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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: EPA Identification No. 069149
90-Day Subchronic Study with
Didecyldimethylammoniumchloride in the Rat

Project No.: 0-0376
Tox. Chem. No.: 331A
Record No.: 256551

From: Brian Dementi, Ph.D., D.A.B.T. Brian Dementi 9/24/91
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You will find appended the Data Evaluation Report for the identified subchronic study of didecydimethylammoniumchloride in the rat. This study has been classified supplementary in view of uncertainies as to the possible effects of the test material upon the lymph nodes (histiocytic aggregates) in females. The study would be upgradeable by the submission of historical control data from this laboratory on the parameter, in addition to results of examination of lymph nodes of females in the low and mid dose groups.

Attachment

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EPA No.: 68D80056
DYNAMAC No.: 279-A
TASK No.: 2-79A
July 26, 1991

DATA EVALUATION RECORD

DIDECYLDIMETHYLAMMONIUMCHLORIDE

Subchronic Dermal Toxicity Study in Rats

APPROVED BY:

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Dynamac Corporation

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Date: 7/26/91

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DATA EVALUATION RECORD

DIDECYLDIMETHYLAMMONIUMCHLORIDE

Subchronic Dermal Toxicity Study in Rats

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DATA EVALUATION RECORD

GUIDELINE § 82-3

STUDY TYPE: Subchronic dermal toxicity study in rats.

MRID NUMBER: 413059-01.

TEST MATERIAL: Didecyldimethylammoniumchloride (DDAC).

SYNONYM: Bardac 2280.

STUDY NUMBER: 51-554.

SPONSOR: Lonza, Inc., Fair Lawn, NJ.

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

TITLE OF REPORT: Ninety-Day Subchronic Dermal Toxicity Study with Didecyldimethylammoniumchloride in Rats.

AUTHORS: Gill, M. W., and Van Miller, J. P.

REPORT ISSUED: October 7, 1988.

CONCLUSIONS:

Groups of 15 male and 15 female Sprague-Dawley rats were dosed dermally with 0, 0.1, 0.3, or 0.6% concentrations of didecyldimethylammoniumchloride (DDAC) for 6 hours, 5 days/week for 13 weeks; this corresponds to dose levels of 0, 2, 6, or 12 mg/kg/day. There were no compound-related effects on mortality, body weight, food consumption, hematology, clinical chemistry, or organ weights. The incidence of skin irritation (erythema, edema) was increased in mid- and high-dose males and females in a dose-related manner from days 5 to 8 in males and days 5 to 33 in females; only high-dose females were affected on study days 12, 19, 22, and 26. The severity of the erythema was slight to well defined; the edema was graded as slight. Incidence and severity of skin irritation decreased with increasing time. Mild exfoliation of the treated skin was exhibited in high-dose males and mid-dose males and females throughout the study. Gross skin lesions (exfoliation, excoriation) occurred primarily in high-dose females. Microscopic lesions of dosed males and females were indicative of minimal to mild irritation, with females more severely affected than males. Dermal effects were observed histologically at all dose levels in females. The most frequent lesions included acanthosis, hyperkeratosis, epidermitis, and dermatitis. Also exhibited less frequently were dermal fibrosis, hemorrhage, ulceration, and vascular degeneration of the epidermis.

Based on the absence of overt systemic effects, the systemic LOEL and NOEL could not be determined; however, the NOEL is ≥12 mg/kg/day, the highest dose tested. On the basis of increased skin irritation at the mid- and high-dose, the LOEL for dermal irritation in males is 6 mg/kg/day, and the NOEL is 2 mg/kg/day DDAC. The LOEL for dermal irritation in females is 2 mg/kg/day, the lowest dose tested, based on acanthosis of the treated skin; the NOEL is <2 mg/kg/day when applied to approx. 45 cm^2 for 6 hrs each day Hext 8/7/

Classification: CORE Supplementary; the highest dose level was not adequate to achieve systemic toxicity. The study may be upgraded when additional requested histological data are submitted (see Reviewers' Discussion and Interpretation of Results).

A. MATERIALS:

1. Test Compound: Didecyldimethylammoniumchloride; description: viscous, honey-colored liquid; batch No.: B-1889 (BRRC No. 50-382); purity: 80.8%.

