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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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JUN 29 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

069/61

SUBJECT: Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC)

TO: John Lee PM-31
Registration Division (H7505C)

6/15/89

FROM: Robert P. Zendzian Ph.D.,
Acting Head, Rev Sec I
Toxicology Branch I
Health effects Division (H7509C)

THROUGH: Edwin Budd
Acting Chief
Toxicology Branch I

*Budd
6/22/89*

Compound; ADBAC

Tox Chem #016

Registration #069105

Registrant; CSMA/ADBAC Quat Joint Venture

MRID # 407466-01

Tox Project #9-0901

Action Requested

Review the following study submitted in reply to a Registration Standard;

Ninety-day dietary toxicity study with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in rats, J.P. Van Miller & E.V. Weaver; Union Carbide, Bushy Run Research Center; Lab Project ID # 51-503; June 20, 1988; MRID # 407466-01.

Conclusion

Core Classification Minimum

Sprague-Dawley rats were dosed with ADBAC at 0, 100, 500, 1000, 4000 and 8000 ppm in the diet. Compound was lethal at doses of 4000 and 8000 ppm. At 1000 ppm decreased body weight gain with no effect on food consumption was observed in the males. No other toxic effects were observed. LEL=1000 ppm, NOEL=500 ppm.

Attachment

DER

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA No.: 68D80056
DYNAMAC No.: 164-B
TASK No.: 1-64B
May 25, 1989

DATA EVALUATION RECORD

ALKYL DIMETHYL
BENZYL AMMONIUM CHLORIDE

Subchronic Toxicity Feeding Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: *Robert J. Weir*

Date: May 25, 1989

EPA No.: 68D80056
DYNAMAC No.: 164-A
TASK No.: 1-64A
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DATA EVALUATION RECORD

ALKYL DIMETHYL
BENZYL AMMONIUM CHLORIDE

Subchronic Toxicity Feeding Study in Rats

REVIEWED BY:

William L. Richards, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. Richards

Date: May 25, 1989

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower

Date: May 25, 1989

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta

Date: May 25, 1989

William Burnam, M.S.
EPA Reviewer and
Acting Chief,
Herbicide/Fungicide/
Antimicrobial Support
Toxicology Branch II (H-7509C)

Signature: William Burnam

Date: 5/31/89

DATA EVALUATION RECORD

STUDY TYPE: Subchronic toxicity feeding
study in rats.

GUIDELINE §82-1

MRID NUMBER: 407466-01.

TEST MATERIAL: Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC).

SYNONYM(S): Benzalkonium chloride, Zephiran chloride, Zephirol,
BTC, Roccal, Benirol, Enuclen, Germitol, Drapolene, Drapolex,
Cequartyl, Paralkan, Germinol, Rodalon, Osvan.

STUDY NUMBER(S): Laboratory Project ID 51-503.

SPONSOR: ADBAC QUAT Joint Venture/Chemical Specialties
Manufacturers Association, Washington, D.C.

TESTING FACILITY: Bushy Run Research Center, Export, PA.

TITLE OF REPORT: Ninety-Day Dietary Toxicity Study with Alkyl
Dimethyl Benzyl Ammonium Chloride (ADBAC) in Rats.

AUTHOR(S): J. P. Van Miller and E. V. Weaver.

REPORT ISSUED: June 20, 1988.

CONCLUSIONS:

When ADBAC was fed to Sprague-Dawley rats for up to 95 (males) or 96 (females) days at dietary concentrations of 0, 100, 500, 1000, 4000, or 8000 ppm, evidence of severe toxicity was observed in rats of both sexes in the 4000- and 8000-ppm groups. All of the rats in the 8000-ppm group died from day 4 to day 8 of the study; in the 4000-ppm groups, 12/15 males died from day 7 to day 19 and 11/15 females died from day 7 to day 11. Other compound-related findings in the 4000- and 8000-ppm groups included cachexia (emaciation, body thinness), loose feces, decreased body weight, and body weight gain, decreased food consumption, decreased organ weights in males (liver, kidneys, spleen, and heart), gross lesions (intestinal ileus consisting of distended fluid- and gas-filled viscera extending from the stomach to the cecum, perineal staining, decreased spleen size, brain hemorrhage, and color change in the lungs), and nonneoplastic histologic lesions [stomach congestion and edema, stomach hemorrhage (only in males), congestion of the small intestine and cecum; mucosal cell degeneration in the duodenum, jejunum (only in males), ileum (only in males), and cecum (only in males), congestion and hepatocellular atrophy in the liver, contracted spleen, brain congestion, and congestion and hemorrhage of the lungs]. Except for a slightly earlier time of death in females in the 8000-ppm group than in males in that group, signs and symptoms of compound-related toxicity, especially nonneoplastic histologic lesions, tended to be more marked in males than females in the 4000- and 8000-ppm groups. Gross pathologic findings and microscopic lesions supported ileus, hypovolemic shock, hemorrhage of the brain and lungs, and, perhaps, brain and liver congestion as the probable cause of death. Changes in serum glucose and phosphorus levels were in the normal range of variation for this rat strain. Slight elevations in SGPT and SGOT activities may have been related to stress. At the lower dietary concentrations (1000, 500, and 100 ppm), the only clearly compound-related findings were decreased body weight and decreased body weight gain, with no effect on food consumption, in males of the 1000-ppm group during part of the period of compound administration. No treatment-related changes were observed in any hematology measurement or in the ophthalmologic examination. Based on the effects of ADBAC on body weight at 1000 ppm, the Lowest-Observed-Effect Level (LOEL) is 1000 ppm, and the No-Observed-Effect Level (NOEL) is 500 ppm.

