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 WASHINGTON, D.C. 20460

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OFFICE OF
 PREVENTION, PESTICIDES AND
 TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Evaluation of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in an Oncogenicity Study in Mice. Guideline Series 83-2. (MRID 417652-01)

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FROM: Brian Dementi, Ph.D., D.A.B.T.
 Review Section III
 Toxicology Branch I
 Health Effects Division (H7509C)

Brian Dementi 7/15/93

TO: Brigid Lowery, PM Team 72
 Reregistration Branch
 Special Review and Reregistration Division (H7508W)

THRU: Karen Hamernik, Ph.D.
 Section Head, Review Section III
 Toxicology Branch I
 Health Effects Division (H7509C)

K. Hamernik 7/16/93
KP 7/20/93

The Data Evaluation Review for the ADBAC oncogenicity study in mice, submitted by the Chemical Specialties Manufacturers Association toward satisfying the Registration Guideline Series 83-2 testing requirement is herewith submitted to SRRD.

The test material was evaluated in CD-1 mice via the dietary route of administration for 78 weeks at dosage levels of 0, 100, 500 and 1500 ppm. There was no evidence of carcinogenicity under the conditions of the study. With respect to systemic toxicity, LOEL = 1500 ppm (decreased body weight and body weight gain); NOEL = 500 ppm. The study is rated Core Guideline. For further details please see the Data Evaluation Review.

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FINAL

DATA EVALUATION REPORT

ADBAC

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Study Type: Oncogenicity in Mice

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

March 30, 1992

Principal Author: *John Liccione* Date 4/26/92
John Liccione, Ph.D.

Reviewer: *Wayne Reichardt* Date 5-26-92
Wayne Reichardt, Ph.D.

QA/QC Manager: *Sharon Segal* Date 5/26/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
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Clement Number: 91-167
Project Officer: Mr. James Scott

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EPA Reviewer: Brian Dementi, Ph.D.
Review Section III, Toxicology Branch I,
Health Effects Division

Signature: [Signature]
Date: 8/31/92

EPA Acting Section Head: Karen Hamernik, Ph.D.
Review Section III, Toxicology Branch I,
Health Effects Division

Signature: [Signature]
Date: 9/26/93

DATA EVALUATION REPORT

STUDY TYPE: Guideline Series 83-2: Chronic dietary oncogenicity study in mice.

TEST MATERIAL: Alkyl dimethyl benzyl ammonium chloride

MRID Number: 417652-01

SYNONYM: ADBAC

STUDY NUMBER: 53-515

SPONSOR: ADBAC QUAT Joint Venture/Chemical Specialties Manufacturers Association, 1913 Eye Street, N.W., Washington, D.C., 20006

TESTING FACILITY: Bushy Run Research Center, 6702 Mellon Road, Export, PA, 15632-8902

TITLE OF REPORT: Chronic Dietary Oncogenicity Study with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in Mice

AUTHORS: M.W. Gill, S.J. Hermansky, and C.L. Wagner

REPORT ISSUED: January 9, 1991

QUALITY ASSURANCE: A quality assurance statement was signed and dated January 8, 1991.

CONCLUSIONS: ADBAC was fed to male and female CD-1 mice at dietary levels of 0, 100, 500, or 1500 ppm. Mean body weights and body weight gains in the high-dose males and females were significantly lower than those of controls throughout most of the study. There was no significant effect of dosing on clinical signs, mortality, hematology, clinical chemistry, food consumption, gross pathology, or histopathology. ADBAC was not oncogenic under the conditions of the study.

The maximum tolerated dose (MTD) was reached in males and females. The LOEL is 1500 ppm based on decreases in body weights and body weight gains in males and females. The NOEL is 500 ppm.

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CORE CLASSIFICATION: This study satisfies the Guideline requirements for a chronic dietary oncogenicity study (#83-2) in mice and is classified Core Guideline.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Alkyl dimethyl benzyl ammonium chloride (ADBAC)

Lot numbers: 7292K; BRRC 50-512 (A-E)

Purity: 79.6-81.5% (sponsor analysis). A purity value of 81.09% was used for diet preparation. The test material contained approximately 10-15% ethanol.

Physical property: Pale yellow, viscous liquid

Stability: Stable up to 14 days

Storage: Room temperature

2. Test Article Analyses for Purity and Stability

Test diets were prepared by adding ADBAC directly to ground rodent feed. A concentrated premix was prepared to ensure maximal loss of ethanol (approximately 12% by weight) from the test substance during the original mixing time of 1 hour. Corrections were made in the preparation of the premix for the ethanol lost during initial mixing. All diets were prepared weekly and stored in polypropylene containers at room temperature.

