years			
Rats			
9 June 1987	0.25	0.249	1.12 ± 0.0[illegible]
	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
dl-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)[f]	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)[f]	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[f]	<0.01		25
β-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 five male and five female rats at 6, 12, and 18 months into the study and from 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 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	0/10	None positive
12 months	0/10	None positive
18 months	0/10	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	0/10	None positive
22 months	7/10	Mouse hepatitis virus
24 months	8/10	Mouse hepatitis virus

[a] Possible *Mycoplasma arthritidis*

NTP Studies

ABSTRACT

A subchronic study was conducted in which ten B6C3F1 mice of each sex were administered 25, 12.5, 6.25, 3.125, 1.563, or 0 mg/kg benzethonium chloride dermally (five times per week) for 13 weeks. No mortality occurred during this study. Chemical administration produced an initial irritation or redness, crustiness, depigmentation of fur, and thickening of the skin in the dosed area predominately in high dose mice of both sexes. All male dose groups and the 12.5 and 25 mg/kg female dose groups exhibited a depression in differential weight gain relative to their respective control group over the course of the study. This effect was dose-related in males (at the 6.25 mg/kg level and above) but not in the female dose groups. At study termination, no consistent or biologically significant alterations were seen between treated and control mice of either sex in mean organ absolute weights or in the organ weight to body weight or brain weight ratios. Upon histopathological examination, chemically-induced microscopic abnormalities were restricted to the dosed area. Epidermal hyperkeratosis and acanthosis occurred in all benzethonium chloride dose groups of both sexes. Necrotizing epidermal inflammation was restricted to one male mouse at the 12.5 mg/kg dose level and to several mice of both sexes at the 25.0 mg/kg dose level. Chronic dermal inflammation occurred mainly at the 6.25 mg/kg dose level and above in mice of both sexes. A summary of the results of this study is presented in Table 1.

TABLE 1. SUMMARY OF TOXICOLOGIC DATA FOR MICE ON THE DERMAL SUBCHRONIC STUDY OF BENZETHONIUM CHLORIDE IN B6C3F1 MICE

Dose Level, mg/kg	Mortality	Mean Body Weight Gain, grams (Percent Change Relative to Control[a])	Clinical Signs	Significant Necropsy Findings	Significant Microscopic Findings
			Males		
25	0/10	6.8 (-26.1)	Alopecia, body ventral (1/10); discoloration, hair (4/10); prolapse, penis (1/10); scaly (9/10), thickened (5/10), irritation (7/10), and discoloration (10/10), site of application	Skin: crust (1/10), and hair, pigment, white (6/10)	Skin, treated: dermis, inflammation, chronic (6/10); epidermis, hyperkeratosis (5/10) and acanthosis (6/10); inflammation, necrotizing (4/10)
12.5	0/10	7.8 (-15.2)	Alopecia, body ventral (1/10); scaly (5/10), thickened (3/10), irritation (1/10), and discoloration (5/10), site of application	Skin: crust (1/10), and hair, pigment, white (1/10)	Skin, treated: dermis, inflammation, chronic (8/10); epidermis, hyperkeratosis (8/10) and acanthosis (8/10); inflammation, necrotizing (1/10)
6.25	0/10	7.9 (-14.1)	Alopecia, body ventral (1/10); thickened, site of application (1/10)	None	Skin, treated: dermis, inflammation, chronic (6/10); epidermis, hyperkeratosis (9/10) and acanthosis (10/10)
3.125	0/10	8.5 (-7.6)	Prolapse, penis (1/10)	None	Skin, treated: dermis, inflammation, chronic (1/10); epidermis, hyperkeratosis (6/10) and acanthosis (8/10)
1.563	0/10	8.3 (-9.8)	None	None	Skin, treated: epidermis, hyperkeratosis (8/10) and acanthosis (7/10)
0	0/10	9.2	None	None	None

TABLE 1. (Continued)

Dose level, mg/kg	Mortality	Mean Body Weight Gain, grams (Percent Change Relative to Control[a])	Clinical Signs	Significant Necropsy Findings	Significant Microscopic Findings
			Females		
25	0/10	7.0 (-7.9)	Alopecia, right rear leg (1/10); alopecia, body ventral (4/10); discoloration, hair (4/10); scaly (6/10), thickened (2/10), irritation (2/10), and discoloration (9/10), site of application	Skin: crust (1/10) and hair, pigment, white (4/10)	Skin, treated: dermis, inflammation, chronic (7/10); epidermis, hyperkeratosis (6/10) and acanthosis (6/10); inflammation, necrotizing (3/10)
12.5	0/10	6.7 (-11.8)	Alopecia, right rear leg (1/10); alopecia, anal area (1/10); alopecia, body ventral (1/10); scaly (2/10) and discoloration (1/10), site of application	Skin: crust (1/10)	Skin, treated: dermis, inflammation, chronic (10/10); epidermis, hyperkeratosis (10/10) and acanthosis (10/10)
6.25	0/10	8.1 (6.6)	None	None	Skin, treated: dermis, inflammation, chronic (7/10); epidermis, hyperkeratosis (10/10) and acanthosis (10/10)
3.125	0/10	7.9 (3.9)	Alopecia, body ventral (1/10)	None	Skin, treated: epidermis, hyperkeratosis (10/10) and acanthosis (10/10)
1.563	0/10	7.7 (1.3)	None	Skin: scar (1/10)	Skin, treated: dermis, inflammation, chronic (2/10); epidermis, hyperkeratosis (9/10) and acanthosis (10/10)
0	0/10	7.6	Clipper injury, site of application (1/10)	None	Skin, treated: dermis, inflammation, chronic (1/10)

[a] Percent Change Relative to Control = $\frac{\text{Mean Value for Dosed Group - Mean Value for Control Group}}{\text{Mean Value for Control Group}} \times 100$

ABSTRACT

A subchronic dermal toxicity study of benzethonium chloride was conducted in young adult Fischer 344 rats. The test chemical, formulated in ethanol, was applied to the dorsal skin of rats (10/sex/group) at dose levels of 25, 12.5, 6.25, 3.125, 1.563, or 0 mg/kg, five times per week for 13 weeks.