2. Test Animals: Species: rat; strain: Sprague-Dawley; age: 8 weeks at study initiation; weight: males--221.3 to 264.6 g, females--166.8 to 219.1 g at study initiation; source: Charles River Breeding Laboratories, Portage, MI.

B. STUDY DESIGN:

1. Animal Assignment: Following 20 days of acclimation, animals were assigned to the following test groups on the basis of body weight using a computer-generated randomization procedure:

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Test Group	Dermal Dose (mg/kg/day)	Test Concentration[a] (%)	Main Study (90 days) Males	Females
1 Control	0	0	15	15
2 Low (LDT)	2	0.1	15	15
3 Mid (MDT)	6	0.3	15	15
4 High (HDT)	12	0.6	15	15

[a]Solution concentrations of the test material were corrected for percentage of active ingredient.

Dose levels were determined based on a preliminary skin irritation screen (BRRC project No. 87-44-25009) in which 0.6% DDAC was determined to be the highest concentration of the test material that could be applied without producing skin irritation, and 2.0 mL/kg was determined to be the largest volume of test solution that could be applied without runoff.

A pretest health screen of 10 animals/sex included clinical laboratory studies (hematology and selected serum chemistry analyses), gross pathological examinations, and histopathological examinations of selected tissues (3 animals/sex). A viral screen and an examination for fecal parasites was conducted on five animals/sex. Animals were housed individually 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.

2. Dose Preparation: Appropriate amounts of DDAC were dissolved (w/w) in water to prepare the high-dose solutions (0.6%); lower dose solutions were prepared by mixing the vehicle with measured volumes of the high-dose solution. Dosing solutions were prepared weekly and stored at room temperature. A trial batch of dose solutions was prepared prior to study initiation to assess homogeneity and stability (0.1 and 0.6%) of the test material in water; stability was evaluated 0, 7, and 14 days following dose solution preparation. Actual dosing solutions of DDAC were analyzed for concentration prior to study initiation and weekly through study week 4; refrigerated samples were analyzed during study weeks 8 and 13.

Results: Trial dosing solutions were reported to be homogeneous; the mean concentrations of DDAC in the 0.1, 0.3, and 0.6 percent solutions were 95.4 ± 0.7, 94.8 ± 1.8, and 96.1 ± 1.2% of nominal, respectively. The test material was stable in the dosing solutions; after storage at room temperature for 14 days, concentrations of test material in the low- and high-dose preparations were 99% and 102.2% of nominal, respectively. Less than 10% deviation from target concentration was noted in dosing solutions for all doses; test material concentrations for weeks 1-13 ranged from 97 to 106%, 96 to 108%, and 94 to 106% of target for low-, mid-, and high-dose preparations, respectively. DDAC was not detected in control dosing solutions.

3. Preparation of Animal Skin and Method of Application: The fur was clipped from the dorsal area (approximately 5 cm wide) of the trunk of the animals 8 days prior to initiation of dosing. Animals were wrapped in the manner used during the study for 6 hours/day for 4 days of the week prior to dosing. Pretreatment animals that did not adapt to the wrapping procedure were not used. The fur was reclipped prior to dosing initiation and as needed thereafter. The dosing solution or vehicle in a constant volume of 2.0 mL/kg was applied to the intact skin and held in contact with the skin for a 6-hour period each day by means of a gauze dressing, which was secured with bandaging tape and overwrapped with elastic tape. At the end of the exposure period, the test site wrappings were removed, and the exposure area was rinsed with water to remove any residual test material. Animals were dosed 5 days/week. The elapsed time from dosing to terminal sacrifice (2 days) was the same for males and females.

4. Food and Water Consumption: Animals received Ground Purina Certified Rodent Chow No. 5002 (Ralston Purina Co.) and water ad libitum.

5. Statistics: The following procedures were utilized in analyzing the numerical data: Levene's test was performed to test for variance of homogeneity. Analysis of variance and t-tests were used to analyze parametric data. Non-parametric data were analyzed by the Kruskal-Wallis test or the Wilcoxon rank sum test, as modified by Mann-Whitney. Fisher's Exact test was used to compare frequency data.