Classification: Core Minimum (see Reviewers' Discussion and Interpretation of Results).

A. MATERIALS:

1. Test Compound: Alkyl dimethyl benzyl ammonium chloride; description: pale yellow viscous liquid, batch No. 6158-59-60 (BRRC No. 50-268), weeks 1-4; lot No. SC 132-65 (BRRC No. 50-328), week 5-termination; purity: 80.51% and 79.7%, respectively. *Composite Sample. 40% C-12, 50% C-14, 10% C*
2. Test Animals: Species: rats; strain: Sprague-Dawley CD[®] *By 6/1*
age: 32 days at study initiation; weight: males--221.6 g to 250.9 g, females--147.7 g to 174.4 g; source: Charles River Breeding Laboratories, Inc., Portage, MI.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated to laboratory conditions for 3 weeks, identified by toe-clipping and ear-notching, and assigned randomly by a weight-stratified procedure to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study (13 weeks)	
		Males	Females
1 Control	0	15	15
2 Low	100	15	15
3 Mid-1	500	15	15
4 Mid-2	1000	15	15
5 Mid-3	4000	15	15
6 High	8000	15	15

The study authors stated that at terminal sacrifice, errors in toe clipping, relative to ear notching, were found in the control group (1 male, 3 females), 100 ppm group (2 males, 1 female), 1000 ppm group (1 female), and 8000 ppm group (1 female).

Rats were individually housed in a single room controlled for temperature (22 ± 3°C), humidity (40-70%), and light (12 hours/day).

Additional rats that were received with the test groups (10/sex, randomly selected) received a pretest health screen consisting of clinical laboratory studies, a viral screen, gross necropsy, examinations for fecal parasites, and histopathological examinations of the liver, kidneys, trachea, lungs, heart, spleen, salivary glands, submandibular lymph nodes, and nasal cavities.

2. Diet Preparation: Dietary mixtures of the test substance at concentrations of 0, 100, 500, 1000, 4000, and 8000 ppm were prepared weekly in Ground Purina Certified Rodent Chow #5002. The time between preparation and initiation of feeding was not indicated. Test compound in the diets was analyzed weekly; for the first 5 weeks, analyses were done prior to the administration of the diets to the animals. Homogeneity was determined for all test concentrations, and stability was determined on low- and high-dose diets prior to study initiation. Test material of lot number 6158-59-60, BRRC No. 50-268, 80.51% active ingredient, was used for study weeks 1-4; lot No. SC 132-65, BRRC No. 50-328, 79.7% active ingredient, was used for study weeks 5-14. All dietary concentrations of both materials were corrected for percentage of active ingredient.

Results: As shown in Table 1, all diets were within 12% of target and ranged from a low of 90.7% (1000 ppm, study weeks 9 and 11) to a high of 111.8 ppm (500 ppm, study week 10). The diets were homogeneous; standard deviations from the nominal concentration were 5.0% for the 100-ppm diet, 2.6% for the 500-, 1000-, and 4000-ppm diets, and 2.9% for the 8000-ppm diet. The test compound was stable in the diet. After storage of low- and high-concentration diets for 21 days at room temperature (not specified), 100 and 101.4% of the nominal concentration were recovered from low- and high-concentration diets, respectively.

3. Food and Water Consumption: Animals received food (Ground Purina Certified Rodent Chow #5002) and water ad libitum.
4. Statistics: The following procedures were utilized in analyzing the numerical data: Data for continuous, parametric variables were intercompared for dose and control groups by Levene's test for homogeneity of variances, by analysis of variance, and by pooled variance t-tests. Nonparametric data were analyzed by the Kruskal-Wallis test or by the Wilcoxon rank sum test as modified by Mann-Whitney. Frequency data were compared using Fisher's exact test.

TABLE 1. Analysis of Alkyl Dimethyl Benzyl Ammonium Chloride in Test Diets

Week		Target Concentration (ppm)				
		100	500	1000	4000	8000 ^a
1	Concentration (ppm)	96	494	1026	4245	8420
	Percent of target	96.0	98.7	102.6	106.1	105.2
2	Concentration (ppm)	104	492	913	4078	8096
	Percent of target	104.0	98.4	91.3	102.0	101.2
3	Concentration (ppm)	100	524	1048	4352	8438
	Percent of target	100.0	104.9	104.8	108.8	105.5
4	Concentration (ppm)	97	506	1045	3837	
	Percent of target	97.0	101.2	104.5	95.9	
5	Concentration (ppm)	96	496	964	3808	
	Percent of target	96.0	99.2	96.4	95.2	
6	Concentration (ppm)	98	498	1000	4310	
	Percent of target	98.0	99.6	100.0	107.7	
7	Concentration (ppm)	104	516	1022	4231	
	Percent of target	104.0	103.3	102.2	105.8	
8	Concentration (ppm)	97	486	986	4402	
	Percent of target	97.0	97.3	98.6	110.0	
9	Concentration (ppm)	100	551	907	4028	
	Percent of target	100.0	110.0	90.7	100.7	
10	Concentration (ppm)	100	559	962	4272	
	Percent of target	100.0	111.8	96.2	106.8	
11	Concentration (ppm)	103	526	907	4274	
	Percent of target	103.0	105.0	90.7	106.9	
12	Concentration (ppm)	100	538	940	4138	
	Percent of target	100.0	107.5	94.0	103.4	
13	Concentration (ppm)	103	519	958	4283	
	Percent of target	103.0	103.8	95.8	107.1	
14	Concentration (ppm)	103	525	1070	4100	
	Percent of target	103.0	104.9	107.0	102.5	

^aAll animals from the 8000-ppm dose group died prior to week 4 analyses.

