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

JUL 21 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Cacodylic Acid: Review of a Repeated Dose Dermal 21 Day Study/Non-Rodent

FROM: Steven L. Malish, Ph.D., Toxicologist S.L. Malish 9/15/93
Tox. Branch II, Review Section IV
HED (H7509C)

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THRU: Jess Rowland, M.S., Acting Section Head Jess Rowland 7/19/93.
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and

management 7/20/93
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Tox Branch II; HED (H7509C)

Task Identifications: Submission: S442011 DP Barcode: D191921
P.C. Code: 012501 Caswell No.: 133

ACTION REQUESTED: Review of a 82-2 Repeated Dose Dermal Toxicity 21 Day Study - Non-Rodent [MRID NO.: 418728-01]

Response:

A Data Evaluation Report for the above referenced study is attached. A summary is provided below.

Cacodylic Acid was applied dermally under an occlusive bandage to 5 rabbits/sex/group at doses of 0 (Control), 100, 300 and 1000 mg/kg once daily, five days per week for 6 hours for a period of 21 days.

Cacodylic Acid did not elicit any effects on the rabbit skin. At 1000 mg/kg/day the test substance caused a decrease in the rate of body weight gain in females and testicular weight decreases and histopathological alterations in the testes in the males.

Under conditions of this study, the following NOEL and LOEL are established. In males, the LOEL is based on the decrease in testicular weights and histopathological lesions in the testes. In the females, the LOEL is based on a decrease in body weight gain.

Dermal Irritation: NOEL: 1000 mg/kg/day [HDT]
LOEL: not achieved

Systemic Toxicity: NOEL: 300 mg/kg/day
LOEL: 1000 mg/kg/day

CORE CLASSIFICATION: - Minimum

This study satisfies the data requirement 82-2 for a repeated dose dermal toxicity in rabbits and is acceptable for regulatory purposes.

7/19/1993

Reviewed by: Steven L. Malish, Ph.D., S.L. Malish 7/15/93
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Jess Rowland, M.S., Jess Rowland 7/19/93
Tox. Branch II, Section IV (H7509C)

DATA EVALUATION REPORT

STUDY TYPE:	82-2 Repeated Dose Dermal Toxicity 21 Day Study - Rabbit
IDENTIFICATIONS:	Submission: S442011 DP Barcode: D191921
MIRD NO.:	418728-01
PC Code:	012501 Caswell No.: 133
TEST MATERIAL:	Cacodylic Acid
SYNONYM:	Dimethylarsenic Acid
REPORT NO.:	PH 430-LI-002-90
SPONSOR:	Dr. E. Koren Luxembourg Industries (Pamol), Ltd. P.O.Box 13, 27 Hamered Street Tel Aviv 61000, Israel
TESTING FACILITY:	Pharmakon Research International, Inc, Waverly, PA 18471
TITLE OF REPORT:	Cacodylic Acid 21 Day Dermal Toxicity Study in Rabbits
AUTHORS:	D. J. Margitich, B.S., L. J. Ackerman V.M.D.
REPORT ISSUED:	March 13, 1991

SUMMARY:

Cacodylic Acid was applied dermally under an occlusive bandage to 5 rabbits/sex/group at doses of 0 (Control), 100, 300 and 1000 mg/kg once daily, five days per week for 6 hours for a period of 21 days.

Cacodylic Acid did not elicit any effects on the rabbit skin. At 1000 mg/kg/day the test substance caused a decrease in the rate of body weight gain in females and testicular weight decreases and histopathological alterations in the testes in the male. Under conditions of this study, the following NOEL and LOEL are established.

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In males, the LOEL is based on the decrease in testicular weights and histopathological lesions in the testes. In females, the LOEL is based on the decrease in body weight gain.

Dermal Irritation: NOEL: 1000 mg/kg/day [HDT]
LOEL: not achieved

Systemic Toxicity: NOEL: 300 mg/kg/day
LOEL: 1000 mg/kg/day

CORE CLASSIFICATION: - Minimum

This study satisfies the data requirements 82-2 for a repeated dose dermal toxicity in rabbits and is acceptable for regulatory purposes.

A. Materials:

1. Test Material

Chemical:	Cacodylic Acid
Synonym:	None available
Purity:	99.95% a.i.
Description:	White Powder
Lot No.:	DM-23-0030101

2. Test Animals

Species:	Rabbit
Strain:	Albino New Zealand White
Source:	Hare-Marland, Hewitt, NJ
Age:	Young adult; age not given
Weight:	2-2.6 Kg.