No unscheduled deaths occurred in this study. A treatment-related reduction in body weight occurred in male rats exposed to 25 mg/kg of benzethonium chloride. No other body weight differences between the treatment and control groups of either sex were observed. The incidence of skin irritation in the dosed-skin region and the time that lesions first appeared were both treatment- and dose-related. Treatment-associated gross changes observed at necropsy were limited to the dosed skin area of all treatment groups. Multiple, irregular, epidermal crusts and thickened skin occurred frequently in rats treated with 25 mg/kg or 12.5 mg/kg of the test material with multiple red foci observed in rats of each dose level. Dosed skin was the only target organ identified by microscopic examination. All rats in the high dose group had necrotizing inflammation, with the incidence and severity of this lesion decreasing through the lower dose groups. Additionally, chronic dermatitis, hyperkeratosis, and acanthosis were present in treated rats of all but the high dose group. A summary of the toxicology results of this study is presented in Table 1.

TABLE 1: SUMMARY OF TOXICOLOGIC DATA FOR RATS ON THE SUBCHRONIC DERMAL STUDY OF BENZETHONIUM CHLORIDE IN FISCHER 344 RATS

Dose Level, mg/kg	Mortality	Mean Body Weight Gain, grams (Percent Change Relative to Control[a])	Clinical Signs	Significant Necropsy Findings	Significant Microscopic Findings
			Males		
25	0/10	101.4 (-25.3)	Irritation (10/10) at site of application; nasal discharge (1/10)	Crusts (10/10), thickening (6/10) at site of application	Skin, treated: necrotizing, inflammation (10/10)
12.5	0/10	123.3 (-6.9)	Irritation (10/10) at site of application	Crusts (4/10), thickening (1/10), red foci (2/10), and lesion (1/10) at site of application	Skin, treated: necrotizing inflammation (8/10), chronic inflammation of dermis (1/10) hyperkeratosis (2/10), and acanthosis (1/10)
6.25	0/10	127.5 (-3.8)	Irritation (7/10) at site of application	Crusts (2/10) and red foci (5/10) at site of application	Skin, treated: necrotizing inflammation (6/10), chronic inflammation of dermis (3/10), hyperkeratosis (2/10), and acanthosis (2/10)
3.125	0/10	134.8 (1.7)	Irritation (3/10) and clipper injury (1/10) at site of application	Red foci (4/10) at site of application	Skin, treated: necrotizing inflammation (3/10), chronic inflammation of dermis (2/10), hyperkeratosis (1/10), and acanthosis (2/10)
1.563	0/10	129.0 (-2.6)	None	None	Skin, treated: necrotizing inflammation (1/10)
0	0/10	132.5	Discharge, right eye (1/10)	None	None

TABLE 1. (Continued)

Dose Level, mg/kg	Mortality	Mean Body Weight Gain, grams (Percent Change Relative to Control[a])	Clinical Signs	Significant Necropsy Findings	Significant Microscopic Findings
			Females		
25	0/10	51.2 (-8.9)	Scaly (1/10); irritation (10/10) and discoloration (1/10) at site of application	Crusts (10/10) and thickening (2/10) at site of application	Skin, treated: necrotizing inflammation (10/10)
12.5	0/10	52.3 (-6.9)	Scaly (2/10), and irritation (9/10) at site of application	Crusts (3/10) and red foci (4/10) at site of application	Skin, treated: necrotizing inflammation (7/10), chronic inflammation of dermis (3/10), hyperkeratosis (1/10), and acanthosis (2/10)
6.25	0/10	52.6 (-6.4)	Irritation (7/10) at site of application	Crusts (3/10) and red foci (3/10) at site of application	Skin, treated: necrotizing inflammation (4/10), chronic inflammation of dermis (4/10), hyperkeratosis (1/10), and acanthosis (3/10)
3.125	0/10	52.9 (-5.9)	Irritation (8/10) at site of application	Crusts (2/10) and red foci (4/10) at site of application	Skin, treated: necrotizing inflammation (5/10), chronic inflammation of dermis (4/10), hyperkeratosis (2/10), and acanthosis (3/10)
1.563	0/10	54.5 (-3.0)	Irritation (1/10) at site of application	Red foci (2/10) at site of application	Skin, treated: chronic inflammation of dermis (3/10), and hyperkeratosis (2/10)
0	0/10	56.2	None	None	None

[a] Percent Change Relative to Control = $\frac{\text{Mean Value for Dosed Group - Mean Value for Control Group}}{\text{Mean Value for Control Group}} \times 100$

Pathology Working Group

Benzethonium Chloride (C61494)
13 Week Subchronic Dermal Toxicity
Study in F344 Rats and B6C3F1 Mice
May 1, 1986

Conflict of Interest Statement

Those individuals involved in testing or evaluation of this chemical (Benzethonium Chloride, C61494) at Pathology Associates, Inc. are listed below.

Pathology - Michael A. Stedham, DVM, MS
Clerical - Janet M. Bromfield
Terri Smith

They have not been involved in the testing or evaluation of this chemical for clients other than the National Toxicology Program.

Body Weight

The difference in weight gain (%) at 13 weeks compared to the controls is listed by dose group.

Group	Male	Female
1.563	-2.6	-3.0
3.125	+1.7	-5.9
6.25	-3.8	-6.4
12.5	-6.9	-6.9
25.0	-23.5	-8.9

The only large decrease in weight gain was in the 25.0 mg/kg males.

Organ Weight

Absolute organ weights, organ to body weight ratios, and organ to brain weight ratios were performed for thymus, liver, kidney, testis, heart, lung, and brain. As noted in the original report, the majority of significant differences were in the high dose groups (25 mg/kg) of male rats. The only significant difference in absolute organ weight was for the thymus (decreased) in the high dose male group.

Clinical Pathology

None performed.

Clinical Signs

"Skin irritation", not further defined, occurred in a dose-related pattern in both males and females as follows:

Group	Male n=10	Female n=10
0.0	0	0
1.563	0	1
3.125	3	8
6.25	7	7
12.5	10	9
25.0	10	10

Scaliness or discoloration was reported in a few females in the two highest dose groups.

