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WASHINGTON, D.C. 20460

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JUL 28 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Evaluation of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Ninety-Day Subchronic Dermal Toxicity Study in Rats. Guideline Series 82-3. (MRID 414996-01)

Tox Chem No.: 016E
EPA ID No.: 069105
DP Barcode No.: D167282
Submission No.: S400536
Case No.: 819070

FROM: Brian Dementi, Ph.D., D.A.B.T.
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Brian Dementi 7/15/93

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

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

The Data Evaluation Review for the ADBAC 90-day subchronic dermal toxicity study, submitted by Chemical Specialties Manufacturers Association toward satisfying the Registration Guideline Series 82-3 testing requirement is herewith submitted to SRRD.

Results of this study are summarized as follows. For further details see the Data Evaluation Review.

The test material as evaluated by the dermal route of administration for 90 days in 15 rats/sex/dose-group at dosage levels of 0, 2, 6 or 20 mg/kg/day did not elicit any toxicological effects that could be ascribed to the test material.

Please be advised that the study is rated Core Supplementary, the reason being that a LOEL was not identified under circumstances where the doses employed were too far below the limit dose.

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FINAL

DATA EVALUATION REPORT

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Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC)

Study Type: Subchronic Dermal Toxicity in Rats

Prepared for:

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

Prepared by:

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9300 Lee Highway
Fairfax, VA 22031-1207

August 17, 1992

Principal Author Carrie Rabe, Ph.D. Date 8/17/92

Reviewer Wayne Reichardt, M.S. Date 8-17-92

QA/QC Manager Sharon Segal, Ph.D. Date 8/17/92

Contract Number: 68D10075
Work Assignment Number: 1-51
Clement Number: 91-165
Project Officer: James E. Scott

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Guideline Series 82-3: Subchronic Dermal Toxicity in the Rat

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

Signature: Brian Dementi
Date: 8/20/92

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

Signature: Karl P. Kotzke for
Date: 4/23/93

DATA EVALUATION REPORT

STUDY TYPE: Subchronic dermal toxicity in rats

TEST MATERIAL: Alkyl dimethyl benzyl ammonium chloride (ADBAC)

Tox. Chem. Number: 016E

P.C. Number: 069105

SYNONYMS: Benzalkonium chloride

CAS Number: 68391-01-5

STUDY NUMBER: 52-623

MRID Number: 414996-01

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

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

AUTHORS: M.W. Gill and C.L. Wagner

REPORT ISSUED: Completion date, May 14, 1990

CONCLUSIONS: Application of ADBAC, at dose levels of 2, 6, and 20 mg/kg/day, to the clipped backs of Sprague-Dawley rats for 6-8 hours/day, 5 days/week, for 13 weeks was associated with no toxicological effects that could be definitively attributed to the test material. The NOELs for dermal and systemic toxicity were 20 mg/kg/day.

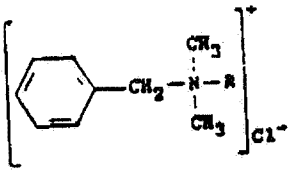
CORE CLASSIFICATION: This study is classified as Core Supplementary because LOELs were not achieved for either dermal or systemic toxicity. The doses used were far below the limit dose.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Alkyl dimethyl benzyl ammonium chloride (ADBAC)

Formula: A mixture of alkyldimethylbenzylammonium chlorides of the general formula in which R represents a mixture of the alkyls from $C_{12}H_{25}$ to $C_{16}H_{33}$.



Lot number: 7293K

Purity: 81.09% active ingredient; the material safety data sheet for this material identifies the other two major constituents as ethanol (5-15%) and water (5-15%).

Physical property: Pale yellow, viscous liquid

Stability: Stable for at least 14 days when stored at room temperature

Storage: Room temperature

Vehicle: Millipore® water

2. Test Article Analyses for Purity and Stability

ADBAC is a mixture of alkyldimethylbenzylammonium chlorides. The purity of the test material was not verified by the testing facility. However, the testing facility identified the three major components as benzyldimethyldidecylammoniumchloride, benzyldimethyltetradecylammoniumchloride and benzylcetyldimethylammoniumchloride using HPLC. The purity of the test material was reported by the sponsor to be 81.09%. Solutions were prepared using corrections for purity.