5. Quality Assurance: A quality assurance statement was signed and dated June 17, 1988.

C. METHODS AND RESULTS:

1. Observations: Observations for mortality and overt clinical signs were made twice daily. Detailed clinical examinations (not described) were performed weekly.

Results: Treatment-related clinical findings, which were restricted to the males and females of the 4000- and 8000-ppm groups, included general cachexia (e.g., emaciation, unkempt appearance) and loose feces. As shown in Table 2, mortality was elevated in the 4000- and 8000-ppm groups of males and females. All of the rats in the 8000-ppm group died from day 4 to day 8 of the study. Twelve of the 15 male rats from the 4000-ppm group were found dead or sacrificed in a moribund condition from day 7 to day 19, and 11/15 females from this group were found dead or were sacrificed in a moribund condition from day 7 to day 11. All of these deaths were attributed to treatment with the test compound. Clinical findings for animals that survived to termination of the study in the 4000-ppm group were similar to those for the animals that died.

A pretest health screen of 10 rats/sex that were received with the test groups indicated that the animals were free of infectious diseases and parasites.

2. Body Weight: Rats were weighed pretest (time not indicated) and once weekly on test.

Results: Table 3 presents representative data on mean body weights of males and females. Body weights of males of the 8000-ppm group were significantly lower ($p < 0.01$) than controls at week 1. Body weights of males of the 4000-ppm group were significantly ($p < 0.01$) lower than controls throughout the period of compound administration. Although mean body weights of males of the 1000-, 500-, and 100-ppm groups were slightly less than those of controls throughout the period of compound administration, and the difference in mean body weight was related to the dose level, differences from controls were significant ($p < 0.05$) only during weeks 2 and 3 in males of the 1000-ppm group.

TABLE 2. Cumulative Mortality and Percent Survival in Rats Fed Alkyl Dimethyl Benzyl Ammonium Chloride for 13 Weeks^a

Dose Group (ppm)	<u>Mortality (Percent Survival) at End of Week</u>			
	1	3	7	13
<u>Males</u>				
0	0(100)	0(100)	0(100)	0(100)
100	0(100)	0(100)	0(100)	0(100)
500	0(100)	0(100)	0(100)	0(100)
1000	0(100)	0(100)	0(100)	0(100)
4000	1 (85)	12 (20)	12 (20)	12 (20)
8000	9 (40)	15 (0)	15 (0)	15 (0)
<u>Females</u>				
0	0(100)	0(100)	0(100)	0(100)
100	0(100)	0(100)	0(100)	0(100)
500	0(100)	0(100)	0(100)	0(100)
1000	0(100)	0(100)	0(100)	0(100)
4000	4 (73)	11 (27)	11 (27)	11 (27)
8000	15 (0)	15 (0)	15 (0)	15 (0)

^aPercent survival was based on 15 rats/sex/dose.

TABLE 3. Representative Results of Mean Body Weights of Rats Fed Alkyl Dimethyl Ammonium Chloride for 13 Weeks

Dose Group (ppm)	Mean Body Weights (g ± S.D.) at Week				
	1	3	5	9	13
	<u>Males</u>				
0	287.3 ± 12.86	372.6 ± 23.87	422.7 ± 33.38	494.5 ± 41.15	536.6 ± 46.32
100	284.0 ± 7.39	362.6 ± 13.02	409.7 ± 17.58	478.8 ± 25.15	519.4 ± 31.41
500	281.2 ± 10.63	359.6 ± 15.51	407.6 ± 19.91	478.8 ± 21.10	517.9 ± 26.66
1000	282.2 ± 9.93	354.7 ± 16.90*	403.8 ± 21.12	474.8 ± 26.67	515.1 ± 35.62
4000	188.2 ± 8.88**	212.8 ± 10.41**	273.4 ± 10.44**	342.5 ± 13.73**	380.5 ± 20.92**
8000	159.5 ± 4.97**	-- ^a	--	--	--
	<u>Females</u>				
0	176.2 ± 7.53	207.3 ± 11.52	224.7 ± 14.12	256.7 ± 14.11	275.7 ± 15.29
100	177.7 ± 10.0	209.7 ± 13.99	226.6 ± 17.67	260.1 ± 20.96	278.1 ± 25.29
500	177.1 ± 7.08	206.7 ± 13.85	227.8 ± 13.69	258.4 ± 19.41	277.4 ± 22.20
1000	179.2 ± 7.11	209.9 ± 13.06	231.2 ± 13.42	257.4 ± 18.09	274.6 ± 22.91
4000	131.8 ± 9.26**	181.2 ± 7.54**	214.7 ± 16.85	230.7 ± 28.98	243.9 ± 19.50
8000	-- ^a	--	--	--	--

^aAll high-dose rats died from day 4 to day 8 of the study.