Necropsy Data

As noted in the original report, multiple irregular, epidermal crusts and thickened skin were the predominant lesions, the highest frequency occurring in the 25.0 and 12.5 mg/kg groups. Red foci were noted in rats in the other dose groups.

Histopathology

The original pathologist (OP) recorded inflammatory (including necrotizing) and lesions of the skin in all dose groups, and proliferative lesions in all dose groups but the highest one. The inflammatory lesions increased in incidence and severity with increased doses. The OP, however, failed to record the proliferative lesions (acanthosis, hyperkeratosis) in the high dose groups (25 mg/kg, male and female) when he rendered a diagnosis of necrotizing inflammation, thus making proper interpretation of the original table impossible with regard to these lesions. As pointed out by the QAP, these lesions were, in fact, present in the 25 mg/kg groups and they were more severe than in the other groups.

The QAP correctly points out that "a thorough description of the lesions noted should have been in the histopathology narrative describing the spectrum of lesions and the sequential reduction in severity and incidences with decreasing dosage." The QAP defines necrotizing inflammation for the purposes of his review as encompassing acanthosis, hyperkeratosis, and chronic inflammation as well. This may have been the intent of the OP as well but it is not stated.

In the QAP comments (diagnoses) column for the most part, additional diagnoses for proliferative epithelial changes are not rendered when he has concurred with the OP diagnosis of necrotizing inflammation. An exception to this format is noted in 25 mg/kg female rat #116, Histology #856356.

Both the OP and QAP apparently considered the bone marrow to be normal where as the PWG chairperson considered that myeloid hyperplasia was present in the high dose (25 mg/kg) male and female rats. Bones from the other dosed groups were not available for review because marrow had not been detected as a target organ. It is suggested that the hyperplasia is

secondary to the inflammatory skin lesions and is not a primary target. The full PWG preferred the term, hypercellularity, for the marrow lesion but concurred that a difference was present between the control and 25 mg/kg rats.

Other differences in diagnoses between the OP, QAP, and the PWG chairman were minor, were related to terminology, or were in non-target tissues. In the last category was inflammation of the nasal mucosa and a fungal colony in the nasal cavity (lumen) of Animal No. 56 in the 25 mg/kg male group.

A rudimentary description of the lesions is offered. In general, all components of the inflammatory and proliferative lesions decreased in incidence and severity with decreasing dose and were roughly equal between sexes.

All components of the epidermis of treated skin proliferated to some degree in many of the more severely affected animals in the higher dose groups. Acanthosis was the most prominent feature, generally, followed by hyperkeratosis and lesser degrees of basal cell hyperplasia or alteration, and an increased stratum granulosum. Parakeratosis was also present as was hyperplasia of the sebaceous glands. These were also considered under hyperplasia, epithelium as diagnosed by the PWG Chairperson.

Inflammation, necrotizing included either necrosis or ulceration (presuming previous necrosis) or both of the epidermis plus varying amounts of chronic and acute inflammatory cells in the dermis. In more severe instances the full depth of the dermis was affected with some involvement of the adjacent subcutis. In addition, some degree of collagen degeneration and/or early fibrosis was noted in some areas. In some instances immature fibroblasts and collagen were oriented with their long axis parallel to each other and to the skin surface (granulation tissue). Also, included with the diagnosis of inflammation was accumulation of neutrophils in the epidermis, primarily between the spiny cell layer and the keratin layer (pustules). The epidermal necrosis was coagulative in nature and was frequently full-depth. In some instances, it was difficult to differentiate between parakeratosis and necrosis. In these instances, the epidermis (progressing through spiny cells, granular cells, and keratin) underlaid plumper, poorly staining "ghost"

cells. In addition, occasional accumulations of neutrophils were noted beneath these overlaying "ghost" cells. This may reflect previous full depth necrosis of the epithelium with neutrophil infiltrate and subsequent regeneration of the epithelium from beneath (residual basal cells or follicular epithelium).

When necrosis or ulceration was not present, inflammation, chronic or chronic, active was rendered. Chronic or chronic, active was also used to modify necrotizing inflammation. Although these differences (chronic versus chronic, active) may be legitimate, they merely reflect the presence or absence of neutrophils in a basically chronic reaction, and for purposes of generating more understandable tabular results, could be consolidated under chronic.

Although the QAP apparently attempted to follow the OPs method of diagnosis, the PWG Chairperson preferred to combine the proliferative lesions into hyperplasia, epithelial and to render this diagnosis even if necrotizing inflammation was also diagnosed. This was done in an attempt to simplify tabular data inasmuch as the OP tables were misleading in this regard. Also, it would be more consistent with diagnoses rendered for the mice in the study. Further, although the QAP and the PWG Chairperson had minor differences in diagnosing the proliferative lesions in the treated skin of the lower dose groups, they were in general concurrence that these lesions were undercalled by the OP in these dose groups.

Summary

The PWG considers the 1.56 mg/kg dose level to be appropriate for the chronic study. Higher dose levels were considered to have risk for a chronic study owing to necrosis, ulcers, and inflammation.

The PWG considered the pathology narrative to be unsatisfactory in that the lesions were not characterized and that the tables do not help substantially in clarifying the lesions. In particular, the tables are misleading in reference to hyperplastic/proliferative lesions. Bone marrow should also be considered a target organ, although probably a secondary target.

Action Items

1. The PWG Chairperson will review again the lesions in the treated skin, classify them under chronic inflammation, necrosis, ulcer, and epithelial hyperplasia, and produce tables useful for dose-setting for the chronic study. The PWG felt that it was important to list necrosis and ulcer separately as these may be life-threatening in a chronic study.

2. The slides will be returned to the OP for reconsideration of the sections of treated skin and of bone marrow.

Action Taken

The following summary tables and individual tables from re-evaluation of treated skin by the PWG Chairperson are provided. It should be noted that the full PWG generally evaluated the lesions to have a slightly lower degree of severity (0 to 1 degree lower) than the Chairperson.