Solutions of ADBAC were prepared by dissolving the test material in Millipore® water. Solutions were prepared each week. Tests for homogeneity, stability, and verification of the concentration were performed by the Bushy Run Research Center using HPLC. The HPLC was run using what was identified only as an "appropriate standard". Prior to initiation of the study, 1.0% and 0.1% solutions were analyzed for stability and 1.0%, 0.3%, and 0.01% solutions were analyzed for homogeneity. The test material was found to be stable for at least 14 days when stored at room temperature. Solutions were also found to be homogeneous. Tests for verification of the

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concentration were performed on the control and test solutions prepared during the first 4 weeks of the study and on the solutions prepared at weeks 8 and 13. The concentrations were between 94.4% and 109.0% of the nominal concentrations.

3. Animals

Rats (90 males and 90 females, Sprague-Dawley CD®) were received from Charles River Laboratories, Inc., Portage, MI. The rats were approximately 32 days old upon arrival and were caged 2 rats/cage for approximately 1 week. Thereafter, rats were individually caged in stainless steel cages with wire mesh floors. The animal room was operated on a 12-hour light/dark cycle, and temperature and relative humidity were maintained between 66°C and 75°C and 40% and 70%, respectively, with 15 room air changes per hour. Water and food (Purina Certified Rodent Chow #5002 ground meal) were provided ad libitum. Within 2 days of arrival, 10 rats of each sex (randomly selected) were subjected to tests for fecal parasites, serology, hematology, clinical chemistry, and gross and histopathologic examination to verify that the shipment of rats was free of infectious diseases and parasites. Rats were randomized by body weight and allocated to study groups (15/sex/dose) using a computerized procedure such that all groups of the same sex had similar mean body weights. The rats were 7-8 weeks old and males and females ranged in weight from 241 to 291 g and from 151 to 196 g, respectively, at the time of the first application of test material.

Ten days prior to the application of test material, a 5 cm wide strip was shaved on the backs (from the scapular region to within 3 cm of the tail) of all rats using a veterinary clipper. Although the report does not indicate the approximate number of square centimeters shaved, the description of the area shaved suggests that approximately 10% of the body surface area was exposed. A 4-day period of acclimation to the wrapping procedure (see below) was allowed during the week prior to administration of the first dose. Any rats that did not acclimate to the procedure were removed from the study. Rats were reclipped prior to the first dose and then at weekly intervals. Rats were also reclipped on an as-needed basis. Only rats whose skin appeared to be intact after shaving were used in this study.

A thin even coat of the test material or vehicle (water) was spread directly on the back of each rat. The site was then covered with a 4" x 4" gauze pad and wrapped with bandaging tape (Vetrap®). After 6-8 hours, the wrapping and gauze were removed and the site was rinsed liberally with water. The area was then blotted dry with a 4" x 4" gauze pad. During the 6-8-hour exposure period, only water was available for consumption.

The dose levels used were as follows:

Dose Level (mg/kg/day)	Number/Sex
0	15
2	15
6	15
20	15

Dose levels were selected based on the results of a preliminary range-finding study in which application of 2 ml/kg of a 0.3% solution (6 mg/kg/day) for 10 days caused no irritation, 2 ml/kg of a 1% solution (20 mg/kg/day) for 10 days caused occasional excoriation, crust, and/or exfoliation, and 2 ml/kg of 3%, 6%, and 10% solutions (60, 120, and 200 mg/kg/day) for 4 or 10 days caused erythema (barely perceptible), excoriation, and skin discoloration. The maximum dose used in the current study (20 mg/kg/day) was selected based on the conclusion that higher concentrations produced skin irritation that could be considered greater than slight. The Pesticide Assessment Guidelines for subchronic dermal toxicty testing (Guideline 82-3) state that the highest dose level should result in a toxic effect but not produce severe skin irritation or fatalities. It is possible that a higher dose could have been tested without resulting in severe skin irritation.