*Significantly different from control group (p<0.05).

**Significantly different from control group (p<0.01)

In females of the 4000-ppm group, body weights were significantly ($p < 0.01$) lower than controls during study weeks 1, 2, 3, and 4 (data for weeks 2, 3, and 4 not shown). Mean body weights of females of the 1000-, 500-, and 100-ppm groups were similar to controls.

Table 4 presents representative data on mean body weight gain in males and females. Mean body weight gain in males of the 8000-ppm group was significantly ($p < 0.01$) lower than controls during week 0 to 1. Mean body weight gain in males of the 4000-ppm group was significantly ($p < 0.01$) lower than controls throughout the period of compound administration. In the 1000-ppm group of males, mean body weight gain was significantly lower than controls for more than half the test intervals (weeks 0 to 2 and 0 to 3, $p < 0.01$; weeks 0 to 1, 0 to 4, 0 to 5, 0 to 6, and 0 to 10, $p < 0.05$). Although mean body weight gains of males of the 500- and 100-ppm groups were slightly less than those of controls throughout the period of compound administration, and the decrement in mean body weight gain was related to the dose level, differences from controls were significant ($p < 0.05$) only during weeks 0 to 2 and 0 to 3 in males of the 500-ppm group.

In females of the 4000-ppm group, mean body weight gains were significantly ($p < 0.01$) lower than controls during all test intervals but two (weeks 0 to 6 and 0 to 7). Mean body weight gains of females of the 1000-, 500-, and 100-ppm groups were similar to controls.

Compared to controls, absolute loss of body weight was found in males of the 8000-ppm group during week 0 to 1; males of the 4000-ppm group during weeks 0 to 1, 0 to 2, and 0 to 3; and females of the 4000-ppm group during weeks 0 to 1 and 0 to 2.

3. Food Consumption and Compound Intake: Food consumption was determined at the same intervals as the weighings. Water consumption was not determined. Mean daily diet consumption and compound intake were calculated. Data were not included for animals with observed food spillage.

Results: Table 5 presents representative data on food consumption and compound consumption. Statistically significant reductions in food consumption compared to controls were observed for males of the 8000-ppm group (week 0 to 1, $p < 0.01$), the 4000-ppm group (weeks 0 to 1 and 2 to 3, $p < 0.01$; weeks 1 to 2, $p < 0.05$), and the 1000-ppm group (weeks 1 to 2, $p < 0.05$; weeks 2 to 3, $p < 0.01$). Statistically significant ($p < 0.01$) reductions in food consumption compared to controls were observed for

TABLE 4. Representative Results of Mean Body Weight Gain of Rats Fed Alkyl Dimethyl Benzyl Ammonium Chloride for 13 Weeks

Dose Group (ppm)	Mean Body Weight Gain (g ± S.D.) at Weeks			
	0-1	0-6	0-10	0-13
	<u>Males</u>			
0	49.3 ± 5.37	201.0 ± 23.92	271.3 ± 34.53	298.6 ± 39.88
100	46.9 ± 3.56	190.6 ± 18.38	258.0 ± 26.89	282.2 ± 29.13
500	45.2 ± 7.16	190.7 ± 18.30	253.0 ± 19.97	281.8 ± 22.40
1000	45.5 ± 4.72*	186.2 ± 19.13*	251.2 ± 27.49*	278.5 ± 32.90
4000	-46.6 ± 8.76**	79.9 ± 2.69**	128.7 ± 19.30**	153.8 ± 16.50**
8000	-79.0 ± 3.80**	-- ^a	--	--
	<u>Females</u>			
0	18.7 ± 4.00	77.2 ± 13.07	104.7 ± 10.81	118.2 ± 12.03
100	18.1 ± 5.03	77.0 ± 13.36	107.8 ± 19.78	118.5 ± 20.47
500	18.2 ± 3.63	77.0 ± 14.66	105.2 ± 17.43	118.5 ± 21.15
1000	19.4 ± 3.23	70.4 ± 21.68	103.0 ± 12.59	114.9 ± 17.58
4000	-29.0 ± 6.76**	60.7 ± 6.74	72.4 ± 21.80**	78.9 ± 10.31**
8000	-- ^a	--	--	--

^aAll high-dose rats died from day 4 to day 8 of the study.

*Significantly different from control group (p<0.05).