SUMMARY TABLE

FEMALE RATS

	mg/kg	0.0	1.56	3.12	6.25	12.5	25.0
Inflammation, Chronic		0/10*	4/10	10/10	7/10	10/10	10/10
		0.0**	1.0	1.5	1.6	1.7	3.2
Necrosis		0/10	0/10	1/10	3/10	5/10	8/10
		0.0	0.0	1.0	1.3	1.0	1.4
Ulcer		0/10	0/10	5/10	3/10	1/10	10/10
		0.0	0.0	1.8	1.7	1.0	2.0
Hyperplasia, Epithelial		0/10	5/10	9/10	9/10	10/10	10/10
		0.0	1.0	1.4	1.6	1.9	3.0

SUMMARY TABLE

MALE RATS

	mg/kg	0.0	1.56	3.12	6.25	12.5	25.0
Inflammation, Chronic		0/10*	2/10	7/10	9/10	10/10	10/10
		0.0**	1.0	1.1	1.7	2.5	3.5
Necrosis		0/10	0/10	1/10	2/10	6/10	9/10
		0.0	0.0	1.0	1.0	1.2	1.9
Ulcer		0/10	0/10	2/10	4/10	8/10	10/10
		0.0	0.0	1.0	1.5	1.9	2.5
Hyperplasia, Epithelial		0/10	4/10	9/10	10/10	10/10	10/10
		0.0	1.0	1.1	1.7	2.6	3.0

* No. affected/No. in group

** Average degree of severity
0-Within Normal Limits
1-Minimal
2-Mild
3-Moderate
4-Marked

INDIVIDUAL ANIMAL TABLE
MALE RAT

Control

ANIMAL #	1	2	3	4	5	6	7	8	9	10
Inflammation, chronic	0	0	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelium	0	0	0	0	0	0	0	0	0	0

1.56 mg/kg

ANIMAL #	11	12	13	14	15	16	17	18	19	20
Inflammation, chronic	0	0	1	0	0	0	0	0	0	1
Necrosis	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelium	0	1	1	0	0	0	1	0	0	1

3.12 mg/kg

ANIMAL #	21	22	23	24	25	26	27	28	29	30
Inflammation, chronic	1	1	2	1	1	1	0	0	0	1
Necrosis	0	0	1	0	0	0	0	0	0	0
Ulcer	0	0	1	0	0	1	0	0	0	0
Hyperplasia, Epithelium	1	1	1	1	1	2	0	1	1	1

*Average degree of severity
0-Within Normal Limits
1-Minimal
2-Mild
3-Moderate
4-Marked

6.25 mg/kg

ANIMAL #	31	32	33	34	35	36	37	38	39	40
Inflammation, chronic	0	1	2	2	1	1	2	2	2	2
Necrosis	0	0	1	1	0	0	0	0	0	0
Ulcer	0	0	0	1	0	0	2	1	0	2
Hyperplasia, Epithelium	1	1	2	2	1	1	2	3	2	2

12.5 mg/kg

ANIMAL #	41	42	43	44	45	46	47	48	49	50
Inflammation, chronic	3	1	4	1	3	3	2	2	2	4
Necrosis	0	0	1	0	2	1	1	1	1	0
Ulcer	1	0	3	0	3	2	1	1	1	3
Hyperplasia, Epithelium	3	2	3	2	3	3	2	3	2	3

25.0 mg/kg

ANIMAL #	51	52	53	54	55	56	57	58	59	60
Inflammation, chronic	3	4	4	4	4	4	3	3	4	2
Necrosis	1	3	2	2	2	2	1	2	2	0
Ulcer	2	2	3	3	3	3	3	2	3	1
Hyperplasia, Epithelium	4	2	3	3	3	3	2	3	4	3

*Average degree of severity
0-Within Normal Limits
1-Minimal
2-Mild
3-Moderate
4-Marked

INDIVIDUAL ANIMAL TABLE
FEMALE

Control

ANIMAL #	61	62	63	64	65	66	67	68	69	70
Inflammation, chronic	0	0	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelium	0	0	0	0	0	0	0	0	0	0

1.56 mg/kg

ANIMAL #	71	72	73	74	75	76	77	78	79	80
Inflammation, chronic	1	1	0	1	0	0	1	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelium	1	1	0	1	0	0	1	0	0	1

3.12 mg/kg

ANIMAL #	81	82	83	84	85	86	87	88	89	90
Inflammation, chronic	2	1	2	2	1	2	1	1	2	1
Necrosis	0	0	1	0	0	0	0	0	0	0
Ulcer	2	0	0	2	0	2	1	0	2	0
Hyperplasia, Epithelium	2	1	2	2	0	1	1	1	2	1

*Average degree of severity
0-Within Normal Limits
1-Minimal
2-Mild
3-Moderate
4-Marked

6.25 mg/kg

ANIMAL #	91	92	93	94	95	96	97	98	99	100
Inflammation, chronic	0	1	0	3	2	1	0	1	1	2
Necrosis	0	1	0	1	2	0	0	0	0	0
Ulcer	0	0	0	1	2	0	0	0	0	2
Hyperplasia, Epithelium	1	2	0	3	2	1	1	1	1	2

12.5 mg/kg

ANIMAL #	101	102	103	104	105	106	107	108	109	110
Inflammation, chronic	2	1	1	2	2	2	2	2	2	1
Necrosis	0	0	0	1	0	1	1	1	1	0
Ulcer	0	0	0	0	0	0	1	0	0	0
Hyperplasia, Epithelium	2	1	2	2	2	2	2	2	2	2

25.0 mg/kg

ANIMAL #	111	112	113	114	115	116	117	118	119	120
Inflammation, chronic	3	3	3	3	4	4	4	2	3	3
Necrosis	0	1	0	1	2	2	2	1	1	1
Ulcer	1	2	2	2	3	3	3	1	2	1
Hyperplasia, Epithelium	3	3	3	3	3	3	3	3	3	3

*Average degree of severity
0-Within Normal Limits
1-Minimal
2-Mild
3-Moderate
4-Marked

B6C3F1 MICE

INTRODUCTION

The study design was:

Dose Level mg/kg	Number of Mice Male	Female
0.0	10	10
1.563	10	10
3.125	10	10
6.25	10	10
12.5	10	10
25.0	10	10

Histotechnique

The QA evaluation of histotechnique was fair to good. The PWG considers it to be acceptable.

