Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 23 Nov 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland, M.S. Jess Rowland 11/23/93
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DATA EVALUATION REPORT

STUDY TYPE: Chronic Oral - Dogs [§83-1(b)]

DP BARCODES: D188679, D189620

SUBMISSION NO.: S436173

P.C. CODE: 069149 **CASWELL NO.:** 331A

MRID NO.: 419704-01

TEST MATERIAL: BARDAC 2280

SYNONYMS: Didecyldimethylammoniumchloride

STUDY NUMBER: 2545-102

SPONSOR: Lonza, Inc., Fair Lawn, NJ

TESTING FACILITY: Hazleton Washington, Inc., Vienna, VA

TITLE OF REPORT: Chronic oral toxicity study of didecyldimethylammoniumchloride in dogs

AUTHOR: G.E. Schulze

REPORT ISSUED: 26 July 1991

CONCLUSIONS: In a chronic, 1-year oral toxicity study, male and female beagle dogs were given test compound at dosages of 0, 3, 10, or 20/30 mg/kg body weight/day (Dosing at 30 mg/kg/day was not tolerated well and was discontinued on Day 31; dosing was resumed on Day 36 at 20 mg/kg/day). No treatment-related deaths occurred during the study. The treatment-related clinical signs (soft/mucoid feces, emesis) were observed frequently in high-dose animals. Hematology or urinalysis results were normal. Total cholesterol levels were significantly decreased in the high-dose females. Gross and histopathological findings did not reveal any treatment-related effects.

	NOEL	LOEL
Males and females	10 mg/kg/day	20 mg/kg/day (HDT)

The LOEL is based on increased incidence of clinical observations (emesis and soft/mucoid feces) in males and females and decreased total cholesterol levels in females.

CORE CLASSIFICATION: Guideline. This study satisfies guideline requirements [§83-1(b)] for a chronic oral study in dogs and is acceptable for regulatory purposes.

A. MATERIALS

1. Test Compound: BARDAC 2280 Description: yellow liquid Batch #: B-1889 Purity: 80.8% [a.i.] Contaminants: not given

2. Test animals: Species: Dog Strain: Beagle Age: 8.5 - 9.5 months Weight (kg): 7.8 - 13.5 (males), 6.8 - 9.7 (females) Source: Hazleton Research Products, Inc., Cumberland, VA Housing: Individually in elevated cages Feed: Purina Certified Canine Diet #5007 Water: Tap water, *ad libitum* Environment: Temperature, 59 - 88°F; Humidity, 11 - 96%; Light cycle, 12 hr light/12 hr dark

B. METHODS

1. Animal Assignments: Animals were assigned randomly to main study test groups as shown in Table 1.

Table 1: Animal Assignment to Study Groups

Test Group		Dosage[a] (mg/kg/day)	Animals/Group Male	Female
Control	(CON)	0	4	4
Low	(LDT)	3	4	4
Mid	(MDT)	10	4	4
High	(HDT)	30/20[b]	4	4

a Dosage adjusted for percent purity of active ingredient

b Because of severe toxicity, dosing at 30 mg/kg/day dose was discontinued on Day 31; on Day 36 dosing was resumed at 20 mg/kg/day.

2. Dose Preparation: A weighed amount test compound was thoroughly mixed with basal diet to form a premix, which was refrigerated until used. An appropriate amount of premix was mixed with distilled water (Polar Distilled Water) to form the dosing slurries. The slurries were prepared fresh daily. The slurries were administered in two divided doses of 10 ml/kg/dose. Control animals received an appropriate amount of basal diet/water slurry.

3. Statistical Evaluations: Levene's test was used to evaluate homogeneity of variances. Homogeneous data were initially analyzed using a one-way analysis of variance (ANOVA). If the ANOVA result was significant (F-test), pair-wise comparisons were carried out using Dunnett's test. Heterogeneous data were transformed ($\log_{10}X$, X^2, $\sqrt{X}$, $1/X$, arcsine X, rank) and reevaluated for homogeneity. If transformed data was found to homogeneous, ANOVA and pair-wise comparisons were carried out.