6. Quality Assurance: A quality assurance statement was signed and dated October 6, 1988.

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C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of morbidity and mortality. Detailed clinical observations were performed weekly; special attention was given to the skin of the dose site. Dermal reactions were scored using the Draize scoring system.

Draize scoring of the treated skin was performed following dosing at the end of the week (Friday) and prior to dosing at the beginning of the following week (Monday); scoring was continued until signs of erythema and edema could not be consistently graded because of decreased severity.

Results: One low-dose female (animal No. 37710B08) and one high-dose female (Animal No. 37729D01) were found dead on study days 77 and 63, respectively, and a mid-dose female (Animal No. 37687C07) was sacrificed moribund on study day 75. None of the deaths were attributed to dosing with DDAC. No male rats died during the study.

The study authors reported that there were no compound-related clinical signs of toxicity. The reviewers noted one emaciated, unkempt, low-dose male (animal No. 37577B03) on study day 29 and one emaciated, high-dose female (animal No. 37729D01) from study days 59 to 62. In addition, one high-dose female (animal No. 37729D01) exhibited slow respiration on study day 58. These findings were not addressed by the study author. Two of these animals were not among those found dead or sacrificed moribund.

Representative incidences and severities of skin irritation are shown in Table 1; the severity of skin irritation was scored as shown in footnote c. In males, elevated incidences of skin irritation (erythema and edema) were exhibited at the mid and high dose on study days 5 (erythema, 2 of 15 mid dose, 5 of 15 high dose; edema, 1 of 15 mid and high dose) and 8 (erythema, 1 of 15 mid dose, 2 of 15 high dose; edema, 1 of 15 mid dose). The severity of the erythema was slight to well defined; the edema was graded as slight. Slight erythema was also observed in 1 of 15 high-dose males at study day 19. Mild exfoliation of the treated skin (not tabulated) was reported in 8 of 15 high-dose males throughout the dosing period. In females, elevated incidences of skin irritation (erythema and edema) were exhibited in dosed females from study initiation to study week 5. The highest incidence of erythema and edema was observed at study days 5 (erythema, 4 of 15 mid dose, 13 of 15 high dose; edema, 2 of 15 mid dose, 9 of 15 high dose), 8 (erythema, 1 of 15 mid dose, 8 of 15 high dose; edema, 3 of 15 high dose), and 12 (erythema, 11 of 15 high dose; edema, 3 of 15 high dose). The severity of the erythema was slight to well defined and that of the edema was slight. Incidence and severity

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TABLE 1. Representative Incidence and Severity of Skin Irritation in Rats Treated Dermally with DDAC for 13 Weeks[a]

Day	Dose Level (mg/kg/day)	Incidence and Severity in Males[b]		Incidence and Severity in Females[b]	
		Erythema	Edema	Erythema	Edema
5	0	0	0	0	0
	2	0	0	0	0
	6	2 (1.5)[c]	1 (1)	4 (1.2)	2 (1)
	12	5 (1.6)	1 (1)	13 (1.5)	9 (1.6)
8	0	0	0	0	0
	2	0	0	0	0
	6	1 (2)	1 (1)	1 (1)	0
	12	2 (1)	0	8 (1)	3 (1)
12	0	0	0	0	0
	2	0	0	0	0
	6	0	0	0	0
	12	0	0	11 (1.3)	3 (1)
15	0	0	0	0	0
	2	0	0	1 (2)	0
	6	0	0	0	0
	12	0	0	3 (1)	0
19	0	0	0	0	0
	2	0	0	0	0
	6	0	0	0	0
	12	1 (1)	0	6 (1.8)	5 (1)
22	0	0	0	0	0
	2	0	0	0	0
	6	0	0	0	0
	12	0	0	5 (1.4)	2 (1)
26	0	0	0	0	0
	2	0	0	0	0
	6	0	0	0	0
	12	0	0	2 (1)	1 (1)
29	0	0	0	0	0
	2	0	0	0	0
	6	0	0	0	0
	12	0	0	0	0
33	0	0	0	0	0
	2	0	0	0	0
	6	0	0	1 (1)	0
	12	0	0	3 (1)	0

(continued)

TABLE 1. (continued)

Day	Dose Level (mg/kg/day)	Incidence and Severity in Males[b]		Incidence and Severity in Females[b]	
		Erythema	Edema	Erythema	Edema
36	0	0	0	0	0
	2	0	0	0	0
	6	0	0	0	0
	12	0	0	0	0

[a]Incidence and severity recorded after dosing on days 5, 12, 19, 26, and 33 and prior to dosing on days 8, 15, 22, 29, and 36.