The stability of the test substance in the diet (stored at ambient temperatures) was determined at 0, 7, and 14 days in stainless steel feeders and 0, 7, 14, and 21 days in closed polypropylene containers prior to initiation of dosing. The homogeneity of the test substance (samples derived from the top, middle, and bottom layers of the mixing vessel) was determined in the 100, 500, and 1500 ppm test diets. The concentrations of the test substance in the diet were determined weekly for the first 4 weeks, and every 4 weeks thereafter.

Results of stability analysis indicated that the test substance, at concentrations of 100 and 1500 ppm was stable in diets for at least 14 days in stainless steel feeders and stable for at least 21 days in closed polypropylene containers when stored at room temperature. Mean concentrations of ADBAC in the diets at dose levels of 100 and 1500 ppm were 96.0-109.3% and 96.6-112.0% of target dose, respectively. Results of samples analyzed to determine homogeneity of the test substance in the diet at levels of 100, 500, and 1500 ppm indicated a homogeneous mix.

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3. Animals

Mice (380 male and 380 female, CD-1 mice) were received from Charles River Breeding Laboratories, Portage, MI. A pretest health screen was conducted on several of the animals. The mice were about 32 days of age upon arrival; and were approximately 8 weeks of age at initiation of treatment. During pretest, 2 mice/section (3 sections/cage made of stainless steel, wire mesh) were housed in a room with temperature and humidity controls set at 66 to 75°F and 40 to 70%, respectively, with a 12-hour light/dark cycle. After pretest, mice were housed individually. Water and food were provided ad libitum. At initiation of treatment, males weighed 27.6-36.0 g and females weighed 19.3-27.2 g.

Animals were assigned by sex to the following test groups using a computer-generated randomization procedure that stratified the animals within each sex by body weight:

Dietary Level (ppm)	Number of Animals	
	Males	Females
0 (1st control)	60	60
100	60	60
500	60	60
1500	60	60
0 (2nd control)	60	60

Mice were fed the test diets for 78 weeks. Doses were selected based on dose range-finding studies (BRRC Report numbers 51-514 and 51-504) which were not available for review. The study report did not discuss the results of the range-finding studies.

4. Statistics

The Levene's test for homogeneity of variances, analysis of variance, and pooled variance t-tests were performed on data for continuous, parametric variables. Non-parametric 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 tests where appropriate. Frequency comparisons were made for the microscopic findings for all tissues examined in the high-dose and control groups, and only for the liver, kidney, and lung, in the low- and mid-dose groups.

Mortality data were examined by life-table analysis. Level of significance was judged at alpha = 0.05.

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5. General Observations

(a) Mortality/morbidity/survival

Cage site examinations were conducted at least once daily throughout the study. The total scheduled (SD) and unscheduled (UD) deaths (excluding cage accidents) of animals of the main groups occurring during the study were reported as follows:

Group	Deaths			
	Male		Female	
	SD	UD	SD	UD
0 ppm (1st control)	45	15	46	12
100 ppm	39	21	42	18
500 ppm	45	14	45	14
1500 ppm	48	12	43	15
0 ppm (2nd control)	42	18	42	18

No statistically significant differences in the incidence of mortality in treated animals compared to controls were observed. The incidence of mortality for male mice (including those sacrificed moribund but excluding cage accidents) in the 0- (first control), 100-, 500-, 1500-, and 0- (second control) ppm groups was 25%, 35%, 23%, 20% and 30%, respectively. The incidence of mortality for female mice (including those sacrificed moribund but excluding cage accidents) in the 0- (first control), 100-, 500-, 1500-, and 0- (second control) ppm groups was 20%, 30%, 23%, 25%, and 30%, respectively. The study authors considered renal amyloidosis as the most common cause of death for male mice. Renal amyloidosis and lymphosarcoma were regarded by the study authors to be the primary cause of death in female mice.

(b) Clinical observations

Detailed clinical observation, including palpations, were performed once each week. Mice were inspected at least once daily for overt signs of toxicity except on days that detailed observations were made. No clinical signs or clinically observed palpable masses were attributed to treatment with ADBAC.

(c) Body weight/food consumption/compound intake

Body weight. Individual body weights were recorded weekly during the first 14 weeks of the study and every 2 weeks thereafter.