C. REGULATORY COMPLIANCE

1. Quality assurance was documented by signed and dated GLP and quality assurance statements.

2. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

3. A statement of "no confidentiality claims" was provided.

D. RESULTS

1. Analytical Chemistry: The prepared diets were analyzed for stability, homogeneity and concentration. Test diets were stable for at least 14 days when refrigerated. Analysis of diet/water slurries, taken from the top, middle and bottom, indicated that the test compound was homogeneously distributed (relative standard deviations, 0.28 to 1.96%) and within 87.0 and 99.4% of nominal concentrations. The concentration of test compound was below the target on Day 1 (low-dose) and Day 2 (low- and middle-dose). Adjustments were made and for the remainder of the study, the concentration of test compound were all within ±10% of the target concentrations (91.0 to 103%, LDT; 91.3 to 106%, MDT; 93.8 to 110%, HDT).

2. Observations: Animals were inspected twice daily for signs of toxicity, mortality and moribundity. Detailed examinations were performed weekly.

a. Clinical observations: Table 2 summarizes the clinical observations noted during the study. Treatment-related effects consisted of soft and/or mucoid feces and emesis, described as frothy, containing food or compound-like material, or a combination of all three. Soft and/or mucoid feces was observed at least once in all of the middle- and high-dose animals, however, the frequency was greatest in the high-dose animals. Similarly, emesis, was observed more frequently in the high-dose animals, particularly during the first four weeks of the study when the animals were dosed at 30 mg/kg/day.

b. Mortality: An accidental death, attributed to gavage error, occurred in one low-dose male. All other animals survived until the scheduled sacrifice.

3. Body Weight and Body Weight Gain: Body weights were measured at the start of the study, at weekly intervals through Week 14, and every other week, thereafter. During the first four weeks of the study, the high-dose animals (dosed at 30 mg/kg/day) all experienced significant weight loss (Table 3).

Table 2: Incidence of Clinical Observations (Data summarized from Table 1 and Appendix 2 of the study)

Observation	Sex	CON	LDT	MDT	HDT
Found dead	♂	0	1	0	0
	♀	0	0	0	0
Soft and/or mucoid feces	♂	3[a] (1,2,3)	2 (2,2)	4 (1,2,3,5)	4 (6,14,20,24)
	♀	2 (1,9)	1 (1)	4 (1,1,7,17)	4 (2,4,8,27)
Emesis	♂	1 (1)	2 (2,3)	4 (2,4,6,7)	4 (12,15,21,24)
	♀	2 (2,7)	3 (1,2,3)	2 (1,5)	4 (2,6,11,33)

[a] Values in parenthesis are the number of days the observation was noted in each animal. For example, (1,4,7) denotes that three animals showed an effect 1, 4, and 7 times during the study.

Table 3: Mean Body Weight Gains (Data summarized from Table 3 of the study)

Sex	Week	CON	LDT	MDT	HDT
Male	0-4	0.3	0.3	0.3	-1.3*
Female	0-4	0.2	0.2	0.3	-0.3*

* $p \leq 0.05$

4. Food Consumption: Food consumption was measured at the at weekly intervals through Week 14 and every other week, thereafter. No treatment-related effects were noted in food consumption during the course of the study. At Week 1, the low- and high-dose females had food consumption values significantly less than that of the control. However, since the middle-dose animals showed no effect, the differences noted did not appear to be compound-related.

5. Ophthalmological examinations: Examinations were performed during the prestudy acclimation period and again before terminal sacrifice. No treatment-related eye lesions were observed.

6. Clinical Pathology: Clinical chemistry, hematology and urinalysis were performed before the start of the study to establish baseline values and at 13, 26, and 52 weeks of treatment.

a. Hematology: The following hematology parameters were examined:

Cell morphology
Leukocyte count (WBC)
Erythrocyte count (RBC)
Leukocyte differential
Platelet count
Reticulocyte count
Hematocrit (HCT)
Corrected leukocyte count
Hemoglobin (HGB)
Mean corpuscular HGB (MCH)
Mean corpuscular HGB conc.(MCHC)
Mean cell volume (MCV)

Results: Although significant differences were noted in some hematology parameters, the magnitude of the responses was slight and judged not to be biologically important.

b. Clinical Chemistry: The following clinical chemistry parameters were examined:

Electrolytes
- Calcium (Ca)
- Chloride (Cl)
- Sodium (Na)
- Phosphorous (P_i)
- Potassium (K)

Enzymes
- γ-Glutamyl transpeptidase (GGT)
- Creatine kinase (CK)
- Alanine aminotransferase (SGPT/ALT)
- Aspartate aminotransferase (SGOT/AST)