**Significantly different from control group (p<0.01).

TABLE 5. Representative Food and Compound Consumption of Rats Fed Alkyl Dimethyl Benzyl Ammonium Chloride for 13 Weeks

Dose Group (ppm)	Mean Food Consumption (g/day \pm S.D.) at Weeks				Mean Compound Consumption (mg/kg body weight/day)
	0-1	4-5	8-9	12-13	
	<u>Males</u>				
0	24.9 \pm 1.79	27.0 \pm 2.92	26.3 \pm 2.09	24.6 \pm 2.36	-- ^a
100	24.6 \pm 1.30	26.4 \pm 1.63	25.4 \pm 1.56	24.0 \pm 2.05	6.29
500	24.1 \pm 1.65	26.2 \pm 2.01	25.3 \pm 1.66	23.6 \pm 1.02	31.23
1000	24.2 \pm 1.55	25.7 \pm 1.79	24.9 \pm 1.94	23.1 \pm 1.91	61.95
4000	6.7 \pm 1.21 ^{**}	23.2 \pm 3.40	-- ^b	23.2 \pm 0.00	-- ^c
8000	2.1 \pm 0.57 ^{**}	-- ^b	-- ^b	-- ^b	-- ^c
<u>Females</u>					
0	16.6 \pm 1.58	18.3 \pm 2.05	18.3 \pm 1.64	17.4 \pm 2.12	-- ^a
100	17.2 \pm 1.70	18.7 \pm 2.26	18.6 \pm 1.85	17.1 \pm 1.88	7.91
500	16.8 \pm 1.22	18.9 \pm 2.49	17.3 \pm 2.15	17.2 \pm 3.25	38.35
1000	16.9 \pm 0.96	18.0 \pm 1.39	18.0 \pm 1.36	16.4 \pm 1.52	76.65
4000	7.2 \pm 2.05 ^{**}	18.5 \pm 2.04	13.5 \pm 4.22 ^{**}	-- ^b	-- ^c
8000	-- ^b	-- ^b	-- ^b	-- ^b	-- ^c

^a Control diets were not analyzed for concentration of ADBAC.

^b Due to excessive mortality and food spillage in the 4000- and 8000-ppm groups, food consumption could not be accurately measured.

^c Due to extensive mortality and food spillage in the 4000- and 8000-ppm groups, an accurate daily dosage could not be calculated.

^{**} Significantly different from control group ($p < 0.01$).

females of the 4000-ppm group during weeks 0 to 1, 1 to 2, 8 to 9, and 9 to 10. The mean intake of the test compound over the entire study was 6.3, 31.2, and 62.0 mg/kg/day for the males and 7.9, 38.3, and 76.7 mg/kg/day for the females from the 100-, 500-, and 1000-ppm groups, respectively. Due to extensive mortality and food spillage in the 4000- and 8000-ppm groups, an accurate daily dosage could not be calculated for these animals.

4. Ophthalmological Examinations: Ophthalmic examinations were performed, using an indirect ophthalmoscope, prior to final sacrifice.

Results: Approximately 80% of the animals (distributed across both sexes and all groups including controls) were observed to have corneal crystals consisting of fine granular mineralized deposits of calcium phosphate located along the corneal epithelial basement membrane. The lesions were considered minimal, and there was no evidence that the lesions were related to treatment with the test material.

5. Hematology and Clinical Chemistry: Blood was collected from 10 randomly selected animals/sex/group just prior to sacrifice (or from the surviving animals in the 4000-ppm group) via the retroorbital sinus for clinical chemistry and hematology analyses. The CHECKED parameters were examined:

a. Hematology:

X Hematocrit (HCT) [†]	X Leukocyte differential count
X Hemoglobin (HGB) [†]	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC) [†]	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC) [†]	X Mean corpuscular volume (MCV)
X Platelet count [†]	Coagulation:thromboplastin time (PT)
X Reticulocyte count (RETIC)	
Red cell morphology	

Results: No treatment-related changes were observed in any hematology measurements for males or females from any treatment group (4000 ppm or lower).

[†]Recommended by Subdivision F (October 1982) Guidelines.

b. Clinical Chemistry:

	<u>Electrolytes</u>		<u>Other</u>
X	Calcium ⁺	X	Albumin ⁺
X	Chloride ⁺	X	Albumin/globulin ratio (calculated)
	Magnesium	X	Blood creatinine ⁺
X	Phosphorus ⁺	X	Blood urea nitrogen ⁺
X	Potassium ⁺		Cholesterol ⁺
X	Sodium ⁺	X	Globulins (calculated)
		X	Glucose ⁺
		X	Total bilirubin ⁺
		X	Direct bilirubin
		X	Total protein ⁺
			Triglycerides
X	<u>Enzymes</u>	X	Indirect bilirubin (calculated)
X	Alkaline phosphatase (ALP)		
	Cholinesterase		
X	Creatinine phosphokinase ⁺		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT) ⁺		
X	Serum aspartate aminotransferase (SGOT) ⁺		
X	Gamma glutamyltransferase (GGT)		

Results: Table 6 summarizes data on serum glucose and phosphorus and the activities of SGPT and SGOT. Statistically significant decreases in glucose concentration were observed in males from all treatment groups [10% (0.01>p>0.001), 10% (0.01>p>0.001), 12% (p<0.001), and 22% (p<0.001) reductions for the 100-, 500-, 1000-, and 4000-ppm groups, respectively]. The study authors stated that due to the small magnitude of these changes and the lack of a dose response, these differences were of questionable biological significance. For the same reasons, the biological relevance of the significantly (p<0.001) elevated serum phosphorus in males of the 4000-ppm group is questionable. The significant (0.01>p>0.001) decrease of serum phosphorus in females of the 500-ppm group, compared to control, is considered to be a spurious result. The reviewers also note that the range of glucose and phosphorus values for control and treated rats fell close to or within the normal range for this strain for a study of this duration (glucose, 0.57-1.42 g/L; phosphorus, 53-115 mg/L).¹

¹Normal values taken from the table of clinical chemistry values of Sprague-Dawley rats. Representative Historical Control Data in Rats and Mice, Hazleton Laboratories, 1984.