Tables 1 and 2 summarize data on mean body weights and mean body weight gains, respectively, at selected intervals. Body weights in the low- and mid-dose males and females remained comparable to

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TABLE 1. Mean Body Weights (g ± SD) at Selected Intervals for Mice Fed ADBAC for 78 Weeks^a

Weeks	Dietary Level (ppm)				
	0	100	500	1500	0
<u>Males</u>					
0	32.0±1.85	31.9±1.77	31.6±1.85	31.4±1.66	31.2±1.70
13	39.5±3.05	38.9±2.90	39.3±2.67	36.9±2.29 ^b	38.6±2.73
26	41.8±3.60	41.6±3.87	41.6±3.62	39.3±2.56 ^b	41.4±3.82
52	43.3±4.13	43.3±4.28	43.3±4.54	40.9±3.11 ^b	44.2±5.04
78	43.7±4.35	42.2±4.09	42.7±3.89	40.6±3.18 ^b	42.5±4.80
<u>Females</u>					
0	24.0±1.30	24.0±1.49	24.0±1.46	23.9±1.30	23.7±1.43
13	31.4±2.36	32.1±3.14	31.6±2.53	29.4±1.93 ^b	31.3±2.67
26	34.2±3.40	35.3±4.41	33.8±3.54	31.5±2.12 ^b	34.5±3.54
52	37.7±4.53	38.6±4.74	37.6±4.32	34.1±2.64 ^b	37.9±3.77
78	36.6±4.06	37.4±4.71	37.8±4.33	34.6±2.50 ^b	37.6±2.82

^aData extracted from Tables 8 and 10 of the study report

^bSignificantly different from the first and second control group (p<0.01)

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TABLE 2. Mean Body Weight Gains (g ± SD) at Selected Intervals for Mice Fed ADBAC for 78 Weeks^a

Months	Dietary Level (ppm)				
	0	100	500	1500	0
<u>Males</u>					
0-13	7.5±2.13	7.0±2.10	7.7±1.83	5.5±1.77 ^b	7.3±2.09
0-26	9.9±2.95	9.7±2.98	10.0±2.91	7.9±2.15 ^b	10.2±3.39
0-52	11.4±3.69	11.5±3.49	11.7±4.02	9.5±2.70 ^b	13.0±4.85
0-78	11.8±3.97	10.6±3.83	11.3±3.59	9.1±2.75 ^b	11.4±4.68
<u>Females</u>					
0-13	7.4±1.95	8.1±2.28 ^c	7.5±1.90	5.5±1.33 ^b	7.6±1.92
0-26	10.2±3.01	11.3±3.65	9.8±3.03	7.6±1.93 ^b	10.7±2.86
0-52	13.7±4.17	14.6±4.02	13.6±3.79	10.3±2.32 ^b	14.1±3.32
0-78	12.7±3.65	13.6±4.11	13.9±3.67	10.8±2.37 ^b	13.9±2.69

^aData extracted from Tables 9 and 11 of the study report.

^bSignificantly different from the first and second control group (p<0.01).

^cSignificantly different from the first control group (p<0.05).

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controls throughout the treatment period. Statistically significant ($p < 0.05$ or $p < 0.01$) reductions (3%-7%) in body weight as compared to both control groups were observed in the high-dose males from week 1 through week 78. Mean body weight gains in the high-dose males were significantly ($p < 0.01$) lower than those of controls throughout the treatment period with the exception of body weight gain at weeks 0-74. Mean body weight gains in the high-dose females were 27% lower than those of controls at the midpoint interval (weeks 0-40) and 15% lower at weeks 0-74.

Mean body weights in the high-dose females were significantly ($p < 0.05$ or $p < 0.01$) lower (4-10%) than controls from week 1 to week 78. Mean body weight gains in the high-dose females were significantly ($p < 0.01$) lower than those of controls at every interval throughout the treatment period. Mean body weight gain was significantly lower (27%) at the midpoint interval weeks 0-40 and 15% at weeks 0-78 as compared to controls. The study authors considered these decreases in mean body weight and mean body weight gain in high-dose males and females to be treatment related.

Food consumption. Individual food consumption was determined weekly for the first 14 weeks and at 2-week intervals thereafter.

Table 3 summarizes selected data on mean food consumption. No treatment-related effects on food consumption were observed. Occasional statistically significant differences in food consumption were noted in all dose groups, but this was considered to be spurious by the study authors.

Compound intake. Mean compound intakes for males receiving 100, 500, or 1500 ppm over the entire study period were 14.9, 73.4, and 229.3 mg/kg body weight/day, respectively; and compound intakes for females receiving the same doses were 17.8, 92.1, and 288.6 mg/kg body weight/day, respectively.

(d) Ophthalmoscopic examination

No ophthalmoscopic examinations were conducted.