[b]Based on 15 animals/sex/dose.

[c]Numbers in parentheses equal mean Draize scores; scores weighted as follows:

Erythema

0	None
1.0	Slight (barely perceptible)
2.0	Well-defined
3.0	Moderate to severe
4.0	Severe

Edema

0	None
1.0	Barely perceptible
2.0	Slight
3.0	Moderate
4.0	Severe

decreased with increasing time of treatment. Mild exfoliation of the treated skin (not tabulated) was reported in 15 of 15 high-dose females and 12 of 15 mid-dose females throughout the dosing period. Skin irritation was not scored following study day 36.

2. Body Weight: Rats were weighed weekly.

Results: Tables 2 and 3 summarize data on mean body weights and body weight gain, respectively. Body weights and body weight gains of dosed males and females were similar to those of concurrent controls. Sporadic increases in the body weight gains of females exposed to 2 and 6 mg/kg/day for study weeks 3 to 5 and 12 to 13 were considered to be a result of random variation.

3. Food Consumption: Consumption was determined and mean daily diet consumption was calculated weekly.

Results: Food consumption of control and dosed males and females was similar throughout the study. Data for animals with significant food spillage were removed from the study results.

4. Ophthalmological Examinations: Ophthalmological examinations were performed prior to study initiation and at study termination.

Results: No compound-related effects on ophthalmology parameters were reported. Findings at study initiation were similar to those seen at study termination and were attributed to congenital abnormalities. Two dosed males (one low dose, one high dose) exhibited a "red-brown" discharge at study termination; this was reported to be an incidental finding attributable to a source of irritation unrelated to dosing.

5. Hematology and Clinical Chemistry: Blood was collected from the retroorbital sinus immediately prior to sacrifice from all fasted surviving animals for hematology and clinical analysis. The CHECKED (X) parameters were examined:

a. Hematology:

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

†Recommended by Subdivision F (November 1984) Guidelines for Subchronic Dermal Toxicity Studies.

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TABLE 2. Representative Mean Body Weights of Rats Exposed to DDAC for 13 Weeks[a]

Exposure Level (mg/kg/day)	Mean Body Weight (g ± S.D.) at Weeks: 1	7	13
		Males	
0	275 ± 13.8	381 ± 29.4	431 ± 32.5
2	278 ± 13.4	387 ± 27.9	439 ± 31.8
6	272 ± 14.1	387 ± 29.3	437 ± 39.2
12	274 ± 16.8	384 ± 28.0	434 ± 35.1
		Females	
0	214 ± 13.7	282 ± 18.6	295 ± 19.4
2	219 ± 15.0	295 ± 18.3	309 ± 19.9
6	214 ± 12.6	287 ± 18.5	314 ± 25.9
12	212 ± 14.3	284 ± 14.5	302 ± 12.3

[a]Based on 15 rats/sex/dose with the exception of 14 low-, mid-, and high-dose females at 13 weeks.

TABLE 3. Representative Mean Body Weight Gain of Rats Exposed to DDAC for 13 Weeks[a]

Exposure Level (mg/kg/day)	Mean Body Weight Gain (g ± S.D.) at Weeks: 0-3	0-7	0-13
		Males	
0	76 ± 17.3	136 ± 22.3	185 ± 25.2
2	80 ± 14.9	140 ± 23.3	191 ± 26.8
6	83 ± 16.1	143 ± 24.0	193 ± 33.6
12	81 ± 11.6	139 ± 20.8	189 ± 27.5
		Females	
0	55 ± 10.2	92 ± 13.1	106 ± 15.4
2	64 ± 7.6*	101 ± 12.3	115 ± 14.8
6	62 ± 9.2*	98 ± 15.9	125 ± 23.9**
12	57 ± 6.9	94 ± 7.2	110 ± 14.5

[a]Based on 15 rats/sex/dose with the exception of 14 low-, mid-, and high-dose females at 13 weeks.