As pointed out by the QAP, the location of the skin sites by slide number was not defined in the original report. It was assumed that #2 and #6 were control sites whereas #4 was the treated site with #7, when present, being an additional section of treated skin.

As in the rat study, multiple slides of skin (duplicates or recuts) were present in many animals. These slides usually had no sub-number differentiation. For example, mouse #14 in the 1.56 mg/kg male group had 4 #2s, 2 #4s, 1 #6, and 1 # 6A.

Mortality

No deaths or early sacrifices were reported.

Body Weight

The difference in weight gain (%) at 13 weeks as compared to the controls is listed by dose groups.

Group	Male	Female
1.563	-9.8	+1.3
3.125	-7.6	+3.9
6.25	-14.1	+6.6
12.5	-15.2	-11.8
25.0	-26.1	-7.9

In the male groups, the 6.25 mg/kg and higher groups had greater than 10% decrease in weight gain whereas in females only the 12.5 mg/kg group exceeded 10%.

Organ Weight

Absolute organ weights, organ to body weight ratios, and organ to brain weight ratios were performed for the liver, thymus, heart, lungs, right kidney, right testis, and brain. There were no significant differences in absolute organ weights (dosed group vs. control group). The few differences in organ weight relative to body weight occurred primarily in the 12.5 and 25.0 mg/kg male groups which had moderate decreases in weight gain compared to controls.

Clinical Pathology

None performed.

Clinical Signs

Abnormalities of the skin at the application site were noted in female mice in the 12.5 and 25 mg/kg dose groups and in male mice in the 6.25 mg/kg dose group and higher. These abnormalities included irritation or redness, thickening, scaliness, and/or bleaching.

Necropsy Data

The only lesion occurring in more than 1 of 10 mice was depigmentation of hair at the application site in 25 mg/kg males and females (6 and 4 of 10 respectively). It should be noted that in the report from the original laboratory, scars, crusts, and depigmentation of hair are interpreted as evidence of skin inflammation and these were attributed to Benzethonium Chloride (p. 30 and 33). However, the table of gross lesions (p. 33) indicates a scar in only 1 female mouse at 1.56 mg/kg and in no others. It would seem

more appropriate to consider this an incidental lesion inasmuch as it occurred in none of the other 99 treated animals at up to 16 times that concentration.

Histopathology

The OP reported significant lesions only in the skin. These were related to application of the test chemical, occurring in increasing incidence and severity with increasing concentrations. The QAP and the PWG are in essential aggreement with this. As for the rats, the OP apparently did not diagnose the proliferative lesions (acanthosis, hyperkeratosis) when necrotizing inflammation was diagnosed. This was not specified in the narrative. It resulted in misleading tabular results showing decreased hyperkeratosis and acanthosis in the 25 mg/kg male and female groups. The QAP rightfully pointed this out and diagnosed the component parts of the lesions in great detail (up to 9 diagnoses for the same section). The PWG Chairperson's initial review combined the proliferative lesions under hyperplasia, epithelial, and the inflammation either as inflammation necrotizing, chronic or chronic, active, or as inflammation, chronic, or chronic, active. In retrospect, the chronic and chronic, active modifiers could be combined under chronic. In general, all lesions occurred with less severity than in rats and, in particular, necrosis and/or ulceration were of lower incidence and severity.

As for rats, there was no description of the microscopic lesions.

Although hypercellularity of the bone marrow in 25 mg/kg rats was noted it was not detected in the mice.

Summary

The PWG considered the 3.12 dose level to be appropriate for the chronic study. This was based primarily on the occurrence of necrosis in the epidermis in 6.25 and 12.5 mg/kg male groups. This occurred, however, in only 1 mouse in each of these groups and was of minimal severity. The possibility of a higher dose might be considered although the male group at 6.25 mg/kg had a 14% decrease in weight gain.

The PWG considered the pathology narrative to be unsatisfactory owing to lack of lesion description or clarification of the decrease of hyperkeratosis and acanthosis in the 25 mg/kg groups.

Action Items

1. The PWG Chairperson will review again the lesions in the treated skin, classify them under chronic inflammation, necrosis, ulcer, and epithelial hyperplasia, and produce tables useful for dose-setting for the chronic.

2. The slides will be returned to the OP for reconsideration of the sections of treated skin.

Action Taken

The following summary tables and individual tables from re-evaluation of treated skin by the PWG Chairperson are provided. It should be noted that the full PWG rated the severity of the lesions somewhat lower than the PWG Chairperson (0 to 1 degree lower)

SUMMARY TABLE

Male Mice

mg/kg	0.0	1.56	3.12	6.25	12.5	25.0
Inflammation, Chronic Dermis	1/10*	2/10	3/10	6/10	9/10	10/10
	0.0**	1.0	1.0	1.0	1.2	2.0
Necrosis	0/10	0/10	0/10	1/10	1/10	5/10
	0.0	0.0	0.0	1.0	1.0	1.6
Ulcer	0/10	0/10	0/10	0/10	0/10	1/10
	0.0	0.0	0.0	0.0	0.0	1.0
Hyperplaisia, Epithelial	0/10	9/10	8/10	9/10	9/10	10/10
	0.0	1.0	1.0	1.1	1.6	2.2

Female Mice

mg/kg	0.0	1.56	3.12	6.25	12.5	25.0
Inflammation, Chronic Dermis	1/10*	6/10	8/10	8/10	10/10	10/10
	1.0**	1.0	1.0	1.1	1.6	1.9
Necrosis	0/10	0/10	0/10	0/10	0/10	2/10
	0.0	0.0	0.0	0.0	0.0	1.0
Ulcer	0/10	0/10	0/10	0/10	0/10	0/10
	0.0	0.0	0.0	0.0	0.0	0.0
Hyperplaisia, Epithelial	0/10	9/10	10/10	10/10	10/10	10/10
	0.0	1.0	1.0	1.0	1.5	2.0