6. Clinical Pathology

Hematology measurements were made for 10 animals per sex from the high dose and control groups during week 52 and all dose groups during week 79. Blood samples for all clinical pathology analyses were collected by retroorbital bleeding from nonfasted mice. Those parameters indicated by an X were examined:

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TABLE 3. Mean Food Consumption (g/animal/day) at Selected Intervals for Mice Fed ADBAC for 78 Weeks^a

Weeks	Dietary Level (ppm)				
	0	100	500	1500	0
<u>Males</u>					
0-1	5.9	5.8	5.7	5.9	5.6
12-13	6.0	6.0	5.9	6.0	5.8
25-26	6.1	6.2 ^d	5.9	5.7 ^c	5.9
51-52	5.9	6.0	5.9	5.7	5.8
77-78	5.6	5.7	5.5 ^e	5.5 ^e	5.9
<u>Females</u>					
0-1	5.7	5.6	5.7	5.6	5.7
12-13	5.9	6.2	6.5	6.5	6.3
25-26	5.8	5.0	5.0	5.5	6.0
51-52	6.1	6.4	5.9	5.7 ^{b,e}	6.2
77-78	5.7	5.6 ^d	5.9	5.4 ^e	5.9

^aData extracted from Tables 4 and 5 or the study report.

^bSignificantly different from the first control group (p<0.05).

^cSignificantly different from the first control group (p<0.01).

^dSignificantly different from the second control group (p<0.05).

^eSignificantly different from the second control group (p<0.01).

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(a) Hematology

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

* = Recommended by Subdivision F (November 1984) Guidelines

No treatment-related differences from controls were observed for any hematology parameters.

(b) Blood (clinical) chemistry

No serum chemistry parameters were examined.

Electrolytes

Calcium*
Chloride*
Magnesium*
Phosphorus*
Potassium*
Sodium*

Enzymes

Alkaline phosphatase (ALP)
Cholinesterase
Creatinine phosphokinase
Lactic acid dehydrogenase
Serum alanine aminotransferase (SGPT)*
Serum aspartate aminotransferase (SGOT)*
Gamma glutamyltransferase (GGT)

Other

Albumin*
Albumin/globulin ratio
Blood creatinine*
Blood urea nitrogen*
Cholesterol*
Globulins
Glucose*
Total bilirubin*
Direct bilirubin
Total protein*
Triglycerides

* = Recommended by Subdivision F (November 1984) Guidelines

(c) Urinalysis

No urinalysis was performed.

7. Sacrifice and Pathology

Following the 78-week treatment period, all surviving animals were sacrificed and necropsied. Animals that died during the study or were sacrificed in a moribund condition received a complete gross examination. Animals were anesthetized with methoxyflurane and killed by severing the brachial vessels and subsequent exsanguination. Tissues were fixed in 10% neutral buffered formalin. Those tissues

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indicated by an X were examined histologically for all control and high-dose mice. In addition, the lungs, liver, kidneys, and all gross lesions were histologically examined for all low- and mid-dose animals. Organs indicated by double-checked (XX) were weighed:

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

* - Recommended by Subdivision F (November 1984) Guidelines

(a) Macroscopic

There were no compound-related gross observations among the animals exposed to ADBAC.

Gross lesions were infrequent in most tissues and those that did occur were considered to be within the normal spectrum of degenerative, inflammatory, or neoplastic lesions expected in animals of this species, strain, and age.

(b) Organ weights and body weight ratios

There were no organ weight changes of toxicological importance in animals sacrificed at termination.

Statistically significant changes in relative organ weights (for example, liver and heart) noted in female mice in the 1500-ppm group were attributable to lowered body weights of the animals

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when compared to the controls. None of the absolute organ weight changes in the animals correlated with histopathological findings.

Although significant differences in mean absolute and relative spleen weights were observed in male mice in the 1500-ppm group, the study authors indicated that the differences in spleen weights of these animals were attributable to variation due to hemodynamic (i.e., congestion) or cellularity (i.e., hematopoiesis secondary to extrasplenic inflammation) alterations. Also, there were no corresponding anatomic pathologic changes observed in the spleen.

(c) Microscopic

Nonneoplastic. There were no nonneoplastic findings in male and female mice that were related to administration of ADBAC.

Neoplastic. There were no neoplastic lesions attributed to administration of ADBAC.

B. DISCUSSION

Based on treatment-related decreases in mean body weight and body weight gain in the high-dose males and females, an MTD was reached. The reductions in body weights and body weight gains in the high-dose males and females were seen throughout most of the study. Therefore, the dose levels selected in this study were appropriate in assessing the oncogenic potential of the test material in mice.

There were no toxicologically significant changes in mortality, hematology, clinical chemistry, organ weights, gross pathology, or histopathology.

The reviewers agree with the study authors' conclusion that the no-observed-effect level for chronic effects at 78 weeks was 500 ppm, and that there was no oncogenic effect under the conditions of this study.