*Significantly different from control group at $p < 0.05$.

**Significantly different from control group at $p < 0.01$.

Results: There were no compound-related hematologic findings. Platelet counts were slightly increased in a dose-related manner in dosed males and females; however, these increases were nonsignificant and were within the range of that found in historical controls.

b. Clinical Chemistry:

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

Results: There were no compound-related effects on clinical chemistry.

6. Urinalysis: Urinalyses were not performed.

†Recommended by Subdivision F (November 1984) Guidelines for Subchronic Dermal Toxicity Studies.

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:

Digestive System	**Cardiovasc./Hemat.**	**Neurologic**
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	**Urogenital**	**Glandular**
XX Liver†	XX Kidneys†	XX Adrenals†
Gallbladder†	X Urinary bladder†	X Lacrimal gland
X Pancreas†	XX Testes†	X Mammary gland
	X Epididymides	X Thyroids†
	X Prostate	X Parathyroids†
	Seminal vesicle	Harderian glands
Respiratory	XX Ovaries	
X Trachea	X Uterus†	
X Lung†	X Vagina	
		Other
		X Bone (sternum and femur)
		X Skeletal muscle
		X Skin (treated and control site)†
		X All gross lesions and masses†

All designated tissues from control and high-dose males and females were examined microscopically; in addition, the skin, lungs, liver, kidneys, and gross lesions of low- and mid-dose animals were examined.

†Recommended by Subdivision F (November 1984) Guidelines for Subchronic Dermal Toxicity Studies.

Results:

a. Organ Weights: There were no effects of dosing on organ weights.

b. Gross Pathology: Table 4 summarizes the incidence of frequently occurring gross findings in rats exposed to DDAC for 13 weeks. Compound-related lesions of the treated skin included exfoliation (flaking and scaling) of the skin of one (7%) high-dose male and seven (50%) high-dose females and excoriation (reddened, crusted, or superficially ulcerated or abraded areas) of the skin of one (7%) mid-dose and five (36%) high-dose females. Other gross findings were infrequent and were not considered by the study authors to be related to dosing.

c. Microscopic Pathology:

1) Nonneoplastic: Table 5 summarizes the incidence and severity of frequently occurring histopathological skin lesions in rats exposed to DDAC for 13 weeks. Table 6 summarizes other microscopic observations for these animals. The incidence of acanthosis was increased in low-, mid-, and high-dose males and females when compared to concurrent controls. The incidence of minimal to mild acanthosis was particularly high in low-dose females. The incidence of hyperkeratosis was increased in high-dose males and all dosed females. The incidence of dermal fibrosis was increased in low-, and mid-dose males and females and high-dose females. The severity of 98% of these findings was minimal or mild. The study authors reported that these changes were not reliable indicators of chemical irritancy when occurring in minimal or mild degree, since they can be observed in untreated animals as a secondary effect of clipping and wrapping procedures or as a result of the increased abrasion of clipped skin compared to haired skin. The study authors considered the moderate acanthosis of two high-dose females to be indicative of a regenerative response to chemical irritation. Compound-related findings included epidermitis, dermatitis, ulceration, vacuolar degeneration of the epidermis, and dermal hemorrhage. Minimal epidermitis was observed in low-, mid-, and high-dose males and females (incidence of 1 to 2 of 15/dose); the increased severity of epidermitis occurred primarily in high-dose females. Minimal dermatitis was observed in

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TABLE 4. Representative Gross Observations in Rats Exposed to DDAC for 13 Weeks[a]