* No. affected/No. in group
** Average degree of severity
0-Within Normal Limits
1-Minimal
2-Mild
3-Moderate
4-Marked

INDIVIDUAL ANIMAL TABLE

MALE MICE

					Control					
Animal *	1	2	3	4	5	6	7	8	9	10
Inflammation, Chronic, Dermis	0	0	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelial	0	0	0	0	0	0	0	0	0	0
					1.56 mg/kg					
Animal *	11	12	13	14	15	16	17	18	19	20
Inflammation, Chronic, Dermis	0	0	1	0	0	1	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelial	1	0	1	1	1	1	1	1	1	1
					3.12 mg/kg					
Animal *	21	22	23	24	25	26	27	28	29	30
Inflammation, Chronic, Dermis	0	1	1	1	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelial	1	1	1	1	1	0	1	1	0	1

* Average degree of severity
0-Within Normal Limits
1-Minimal
2-Mild
3-Moderate
4-Marked

	6.25 mg/kg									
Animal *	31	32	33	34	35	36	37	38	39	40
Inflammation, Chronic, Dermis	1	0	1	1	1	0	1	1	0	0
Necrosis	0	0	0	1	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelial	1	1	1	1	2	0	1	1	1	1

	12.5 mg/kg									
Animal *	41	42	43	44	45	46	47	48	49	50
Inflammation, Chronic, Dermis	1	2	1	1	0	1	1	1	1	2
Necrosis	0	0	0	0	0	0	0	1	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelial	2	2	1	1	0	1	1	2	2	2

	25 mg/kg									
Animal *	51	52	53	54	55	56	57	58	59	60
Inflammation, Chronic, Dermis	2	2	2	2	2	2	2	3	1	2
Necrosis	0	0	2	2	0	2	0	1	0	1
Ulcer	0	0	0	0	1	0	0	0	0	0
Hyperplasia, Epithelial	2	3	2	3	2	2	2	2	2	2

* Average degree of severity
0-Within Normal Limits
1-Minimal
2-Mild
3-Moderate
4-Marked

FEMALE MICE

Control

Animal #	61	62	63	64	65	66	67	68	69	70
Inflammation, Chronic, Dermis	0	0	0	0	0	0	0	0	0	1
Necrosis	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelial	0	0	0	0	0	0	0	0	0	0

1.56 mg/kg

Animal #	71	72	73	74	75	76	77	78	79	80
Inflammation, Chronic, Dermis	1	1	0	0	0	1	1	1	1	0
Necrosis	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelial	1	1	0	1	1	1	1	1	1	1

3.12 mg/kg

Animal #	81	82	83	84	85	86	87	88	89	90
Inflammation, Chronic, Dermis	1	0	1	1	1	1	1	0	1	1
Necrosis	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelial	1	1	1	1	1	1	1	1	1	1

* Average degree of severity
  0-Within Normal Limits
  1-Minimal
  2-Mild
  3-Moderate
  4-Marked

	6.25 mg/kg									
Animal #	91	92	93	94	95	96	97	98	99	100
Inflammation, Chronic, Dermis	1	1	0	1	1	1	0	1	1	2
Necrosis	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelial	1	1	1	1	1	1	1	1	1	1

	12.5 mg/kg									
Animal #	101	102	103	104	105	106	107	108	109	110
Inflammation, Chronic, Dermis	1	2	2	2	1	2	2	2	1	1
Necrosis	0	0	0	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelial	1	2	2	1	1	2	2	2	1	1

	25 mg/kg									
Animal #	111	112	113	114	115	116	117	118	119	120
Inflammation, Chronic, Dermis	2	2	2	1	2	1	2	2	2	3
Necrosis	0	0	0	0	0	0	0	0	1	1
Ulcer	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelial	3	2	3	1	2	1	2	2	2	2

* Average degree of severity
  0-Within Normal Limits
  1-Minimal
  2-Mild
  3-Moderate
  4-Marked

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute of
Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, N.C. 27709

May 2, 1994

Mr. Elliot Harrison
Delta Analytical Corporation
7910 Woodmont Ave., Suite 1000
Bethesda, MD 20814

Dear Mr. Harrison,

Enclosed are the NTP/NIEHS mutagenicity data on Benzethonium chloride (CAS # 121-54-0). We have tested this chemical as a coded sample in the Salmonella and in vitro cytogenetics assays. The names of the test laboratories and results appear on the data forms. I have enclosed code sheets and summary protocols for each test system.

The in vitro cytogenetics data have not been published. I request that you not publish these data or include them in any formal report. You may reference the conclusion for these tests as "NTP unpublished results." The references for the published data are as follows:

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., and Speck, W. (1987) Salmonella mutagenicity tests. III. Results from the testing of 225 chemicals. Environ. Mutagen. 9(Suppl 9): 1-109.

I hope that this information is of use to you.

Sincerely,

Errol Zeiger, Ph.D., J.D.
Head, Chemical Selection and
Information Management Office
Environmental Toxicology Program

SALMONELLA PROTOCOL

All chemicals are tested and evaluated as unknowns for their ability to induce gene mutations in Salmonella typhimurium. A detailed protocol of the test system is presented in Haworth et al., Environ. Mutagen. 5(Suppl. 1): 3-142, 1983. Chemicals tested most recently have been tested in this system with minor modifications as indicated below. A preincubation modification of the Salmonella test is used; the test chemical is incubated with the tester strain either in buffer or S9 plus cofactor mix, for 20 minutes at 37°C prior to the addition of soft agar and plating on minimal agar plates. All chemicals are tested both in the absence of metabolic activation and with exogenous metabolic activation (S9) from Aroclor 1254-induced Sprague-Dawley rats and Syrian hamsters, in Salmonella strains TA98, TA100, TA1535, TA1537 and/or TA97. Testing is done either using a series of 4 strains or using a hierarchy of strains. When tested in series all negatives are repeated; all positives are repeated for conditions that elicited the positive response. When tested as a hierarchy, chemicals are tested initially in TA100 and TA98 and repeated if positive. If negative, the chemical is then tested in 2 or 3 of the other strains. If still negative, all strains are retested with a change in the S9 concentration. Each test consists of triplicate plating of concurrent positive and solvent controls and of at least 5 doses of test chemical; the high dose is limited by toxicity or solubility, but not exceeding 10 mg/plate. A positive response is defined as a reproducible, dose related increase in histidine-independent (revertant) colonies. An equivocal response (?) is either a non-dose-related increase or a response that is not reproducible. A chemical is judged positive if a reproducible positive response is observed in any strain/activation combination.