4. Statistical Analyses

Data with homogeneous variances (as determined using Levene's test) were analyzed using analysis of variance. If the result was significant ($p \leq 0.05$), pooled variance t-tests were used to analyze for differences between the control and other study groups. Data with heterogeneous variances were analyzed using analysis of variance for unequal variances, followed by variance t-tests. Nonparametric data were analyzed using the Kruskal-Wallis test or the Mann-Whitney U test. Fisher's exact test was used to analyze incidence data. The limit for statistical significance was set at 0.05 for all tests.

5. General Observations

(a) Mortality/moribundity/survival

Animals were observed twice daily (once in the morning and once in the afternoon) for mortality/moribundity.

One female from the high-dose group died on the first day of dosing and was replaced by an extra animal on the second day of dosing. The death was attributed to stress from wrapping rather than from the test material itself. No necropsy data were

presented for the female that died. Therefore, it is difficult to eliminate other possible causes of death. However, based on the absence of mortalities in the range-finding study at doses as high as 200 mg/kg/day, it is unlikely that the death was related to exposure to the test material. No other animals died during the study.

(b) Clinical observations

Animals were observed daily for overt adverse clinical signs. In addition, once per week, detailed clinical observations were made with special attention to the treated skin.

The only abnormal observation that may have been treatment related was a single incidence of exfoliation noted on the day of sacrifice of 1 of the 20-mg/kg/day females. Excoriation of the abdomen of 1 of the 6-mg/kg/day females and ulceration on the back (not the treatment site) of 1 of the 20-mg/kg/day males were not considered to be treatment related.

(c) Body weights/food consumption

Body weights--Individual body weights were determined at the start of the study and then weekly thereafter. The study protocol indicates that terminal body weights were to be obtained prior to sacrifice. The body weights recorded at termination were approximately 20-25 g less than the body weights recorded at week 13. It is unclear whether the 20-25-g loss represents weight loss due to fasting overnight or whether termination body weights were of exsanguinated animals.

No significant effects on body weight or body weight gain were observed during the study or at termination. The study authors concluded that the termination body weight of the 20-mg/kg/day females was significantly elevated. However, analysis of the data by the reviewer could not verify this statement.

Food consumption--Individual food consumption values were determined weekly.

A single instance of significantly decreased food consumption was observed during the second week of the study in the 6-mg/kg/day males. However, because the decrease was not dose related, the effect was not attributed to treatment with ADBAC. A number of instances of significant increases in food consumption among treated females were also observed. These increases were also considered not to be treatment related.

(d) Ophthalmoscopic examination

Eye examinations were conducted prior to the first exposure and at the end of the test period. 7

No treatment-related effects on the appearance of the eyes were observed.

6. Clinical Pathology

Hematology and clinical chemistries were performed on blood samples obtained from rats that had been fasted overnight. Blood was collected just prior to the terminal sacrifice and was obtained from the retroorbital sinus of anesthetized rats.

(a) Hematology

The parameters marked with an "X" were examined.

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Reticulocyte count (RETIC)
X Leukocyte differential count*
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)

* Recommended by Subdivision F (November 1984) Guidelines

A small, but statistically significant dose-related decrease in reticulocyte count was observed in the 6- and 20-mg/kg/day females (Table 1). The study authors concluded that this decrease was not biologically significant. A significant increase in MCV in the 6-mg/kg/day males and a significant increase in eosinophil count in the 6-mg/kg/day females appeared to be incidental findings.

(b) Blood (clinical) chemistry

The parameters marked below with an "X" were examined.