Organ/Finding	Exposure Level (mg/kg/day): Males 0	2	6	12	Females 0	2	6	12
Liver	(15)[a]	(15)	(15)	(15)	(15)	(14)	(14)	(14)
Anomaly	1	1	1	2	0	0	0	0
Color change, focal/multifocal	0	1	0	2	2	4	0	0
Skin, treated	(15)	(15)	(15)	(15)	(15)	(14)	(14)	(14)
Exfoliation	0	0	0	1	0	0	0	7
Excoriation	0	0	0	0	0	0	0	5
Lymph node, submandibular	(15)	(15)	(15)	(15)	(15)	(14)	(14)	(14)
Color change, diffuse	1	6	2	3	1	2	3	4
Size increase	2	3	1	3	0	0	0	1
Thymic region	(15)	(15)	(15)	(15)	(15)	(14)	(14)	(14)
Color change, focal/multifocal	3	3	1	5	10	4	0	5
Lung	(15)	(15)	(15)	(15)	(15)	(14)	(14)	(14)
Color change, focal/multifocal	2	0	1	3	0	1	2	0
Urinary bladder	(15)	(15)	(15)	(15)	(15)	(14)	(14)	(14)
Calculus	1	0	0	2	0	0	0	0
Contents abnormal	1	0	0	2	0	0	0	0

[a]Numbers in parentheses equal number of animals examined.

TABLE 5. Representative Microscopic Observations of Skin Lesions (Incidence and Severity) in Rats Exposed to DDAC for 13 Weeks[a]

Finding	Dose Group (mg/kg/day): Males 0	2	6	12	Females 0	2	6	12
Acanthosis								
Minimal	3	8	9	2	0	8	4	2
Mild	0	0	0	4	0	1	0	2
Moderate	0	0	0	0	0	0	0	2
Hyperkeratosis								
Minimal	3	1	2	7	0	2	3	4
Mild	2	0	0	3	0	0	0	8
Dermatitis								
Minimal	0	0	1	1	0	0	2	6
Mild	0	0	0	0	0	0	4	1
Moderate	0	0	0	0	0	0	0	2
Epidermitis								
Minimal	0	1	2	1	0	1	2	2
Mild	0	0	0	1	0	0	0	3
Moderate	0	0	0	0	0	0	0	2
Marked	0	0	0	0	0	0	0	1
Folliculitis								
Minimal	0	0	1	0	0	0	0	0
Dermal fibrosis								
Minimal	0	1	2	0	0	4	2	1
Mild	0	1	0	0	0	0	0	0
Hemorrhage								
Minimal	0	0	0	0	0	0	0	1
Vacuolar degeneration, epidermis								
Mild	0	0	0	0	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Marked	0	0	0	0	0	0	0	1
Ulceration								
Moderate	0	0	0	0	0	0	0	1
Marked	0	0	0	0	0	0	0	1

[a]Based on 15 control males and females, 15 DDAC-dosed males and 14 DDAC-dosed females.

TABLE 6. Representative Microscopic Observations in Rats Exposed to DDAC for 13 Weeks

Organ/Finding	Exposure Level (mg/kg/day) Males 0	2	6	12	Females 0	2	6	12
Liver	(14)[a]	(15)	(15)	(15)	(15)	(14)	(14)	(14)
Hemosiderosis	6	8	10	10	9	11	10	7
Hepatocellular necrosis	0	0	1	1	3	3	1	0
Hepatitis	0	3	3	2	4	7	3	0
Fibrosis	8	7	7	11	11	9	10	6
Lymph node, submandibular	(14)	(9)	(1)	(15)	(15)	(2)	(3)	(14)
Sinus erythrocytosis	3	6	1	6	2	2	0	6
Hemosiderosis	2	6*	0	6	3	1	2	6
Lymphoid hyperplasia	5	6	1	6	4	1	0	8
Plasmacytosis	4	3	0	6	6	0	0	7
Lymph node, mesenteric	(13)	(0)	(0)	(14)	(15)	(0)	(0)	(14)
Nodular Histiocytic aggregates	0	0	0	2	3	0	0	9*
Thymus	(15)	(3)	(2)	(15)	(15)	(5)	(0)	(14)
Hemorrhage	9	3	2	11	14	5	0	9
Eye	(15)	(0)	(0)	(15)	(15)	(0)	(0)	(14)
Corneal mineralization	3	0	0	6	3	0	0	11**
Lung	(15)	(15)	(15)	(15)	(15)	(14)	(14)	(14)
Alveolar histiocytosis	0	2	1	1	6	2	1	0*
Atelectasis	0	0	1	1	0	1	0	0
Perivascular infiltrates	0	0	0	2	0	1	0	0
Pneumonitis, interstitial	1	0	1	3	1	1	0	0

[a]Numbers in parentheses equal number of tissues examined.