CELLULAR AND GENETIC TOXICOLOGY BRANCH, NTP
SALMONELLA TESTING RESULTS

CAS #: 121-54-0 Benzethonium chloride
ALIQUOT: 397650 LAB: CASE WESTERN RESERVE UNIVERSITY
MUTAGENICITY CONCLUSION: -

TA100 SOLVENT: H2O PROTOCOL: PREINCUBATION

DOSE	1 NA (-)		2 NA (-)		1 10% HLI (-)		2 10% HLI (-)		1 10% RLI (-)		2 10% RLI (-)	
ug/PLATE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
0.000	90	1.8	101	9.0	109	7.1	152	5.2	128	9.3	142	4.7
0.010	87	0.3	92	3.8								
0.030	85	4.2	107	0.3								
0.100	82	5.2	103	5.3								
0.300	94	4.7	99	4.3								
1.000	92	5.0	66	1.7	105	1.5	130	5.9	109	7.4	122	8.0
3.300					100	3.2	133	16.3	121	6.1	132	10.1
10.000					94	2.3	140	14.2	118	13.0	144	6.9
33.000					84	4.1	144	8.8	123	8.7	141	1.3
100.000					34	3.8	143	15.2	128	6.0	t	
POS	936	11.1	1024	18.1	1636	125.5	1021	63.2	1264	205.4	2105	62.1

TA1535 SOLVENT: H2O PROTOCOL: PREINCUBATION

DOSE	1 NA (-)		2 NA (-)		1 10% HLI (-)		2 10% HLI (-)		1 10% RLI (-)		2 10% RLI (-)	
ug/PLATE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
0.000	18	1.2	4	1.5	17	1.5	6	0.0	25	1.5	7	1.7
0.010	14	1.9	4	0.7								
0.030	16	3.7	4	1.2								
0.100	14	3.2	5	0.7								
0.300	8	1.5	2	1.2								
1.000	8	0.6	2	0.6	18	3.3	4	1.8	16	0.9	5	1.9
3.300					26	1.3	5	1.5	20	2.0	6	2.5
10.000					15	4.2	3	0.9	17	1.5	5	1.7
33.000					9	0.9	4	0.7	8	1.5	4	0.7
100.000					t		t		t		t	
POS	942	36.6	446	18.0	125	10.7	97	15.5	184	15.0	60	8.4

TA1537 SOLVENT: H2O PROTOCOL: PREINCUBATION

DOSE	1 NA (-)		2 NA (-)		1 10% HLI (-)		2 10% HLI (-)		1 10% RLI (-)		2 10% RLI (-)	
ug/PLATE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
0.000	12	1.5	10	1.2	24	2.4	15	1.5	19	0.3	12	1.5
0.010	9	0.6	13	0.7								
0.030	10	2.8	9	1.5								
0.100	14	1.0	7	0.9								
0.300	16	0.7	7	2.3								
1.000	11	3.8	7	1.2	22	2.4	13	1.0	23	3.0	12	1.5
3.300					26	4.3	10	2.1	20	3.4	15	1.7
10.000					14	0.6	9	2.4	13	2.0	7	2.1
33.000					13	1.2	15	2.6	13	2.5	12	1.7
100.000					1	0.7	13	2.5	t		t	
POS	207	94.2	177	104.5	356	21.3	197	15.3	432	29.1	122	43.3

TA98 SOLVENT: H2O PROTOCOL: PREINCUBATION

DOSE	1 NA (-)		2 NA (-)		1 10% HLI (-)		2 10% HLI (-)		1 10% RLI (-)		2 10% RLI (-)	
ug/PLATE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
0.000	20	1.8	14	4.1	19	2.5	28	3.2	22	3.0	21	0.9
0.010	21	0.7	7	1.2								
0.030	18	1.8	6	0.7								
0.100	19	2.1	7	0.3								
0.300	18	0.9	10	1.8								
1.000	22	1.5	10	4.2	15	1.9	28	4.1	21	2.0	17	1.9
3.300					16	2.9	20	0.7	24	3.2	20	2.4
10.000					16	1.7	20	1.3	24	1.5	16	1.8
33.000					10	2.1	19	2.9	16	0.7	24	1.2
100.000					7	1.2	21	0.9	18	0.7	18	3.5
POS	180	63.8	123	9.0	1152	40.1	719	45.8	872	70.4	1150	21.3

END OF ALIQUOT

IN VITRO CYTOGENETICS PROTOCOL SUMMARY

Chemicals are tested as unknowns for their ability to induce sister chromatid exchanges (SCEs) and chromosome aberrations in Chinese hamster ovary cells. A detailed protocol is presented in Galloway et al., Environ Mutagen. 7:1-51, (1985). Chemicals are tested for both endpoints with and without metabolic activation. The metabolic activation (S9) is derived from the livers of Aroclor 1254-induced male Sprague-Dawley rats. Each test consists of concurrent solvent and positive controls and of at least 3 doses of test chemical; the high dose is limited by toxicity or solublity, but does not exceed 5 mg/ml. The data are statistically analyzed for both trend and peak response. A positive response requires that 2 doses produce a significant effect (SCEs: 20% over control; aberrations: $P<0.01$); if only one dose or only the trend test is significant, the response is considered weakly positive or equivocal, respectively.