[†]Recommended by Subdivision F (October 1982) Guidelines.

TABLE 6. Representative Clinical Chemistry Parameters (Mean \pm S.D.) in Rats Fed Alkyl Dimethyl Benzyl Ammonium Chloride for 13 Weeks^a

Dose Group (ppm)	Glucose g/L	SGPT U/L	SGOT U/L	Phosphorous mg/L
<u>Males</u>				
0	1.21 \pm 0.09	52 \pm 4	14 \pm 3	59 \pm 6
100	1.09 \pm 0.09**	51 \pm 7	15 \pm 3	59 \pm 3
500	1.09 \pm 0.09**	52 \pm 5	16 \pm 2	60 \pm 5
1000	1.06 \pm 0.10***	51 \pm 9	14 \pm 4	61 \pm 7
4000	0.95 \pm 0.08***	62 \pm 11	21 \pm 5**	80 \pm 8***
<u>Females</u>				
0	1.03 \pm 0.14	55 \pm 9	18 \pm 5	55 \pm 7
100	1.13 \pm 0.09	56 \pm 6	16 \pm 3	52 \pm 6
500	1.12 \pm 0.11	55 \pm 10	14 \pm 5	46 \pm 9**
1000	1.09 \pm 0.12	52 \pm 5	16 \pm 3	52 \pm 7
4000	0.92 \pm 0.14	61 \pm 3	18 \pm 3	63 \pm 6

^aAll animals fed 8000 ppm ADBAC died during the study.

**Significantly different from control group (0.01 > p > 0.001).

***Significantly different from control group (p < 0.001).

Activities of SGPT and SGOT were slightly elevated in males and females of the 4000-ppm groups, but only the value for SGOT in males of this group was significantly ($0.01 > p > 0.001$) greater than the control value. Although elevation of SGOT and SGPT activities may be indicative of liver damage, the absence of correlative liver pathology suggests that the small changes in these enzymes are more likely the result of stress.

6. Urinalysis: Urinalyses were not performed.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

<u>Digestive System</u>	<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
Tongue	X Aorta ⁺	XX Brain ⁺
X Salivary glands ⁺	XX Heart ⁺	X Peripheral nerve (sciatic nerve) ⁺
X Esophagus ⁺	X Bone marrow ⁺	X Spinal cord (3 levels)
X Stomach ⁺	X Lymph nodes ⁺	X Pituitary ⁺
X Duodenum ⁺	XX Spleen ⁺	X Eyes (optic nerve) ⁺
X Jejunum ⁺	X Thymus ⁺	
X Ileum ⁺		
X Cecum ⁺		
X Colon ⁺		
X Rectum ⁺		
XX Liver ⁺	<u>Urogenital</u>	<u>Glandular</u>
Gallbladder ⁺	XX Kidneys ⁺	XX Adrenals ⁺
X Pancreas ⁺	X Urinary bladder ⁺	X Lacrimal gland (Exorbital)
	XX Testes ⁺	X Mammary gland ⁺
	X Epididymides	X Thyroids ⁺
	X Prostate	X Parathyroids ⁺
	Seminal vesicle	Harderian glands
<u>Respiratory</u>	XX Ovaries	
X Trachea ⁺	X Uterus ⁺	<u>Other</u>
XX Lung ⁺	X Vagina	X Bone (sternum and femur) ⁺
		X Skeletal muscle ⁺
		X Skin
		X All gross lesions and masses
		X Femur

⁺Recommended by Subdivision F (October 1982) Guidelines.

Results:

- a. Organ Weights: Absolute and relative liver and spleen weights are presented in Table 7. Absolute and relative (to brain) weights of the liver, kidneys, spleen, and heart in males of the 4000-ppm group were significantly lower ($p < 0.01$) and relative (to body) weights of the brain and testes were significantly ($p < 0.01$) higher than concurrent controls. The study authors considered these differences due primarily to the significantly ($p < 0.01$) lower body weights in males of this group, compared to controls; however, the reviewers consider the nonneoplastic histologic findings of hepatocellular atrophy and contracted spleen (see below) to correlate with the diminished absolute weights of the liver and spleen. Absolute and relative organ weights in males fed 1000, 500, or 100 ppm ADBAC were, in general, similar to controls. Because of the absence of a dose response, a significantly ($p < 0.05$) lower relative (to brain) liver weight in males of the 100-ppm group was not considered by the study authors to be biologically relevant.

In females, absolute and relative (to brain) weights of the liver, kidneys, spleen, heart, brain, and ovaries in animals fed 4000, 1000, 500, or 100 ppm ADBAC were similar to controls. Significantly ($p < 0.01$) higher relative (to body) weights of the liver and brain in females of the 4000-ppm group are considered due primarily to the lower (13%, not significant) body weight of these animals compared to controls.

Organ weight data were not available for males and females of the 8000-ppm group because of early death.