*Significantly different from control group at $p < 0.05$.

**Significantly different from control group at $p < 0.01$.

1 of 15 mid- and high-dose males and 2 of 15 mid- and 6 of 15 high-dose females. Mild (4 mid- and 1 high-dose females) and moderate (2 high-dose females) dermatitis, hemorrhage, vacuolar degeneration of the epidermis, and ulceration (1 of 15 high-dose females for each latter finding) occurred only in mid- and high-dose females.

The increased incidence of hepatocellular lesions (Table 6) was considered to be a result of handling and wrapping procedures and was not considered to be compound related. Lesions of the lymph nodes, eyes, thymus, and lungs were considered to be common occurrences for the rat strain and were not considered to be related to dosing. Nodular histiocytic aggregates of the mesenteric lymph nodes were increased in high-dose females (9/14) when compared to concurrent controls (3/15); however, these lesions were of minimal or mild severity and were not considered to be of biological significance by the study authors. The incidence of corneal mineralization of the eyes of these same females (11/14) were increased when compared to concurrent controls (3/15), however, these corneal lesions were reported to be of minimal severity, historical incidence of this finding is high in Sprague-Dawley rats, and ophthalmologic examinations of the corneal surface of the eyes of control and dosed animals conducted prior to study termination revealed a high incidence of corneal crystals (minimal severity) in control and low-, mid-, and high-dose males and females. Since only comparatively few corneas were examined histologically, the increased incidence of mineralization in high-dose females was reported to be due to an artifact of sectioning. The cause of death of the females that died or were sacrificed moribund during the study included trauma, incidental neoplasia, and possibly gastritis.

2) Neoplastic: There were no compound-related neoplastic findings. One low-dose female that died during the study was found to have lymphosarcoma and one high-dose male was found to have an adrenal adenoma; these lesions were considered to be incidental.

D. STUDY AUTHORS' CONCLUSIONS:

Solutions of DDAC were administered daily for 13 weeks to the intact dorsal skin of Sprague-Dawley rats at dosage levels of 0, 2, 6, and 12 mg/kg/day [corresponding to concentrations of 0, 0.1, 0.3, and 0.6% (w/w)]. No systemic toxicity resulted.

Mild skin irritation was exhibited grossly and microscopically at the high dose in all animals and grossly at the mid and low dose in some animals. The maximum dosage level that could be evaluated was 12 mg/kg/day.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The design and conduct of the study were acceptable. Even though the skin irritation of the dosed animals was graded for only 36 days of the study, it was reported by the study author that the skin was examined for the 90-day study duration. A trial batch of dosing solutions was prepared prior to study initiation to assess homogeneity and stability; the actual dosing solutions should have been analyzed for these parameters. The results of many hematology parameters and one clinical biochemistry parameter were reported as medians rather than means. This may have been done because of data scatter and large standard deviations; the study authors did not comment on the cause of these large deviations. The column headings on study report page 154 are incorrect; they should be replaced by alkaline phosphatase, calcium, phosphorous, sodium, potassium, and chloride.

The incidence of histiocytic aggregates of the lymph nodes ~~and corneal mineralization of the eyes~~ of high-dose females were increased; increases were considered to be of statistical but not biological significance since the findings were common occurrences for the rat strain. However, in order to validate these opinions of the study authors, the ~~eyes and~~ lymph nodes of low-, and mid-dose animals should be examined by the study laboratory and historical laboratory data on these findings should be submitted.

The reviewers agree with the study authors that no systemic changes occurred in the test animals. Based on the preliminary skin irritation screen, the study authors reported that they were limited as to the concentration and volume of the test compound that could be used. However, the reviewers consider these dose levels to be ineffective in achieving systemic toxicity; the test animals should have been able to tolerate higher dose levels of DDAC. On the basis of increased skin irritation at the mid- and high-dose levels, the LOEL for dermal irritation in males is 6 mg/kg/day, and the NOEL is 2 mg/kg/day DDAC. The LOEL for dermal irritation in females is 2 mg/kg/day, the lowest dose tested, based on acanthosis of the treated skin; the NOEL is <2 mg/kg/day when exposed to approx. 45 cm² for 6 hr periods each day. 8/7/91