In the SCE assay without S9, the cells are treated with the chemical for 26 hours in McCoy's 5A medium; two hours after the chemical treatment begins bromodeoxyuridine (BU) is added to the culture. After 26 hours the treated medium is removed and replaced with medium containing BU and Colcemid and incubated for 2 hours. Cells are then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. If insufficient cells are obtained for scoring, later harvest times are used. In the assay with S9, the cells are incubated with the chemical, serum-free medium, and S9. After 2 hours, the treatment is removed and replaced with BU containing medium for 26 hours. Colcemid is present for the final 2 hours. The same procedure is followed for harvesting and staining as for treatment without S9.

In the chromosome aberration assay without S9, the test chemical is incubated with the cells in McCoy's 5A medium for 8 hours followed by a 2 hour incubation with colcemid. The cells are then harvested, fixed and stained with Giemsa. For the assay with S9, the cells are treated with chemical and S9 for 2 hours. The treatment mixture is removed and the cells are then incubated for 10 hours, with colcemid present for the last 2 hours. The cells are harvested in the same manner as for the treatment without S9. If significant cell cycle delay is seen in either procedure, the cells can be incubated longer prior to addition of colcemid.

CAS #: 121-54-0 CHEMICAL NAME: Benzethonium chloride
ALIQUOT #: 173525 LABORATORY: Columbia University

PUBLICATION:

CHROMOSOME ABERRATIONS (ABS)
CONCLUSION: -

TRIAL #: 1 ACTIVATION: NA DATE: 08/31/83 HARVEST TIME: 14.0 PROGRAM CALL: - STAT CALL: - LAB CALL: (-)

			TOTAL ABERRATIONS			COMPLEX ABERRATIONS			SIMPLE ABERRATIONS			OTHER ABS.	
	DOSE UG/ML	TOTAL CELLS	NO OF ABS	ABS PER CELL	%CELLS WITH ABS	NO OF ABS	ABS PER CELL	%CELLS WITH ABS	NO OF ABS	ABS PER CELL	%CELLS WITH ABS	NO OF ABS	%CELLS WITH ABS
SOL: H2O		100	4	0.04	4.0	1	0.01	1.0	3	0.03	3.0	0	0.0
TEST	0.9600	100	11	0.11	10.0	1	0.01	1.0	10	0.10	9.0	0	0.0
CONC:	3.0000	100	10	0.10	10.0	0	0.00	0.0	9	0.09	9.0	1	0.0
	9.6000	100	8	0.08	8.0	3	0.03	3.0	5	0.05	5.0	0	0.0
POS: MMC	0.1500	50	34	0.68	42.0	7	0.14	12.0	27	0.54	36.0	0	0.0

	Total ABS	Complex ABS	Simple ABS
TREND:	0.986684	1.012527	0.540100
PROBABILITY:	0.161899	0.155643	0.294564

REMARKS: CONTROL ALSO FOR 113918.

TRIAL #: 1 ACTIVATION: RLI DATE: 08/31/83 HARVEST TIME: 14.0 PROGRAM CALL: - STAT CALL: - LAB CALL: (-)

			TOTAL ABERRATIONS			COMPLEX ABERRATIONS			SIMPLE ABERRATIONS			OTHER ABS.	
	DOSE UG/ML	TOTAL CELLS	NO OF ABS	ABS PER CELL	%CELLS WITH ABS	NO OF ABS	ABS PER CELL	%CELLS WITH ABS	NO OF ABS	ABS PER CELL	%CELLS WITH ABS	NO OF ABS	%CELLS WITH ABS
SOL: H2O		100	3	0.03	3.0	0	0.00	0.0	3	0.03	3.0	0	0.0
TEST	3.0000	100	5	0.05	5.0	1	0.01	1.0	4	0.04	4.0	0	0.0
CONC:	9.6000	100	6	0.06	5.0	1	0.01	1.0	5	0.05	4.0	0	0.0
	30.0000	100	6	0.06	6.0	0	0.00	0.0	6	0.06	6.0	0	0.0
POS: CPA	15.0000	50	16	0.32	28.0	4	0.08	8.0	12	0.24	22.0	0	0.0

	Total ABS	Complex ABS	Simple ABS
TREND:	0.945685	0.003262	0.996012
PROBABILITY:	0.172155	0.498699	0.159622

REMARKS: CONTROL ALSO FOR 113918.

IN VITRO CYTOGENETICS RESULTS
CHINESE HAMSTER OVARY CELLS

CAS #: 121-54-0 CHEMICAL NAME: Benzethonium chloride
ALIQUOT #: 173525 LABORATORY: Columbia University

PUBLICATION:

SISTER CHROMATID EXCHANGES (SCE)
CONCLUSION: -

TRIAL #: 1 ACTIVATION: NA DATE: 08/31/81 PROGRAM CALL: - STAT CALL: - LAB CALL:

	DOSE UG/ML	TOTAL CELLS	NO. OF CHROMO	NO. OF SCES	SCE / CHROMO	SCE / CELL	HRS IN BRDU	% INCR OVER SOL
SOL: H2O		50	1047	434	0.41	8.68	26.0	
TEST	0.9600	50	1049	458	0.44	9.16	26.0	5.33
CONC:	3.0000	50	1050	457	0.44	9.14	26.0	5.00
	9.6000	50	1048	473	0.45	9.46	26.0	8.88
POS: MMC	0.0050	25	523	639	1.22	25.56	26.0	194.76

TREND: 1.198638
PROBABILITY: 0.115334

REMARKS: CONTROL ALSO FOR 113918.

TRIAL #: 1 ACTIVATION: RLI DATE: 08/31/83 PROGRAM CALL: - STAT CALL: - LAB CALL:

	DOSE UG/ML	TOTAL CELLS	NO. OF CHROMO	NO. OF SCES	SCE / CHROMO	SCE / CELL	HRS IN BRDU	% INCR OVER SOL
SOL: H2O		50	1043	369	0.35	7.38	26.0	
TEST	3.0000	50	1048	347	0.33	6.94	26.0	-6.41
CONC:	9.6000	50	1050	359	0.34	7.18	26.0	-3.36
	30.0000	50	1047	367	0.35	7.34	26.0	-0.92
POS: CPA	1.0000	50	1045	790	0.76	15.80	26.0	113.69

TREND: 0.013273
PROBABILITY: 0.494705

REMARKS: CONTROL ALSO FOR 113918.