- b. Gross Pathology: Frequently occurring gross lesions are summarized for animals that died prior to terminal sacrifice (Table 8) or were terminally sacrificed (Table 9). Treatment-related gross lesions were confined to males and females of the 4000- and 8000-ppm groups and included body thinness or emaciation, fecal staining of the perineal skin, color change in the lungs (treatment related in rats that died), brain hemorrhage (rats that died), decreased spleen size (rats that died), and intestinal ileus (marked in rats that died) characterized by distended gas- and fluid-filled viscera extending from the stomach to the cecum. Color change in lymph nodes in rats at terminal sacrifice was not considered to be treatment related since this lesion was found in both control and treated animals and incidences were not dose related.

TABLE 7. Absolute and Relative Liver and Spleen Weights of Rats Fed Alkyl Dimethyl Benzyl Ammonium Chloride for 13 Weeks

Dose Group (ppm)	Liver			Spleen		
	Absolute (g)	Relative		Absolute (g)	Relative	
		Body (%)	Brain (%)		Body (%)	Brain (%)
<u>Males</u>						
0	13.25 ± 1.47	2.60 ± 0.17	634.18 ± 67.58	0.70 ± 0.07	0.14 ± 0.01	33.44 ± 3.40
100	12.33 ± 1.17	2.52 ± 0.15	581.40 ± 60.56*	0.66 ± 0.09	0.14 ± 0.02	31.22 ± 4.72
500	13.08 ± 1.24	2.66 ± 0.21	628.56 ± 60.63	0.66 ± 0.08	0.13 ± 0.01	31.67 ± 4.18
1000	12.74 ± 1.69	2.59 ± 0.24	609.27 ± 77.29	0.70 ± 0.05	0.14 ± 0.01	33.56 ± 2.15
4000	9.12 ± 0.37**	2.68 ± 0.09	455.67 ± 4.01**	0.92 ± 0.06**	0.15 ± 0.02	26.07 ± 2.97**
<u>Females</u>						
0	6.60 ± 0.39	2.57 ± 0.15	347.61 ± 21.70	0.45 ± 0.07	0.18 ± 0.03	23.86 ± 3.69
100	6.53 ± 0.51	2.52 ± 0.18	348.83 ± 26.18	0.45 ± 0.11	0.17 ± 0.04	23.85 ± 5.60
500	6.55 ± 0.46	2.53 ± 0.18	353.84 ± 26.45	0.42 ± 0.53	0.16 ± 0.02	22.71 ± 2.61
1000	6.57 ± 0.68	2.56 ± 0.23	354.98 ± 39.02	0.42 ± 0.04	0.16 ± 0.02	22.85 ± 2.14
4000	6.59 ± 0.46	3.15 ± 0.33**	354.96 ± 17.57	0.42 ± 0.09	0.21 ± 0.07	23.13 ± 6.47

**Significantly different from control group (0.01 > p > 0.001).

***Significantly different from control group (p < 0.001).

TABLE 8. Representative Gross Pathology Findings in Rats Fed Alkyl Dimethyl Benzyl Ammonium Chloride for 13 Weeks^a

Organ/Finding	Dose Group (ppm)											
	Males						Females					
	0	100	500	1000	4000	8000	0	100	500	1000	4000	8000
No. Animals Examined	0	0	0	0	12	15	0	0	0	0	11	15
Total body emaciation	0	0	0	0	10	14	0	0	0	0	8	13
Stomach fluid	0	0	0	0	3	11	0	0	0	0	1	6
Jejunum												
Contents abnormal	0	0	0	0	7	14	0	0	0	0	5	7
Fluid	0	0	0	0	2	1	0	0	0	0	5	1
Ileum												
Contents abnormal	0	0	0	0	7	14	0	0	0	0	5	7
Fluid	0	0	0	0	2	1	0	0	0	0	5	2
Cecum												
Fluid	0	0	0	0	11	13	0	0	0	0	11	12
Skin untreated												
Stained	0	0	0	0	2	5	0	0	0	0	3	4
Spleen												
Size decrease	0	0	0	0	5	1	0	0	0	0	0	1
Brain												
Hemorrhage	0	0	0	0	2	3	0	0	0	0	4	8
Lungs												
Color change, Focal/Multifocal	0	0	0	0	6	7	0	0	0	0	1	10

^aIncludes only animals which died prior to study termination.

TABLE 9. Representative Gross Pathology Findings in Rats Fed Aklyl Dimethyl Ammonium Chloride for 13 Weeks

Organ/Findings	Dose Group (ppm)											
	Males						Females					
	0	100	500	1000	4000	8000	0	100	500	1000	4000	8000
No. Animals Examined	15	15	15	15	3	0	15	15	15	15	4	0
Body/Thin	0	0	0	0	2	0	0	1	0	0	4	0
Cecum												
Fluid	0	0	0	0	2	0	0	0	0	0	3	0
Dilatation/distention	0	0	0	0	2	0	0	0	0	0	3	0
Skin, untreated												
Stained	0	0	0	0	2	0	1	0	0	1	2	0
Lymph node, S-mandibular												
Color change--												
Diffuse	2	1	1	0	0	0	0	0	1	0	0	0
Focal/Multifocal	0	0	0	1	0	0	2	0	0	1	0	0
Lymph node, med												
Size increase	1	0	2	2	0	0	2	1	1	3	0	0
Color change/diffuse	0	1	3	2	0	0	1	0	2	0	0	0
Brain												
Hemorrhage	0	1	0	0	0	0	0	0	0	0	0	0
Lungs												
Color change												
Focal/multifocal	1	1	0	2	1	0	2	1	1	2	0	0

c. Microscopic Pathology:

- 1) Nonneoplastic: Table 10 summarizes representative nonneoplastic lesion incidence data for rats that were sacrificed at week 13, found dead, or sacrificed moribund. Treatment-related nonneoplastic lesions were restricted to rats of the 4000- and 8000-ppm groups. These lesions, which generally occurred at higher incidences in males than females, included stomach congestion (significant in 8000-ppm males); stomach edema (significant in 4000- and 8000-ppm males); stomach hemorrhage (only in males); congestion of the duodenum, jejunum (significant in 8000-ppm males), ileum, and cecum; mucosal cell degeneration in the duodenum, jejunum (only in males), ileum (only in males), and cecum (significant in 8000-ppm males, not seen in females); congestion and hepatocellular atrophy in the liver (significant in 8000-ppm males and females and 4000-ppm males); contracted spleen (significant in 4000-ppm males); brain congestion (significant in 8000-ppm males and females); and congestion and hemorrhage of the lungs (significant in 8000-ppm males and females). The mucosal cell degeneration in the intestinal wall consisted of degeneration or necrosis affecting the villus tips of the small intestine and cecum.

The Anatomic Pathology Report stated that hemorrhages (seen primarily in the stomach, brain, and lungs) may have multiple causes, including agonal death, a possible terminal coagulation disorder (diffuse intravascular coagulation) which can occur in animals in shock, or autolytic degeneration of blood vessels permitting blood leakage into the tissues. Rats described grossly to have hemorrhage on the brain surface were not found to have any hemorrhage within the brain or any other brain lesion except vascular congestion. The study authors indicated that splenic contraction was considered likely to be a response to hypovolemic shock due to fluid pooling in the intestines and is supportive of the theory that shock was the immediate cause of death. Mild hepatocellular atrophy was suggested to be due to inanition as reflected in the emaciated body condition. The pathology report also indicated that two lesions, mononuclear cell infiltration of the liver and tubular basophilia of the kidneys, were seen less frequently in rats at the higher doses than in controls.

TABLE 10. Representative Nonneoplastic Histologic Findings in Rats Fed Alkyl Benzyl Ammonium Chloride for 13 Weeks

Organ/Finding	Dose Group (ppm)											
	Males					Females						
	0	100	500	1000	4000	8000	0	100	500	1000	4000	8000
Stomach	(10) ^a	(10)	(10)	(10)	(9)	(9)	(10)	(10)	(10)	(10)	(10)	(10)
Congestion	0	0	0	0	4	6	0	0	0	0	0	1
Hemorrhage	0	0	0	0	1**	1**	0	0	0	0	0	0
Edema	0	0	0	0	6	6	1	0	0	0	1	2
Liver	(10)	(10)	(10)	(10)	(10) ^a	(10) ^a	(10)	(10)	(10)	(10)	(10)	(10) ^a
Congestion	0	0	0	0	7**	10**	0	0	0	0	4	10**
Hepatocellular atrophy	0	0	0	0	7	8	0	0	0	0	4	7
Duodenum	(10)	(10)	(10)	(10)	(9)	(7)	(10)	(10)	(10)	(10)	(9)	(9)
Mucosal cell degeneration	0	0	0	0	4	1	0	0	0	0	1	2
Congestion	0	0	0	0	3	3	0	0	0	0	1	0
Jejunum	(10)	(0)	(0)	(10)	(4)	(2)	(10)	(0)	(0)	(10)	(4)	(0)
Congestion	0	0	0	0	1	2	0	0	0	0	1	0
Mucosal cell degeneration	0	0	0	0	1	0	0	0	0	0	0	0
Ileum	(10)	(0)	(0)	(10)	(3)	(4)	(10)	(0)	(0)	(10)	(4)	(1)
Congestion	0	0	0	0	2	2	0	0	0	0	2	0
Mucosal cell necrosis	0	0	0	0	1	0	0	0	0	0	0	0
Cecum	(10)	(0)	(0)	(10)	(4)	(5)	(10)	(0)	(0)	(10)	(9)	(2)
Congestion	0	0	0	0	0	2**	0	0	0	0	0	1
Mucosal cell degeneration	0	0	0	0	1	4	0	0	0	0	0	0
Spleen	(10)	(0)	(0)	(10)	(4) ^a	(1) ^a	(10)	(0)	(0)	(10)	(0)	(1)
Contracted spleen	0	0	0	0	4	1	0	0	0	0	0	1
Brain	(10)	(1)	(0)	(10)	(1)	(2) ^a	(10)	(0)	(0)	(10)	(1)	(7) ^a
Congestion	0	0	0	0	1	2	0	0	0	0	1	7
Lungs	(10)	(10)	(9)	(10)	(10)	(10) ^a	(10)	(10)	(10)	(10)	(10)	(10) ^a
Congestion	0	0	0	0	5	8**	0	0	0	1	4	9**
Hemorrhage	1	1	1	1	2	7	0	0	1	1	2	6

a Number in parentheses represents the number of tissues examined for animals that were sacrificed at week 13, found dead, or sacrificed moribund; autolyzed tissues were excluded from analysis.

* Significantly different from control group (p<0.05).

** Significantly different from control group (p<0.01).