Electrolytes

X Calcium*
X Chloride*
X Sodium*
X Phosphorus*
X Potassium*

Enzymes

X Alkaline phosphatase (ALP)
X Gamma glutamyl transferase (GGT)
X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*

Other

X Albumin*
X Albumin/globulin ratio
X Blood creatinine*
X Blood urea nitrogen*
X Total protein*
X Globulins
X Glucose*
X Total bilirubin*
X Direct bilirubin
X Indirect bilirubin
X Total protein*

* Recommended by Subdivision F (November 1984) Guidelines

No significant effect on any of these parameters was observed among the treated animals.

(c) Urinalysis

No urinalysis was performed. Urinalysis is not suggested by Subdivision F (November 1984) Guidelines for Subchronic Dermal Toxicity unless there is a need based on expected or observed toxicity.

7. Sacrifice and Pathology

With the exception of the 1 high-dose female that died after 1 day on study, all rats were sacrificed by exsanguination and received a complete gross examination. The tissues marked with an "X" below were collected for possible histopathological examination and those marked with a "XX" were also weighed at necropsy. All tissues were preserved in neutral buffered 10% formalin.

Digestive System

Tongue
X Salivary glands*
X Esophagus*
X Stomach*
X Duodenum*
X Jejunum*
X Ileum*
X Cecum*
X Colon*
X Rectum*
XX Liver*
Gallbladder
X Pancreas*

Respiratory

X Trachea
X Lung*

Cardiovascular/Hematologic

X Aorta*
XX Heart*
X Bone marrow
X Lymph nodes*
XX Spleen*
X Thymus*

Urogenital

XX Kidneys*
X Urinary bladder*
XX Testes*
X Epididymides*
X Prostate*
X Vagina*
XX Ovaries*
X Uterus*

Neurologic

XX Brain*
X Peripheral nerve* (sciatic nerve)
X Spinal cord (three levels)
X Pituitary*
X Eyes

Glandular

XX Adrenals*
X Lacrimal gland
X Mammary gland
X Thyroids*
X Parathyroids*

Other

X Bone (sternum and femur)*
X Skeletal muscle
X Treated and untreated skin*
X All gross lesions and masses*

* Recommended by Subdivision F (November 1984) Guidelines

(a) Macroscopic

A complete necropsy was performed on each animal. Very few gross lesions were observed in either the control or treated animals. No treatment-related lesions were apparent.

(b) Organ weights and organ-to-body weight ratios

Weights for the organs identified with a "XX" were obtained from all animals. No treatment related changes were observed in the organ weights or organ-to-body weight ratios. The only statistically significant difference between organ weights of treated rats and those of the corresponding controls was a significant increase (4%) in the brain weight in high-dose female rats. This was considered to be an incidental finding because the brain-to-body weight ratio was not similarly elevated relative to controls.

(c) Microscopic Examination

All tissues from control and high-dose rats that were collected for histopathology were examined. In addition, gross lesions, skin, lungs, liver, and kidneys of low- and mid-dose animals were examined.

A significant increase in hyperkeratosis was observed in treated skin of high-dose females (Table 2). However, a high incidence of hyperkeratosis of treated skin was observed in all of the male groups, including controls. The study authors suggested that the hyperkeratosis may be the result of frequent shaving and daily application of an occlusive wrap rather than a toxicologic response to application of ADBAC. An increased incidence of hemosiderosis of the submandibular lymph node in high-dose females was judged to be an incidental finding.

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The reviewers have no other comments regarding the materials and methods sections.

A description of the statistical analysis employed was included in the report.

A signed Good Laboratory Practice Compliance Statement, a signed Quality Assurance Statement, and a list of Quality Assurance Inspections were included.

B. DISCUSSION

Except that higher concentrations of test material could have been used, no deficiencies were noted in the design and conduct of the study. In addition, the reporting of the results was, for the most part, accurate. Only one minor error was detected in reporting. The body weight of

high-dose females was reported as being significantly increased; however, the absolute brain weight was the parameter that was significantly increased in high-dose females.