Other
- Glucose (GLU)
- Blood creatinine (CREAT)
- Blood urea nitrogen (BUN)
- Total bilirubin (TBIL)
- Protein, total (PROT)
- Albumin (ALB)
- Total cholesterol (CHOL)
- Globulin (GLOB)

Results: Statistically significant differences were noted in high-dose males (total protein, albumin and creatine kinase) and females (sodium), however, the values were within historical control ranges (reviewer's reference, historical control values were not included with the study) and therefore of questionable biological significance. In the high-dose females, total cholesterol levels were lower than the control values at each of the time points, with a significanct difference occurring at Week 13 (Table 4).

Table 4: Total Cholesterol Levels (mg/dl) for Female Dogs (Data summarized from Table 7 of the study)

Week	CON	LDT	MDT	HDT
-2	143	163	157	158
13	169	189	127	102*
26	142	156	141	112
52	153	248	167	136

* $p \leq 0.05$

c. Urinalysis: The following urinalysis parameters were examined:

Appearance	Glucose
Specific gravity	Ketone Bodies
Protein	Bile Pigments
Volume	Urobilirubin
Sediment	Total Bilirubin
pH	Occult Blood
Fecal flotation	Reducing substances
Nitrites	

Results: No treatment-related effects were noted in any of the animals.

8. Sacrifice and Pathology: Detailed pathological examination was performed on male and female animals in the control and treatment groups. The tissues listed below were fixed in 10% neutral buffered formalin and examined histologically. Selected organs (CAPITAL LETTERS) were also weighed.

Digestive system	Cardiovas./Hematol	Neurologic
Pancreas	Aorta	BRAIN
Salivary glands	HEART	Periph. nerve
Esophagus	Bone marrow	Spinal cord
Stomach	Lymph nodes	PITUITARY
Duodenum	SPLEEN	Eyes + optic nerve
Jejunum	Thymus	Glandular
Ileum	Urogenital	ADRENALS
Cecum	KIDNEYS	THYROIDS/Parathyroids
Colon	Urinary bladder	Mammary gland
Rectum	TESTES/Epididymides	Other
LIVER	OVARIES	Gross lesions
Gallbladder	Prostate	Skin
Respiratory	Uterus	Bone
Lungs		Skeletal muscle
Trachea		

a. Organ Weights: No treatment-related effects were noted in either the absolute or relative organ weights. All values were comparable to controls.

b. Gross Pathology: Gross examination of tissues taken at the terminal sacrifice did not reveal any treatment-related abnormalities.

c. Histopathology: Histopathological examination at terminal sacrifice did not reveal any changes which could be attributable to treatment.

E. DISCUSSION: In a chronic, 1-year oral toxicity study, male and female beagle dogs were gavaged with test compound at dosages of 0, 3, 10 or 20/30 mg/kg body weight/day. The high-dose (30 mg/kg/day exceeded the maximum tolerated dose, since after approximately four weeks of dosing, some animals experienced significant decreases in body weight and a high incidence of emesis and soft feces. Dosing at 30 mg/kg/day was discontinued on Day 31 and resumed on Day 36 at 20 mg/kg/day. This change did not appear to affect the outcome of the study.

With the exception of the accidental death of one low-dose male (attributed to gavage error), all animals survived to terminal sacrifice. The treatment-related clinical signs observed in the high-dose animals included a high incidence of soft/mucoid feces and emesis. These clinical signs persisted throughout the study. Although these clinical signs were noted in other treatment groups, the occurrence was sporadic.

Clinical pathology did not reveal any treatment-related effects in either hematology or urinalysis. Clinical chemistry effects were considered slight and not biologically significant. A possible treatment-related effect was a significant decrease in total cholesterol in the high-dose females at Week 13. Gross and histopathological findings did not reveal any treatment-related effects. No neoplastic lesions were noted.

	NOEL	LOEL
Males and females	10 mg/kg/day	20 mg/kg/day)HDT)

The LOEL is based on increased incidence of clinical observations (emesis and soft/mucoid feces) in males and females and decreased total cholesterol levels in females.

CORE CLASSIFICATION: Guideline. This study satisfies guideline requirements [§83-1(b)] for a chronic oral toxicity study in dogs and is acceptable for regulatory purposes.