The doses selected for this study were based on the results of a range-finding study in which a 4-day application of the highest dose tested, 2 ml/kg of a 10% solution (200 mg/kg/day), caused barely perceptible erythema and skin discoloration for all males and 1 female, and exfoliation for all 3 males and females tested at this dose. These effects (especially the skin discoloration) were considered by the study authors to be too severe to allow use of this dose in a subchronic dermal study. Therefore, 2 ml/kg of a 1% solution (20 mg/kg/day) was used as the highest dose in the current study. In the range-finding study, application of this concentration for 5 days/week for 2 weeks caused excoriation and crust in 1 out of 3 males and exfoliation in 2 out of 3 females.

According to the Subdivision F 1984 Guidelines, the highest dose tested in a subchronic dermal study should not produce severe irritation or increase the incidence of fatalities. It is likely that the 200-mg/kg/day dose would have been tolerated without producing severe irritation (severe erythema and/or edema) since a 4-day exposure to this dose caused only barely perceptible erythema with skin discoloration, exfoliation, and excoriation. This is supported by the absence of dermal toxicity following 90 days of exposure to 20 mg/kg/day in the current study. The only dermal effect noted in this study was a significant increase in hyperkeratosis of treated skin in high-dose females. However, the relationship of this effect with exposure to test material is doubtful considering the high incidence of this effect in control males that were treated only with vehicle.

A significant and dose-related decrease in reticulocyte count was observed in the 6- and 20-mg/kg/day females. Decreases in reticulocyte count are normally associated with regenerative responses to anemia. However, no evidence of anemia was seen in other hematological parameters. Furthermore, the decreased levels in treated females were similar to the levels observed in control males. Thus, the decreased reticulocyte count was most likely not a biologically significant finding.

Based on the absence of any significant dermal or systemic effects at the highest dose tested, 20 mg/kg/day, this study is classified as Core Supplementary. The NOEL for dermal effects and the NOEL for systemic effects were 20 mg/kg/day.

TABLE 1. Mean Reticulocyte Counts (% of RBCs ± S.D.) of Rats Dermally Exposed to Alkyl Dimethyl Benzyl Ammonium Chloride for 13 Weeks[a]

Dose Level (mg/kg/day)	Males	Females
0	1.9 ± 0.65	2.6 ± 0.28
2	2.0 ± 0.41	2.4 ± 0.41
6	1.9 ± 0.73	2.2 ± 0.39**
20	2.4 ± 0.66	1.9 ± 0.34**

[a]Data extracted from Study # 52-623, Appendix 3, Tables 1 and 2

**Significantly different from controls; $p<0.01$

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TABLE 2. Incidence of Hyperkeratosis of Treated Skin in Rats Dermally Exposed to Alkyl Dimethyl Benzyl Ammonium Chloride for 13 Weeks[a,b]

	Group (mg/kg/day)			
	0	2	6	20
		Males		
Hyperkeratosis (total)	12/15 (80%)	12/15 (80%)	15/15 (100%)	15/15 (100%)
Minimal	0/15 (0%)	1/15 (7%)	3/15 (20%)	0/15 (0%)
Mild	8/15 (53%)	10/15 (67%)	8/15 (53%)	10/15 (67%)
Moderate	4/15 (27%)	1/15 (7%)	4/15 (27%)	5/15 (33%)
		Females		
Hyperkeratosis (total)	4/13[c] (31%)	9/15 (60%)	11/15 (73%)	14/14* (100%)
Minimal	2/13 (15%)	9/15 (60%)	8/15 (53%)	6/14 (43%)
Mild	2/13 (15%)	0/15 (0%)	3/15 (20%)	8/14 (57%)
Moderate	0/13 (0%)	0/15 (0%)	0/15 (0%)	0/14 (0%)

[a]Data extracted from Study # 52-623, Appendix 2, Tables 11 and 12
[b]Incidence shown over the number examined. Percent incidence calculated by reviewer is presented in parentheses.
[c]The treated skin of 2 control females was reported as missing.
*Significantly different than control, $p \leq 0.05$.

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