B. STUDY DESIGN:

1. Treatment

Animals were acclimated to laboratory conditions for 10 days, identified by Gey Band ear tags and assigned randomly by weight to the test groups. Groups of 5 males and 5 females received 100, 300 and 1000 mg/kg/day of the test compound applied to the shaven skin once daily five days per week for 6 hours for a period of 21 days (Table 1).

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Table 1

Group Treatments

Group	Dose mg/kg/ day	Animals M/F
Control	0	5/5
Low	100	5/5
Mid	300	5/5
High	1000	5/5

2. Skin Preparation

All animals were collared throughout the study. Pelage on the dorsal area of the back was clipped prior to the start of dosing. Clipping was repeated if necessary during the study. The test material or vehicle were administered to each animal (10% of body surface area) over a 21 day period, 5 days/week, 6 hours/day. Gauze patches moistened with deionized water were then applied to the skin. Deionized water was used for the vehicle control. A rubber dam was wrapped around the torso of the rabbits from all groups and secured with an elastic bandage to retard evaporation.

Approximately 6 hours after the application, the dressing was removed and the application site was wiped (but not washed) to remove any of the test material. Doses were adjusted weekly based on the latest body weight.

3. Statistics

Body weight, body weight gains, food consumption and hematology and clinical chemistry parameters were analyzed with Dunnett's analysis of variance.

4. Regulatory Compliances

A quality assurance statement, a statement of compliance with Good Laboratory Practice Standards and a statement of no data confidentiality claims was signed and dated.

C. METHODS and RESULTS:

1. Observations

Cage-side examinations were conducted once daily for pharmacological and toxicological signs. Mortality checks were performed twice a day. Signs of toxicity were recorded including time of onset, severity and duration.

No mortality or clinical signs occurred during the study in the treated versus the control animals.

2. Body Weight

Animals were weighed prior to dosing, day 7, 14 and 20. No treatment related effects were seen in absolute weights at any dose level. Body weight gain was decreased (11-25%) in females at 1000 mg/kg/day as shown below. Treated males exhibited a body weight gain increase when compared to the controls (Table 2).

Table 2

Mean Body Weight Gain (gm) in Female Animals[1,2]

	Dose Levels (mg/kg/day)			
Day	0	100	300	1000
7	98(-[3])	137(40)	137(40)	87(-11)
14	214(-)	238(11)	243(14)	160(-25)
20	363(-)	306(-16)	371(2)	279(-23)

[1]Calculated by the reviewer from the absolute weight, p. 25.
[2]No statistical significance seen.
[3]( ) = percent change from the 0 day value compared to the concurrent control.

3. Food Consumption

Animals received food (Purina Certified Rabbit Chow #5322) and tap water ad libitum. Food consumption was measured every second day from days 0 thru 20.

Mean food consumption was increased [7 to 18%] in males at all dose levels when compared to the concurrent controls. The increase, however, was not attributed to treatment. Females showed a mean decrease of 9% at 1000 mg/kg/day throughout the study compared to the concurrent control. None of the differences showed statistical significance.

4. Clinical Pathology

(a) Hematology and Clinical Chemistry

Blood samples for hematology and clinical chemistry were obtained prior to the start of the study and immediately prior to sacrifice on day 20. Animals were fasted overnight prior to blood sampling and sacrifice on day 20. Blood was collected by sampling from the central ear artery. Terminal blood samples were collected by percutaneous cardiocentesis. The following parameters were measured.

Hematology

x Hematocrit (HCT)[a]	x Leukocyte count (WBC)[a]
x Hemoglobin (HGB)[a]	x Platelet count[a]
x Erythrocyte count (RBC)[a]	x Leukocyte differential[a]
Mean corpuscular HGB (MCH)	Mean corpuscular HGB Concentration (MCHC)
Mean corpuscular volume (MCV)	Blood clotting measurements
Corrected leukocyte count (COR WBC)	x Erythrocyte morphology

Clinical Chemistry

Electrolytes:	Other
x Calcium[a]	x Albumin[a]
x Chloride[a]	Blood creatinine[a]
Magnesium[a]	x Blood urea nitrogen[a]
Phosphorus[a]	x Total cholesterol[a]
x Potassium[a]	x Globulins
x Sodium	x Glucose[a]
	x Total bilirubin[a]
Enzymes:	x Direct bilirubin
	x Total protein[a]
x Alkaline phosphatase	Triglycerides
x Alanine aminotransferase (SGPT)[a]	Serum protein electrophoresis
x Aspartate aminotransferase (SGOT)[a]	Triiodothyronine (T_3)
Cholinesterase[b]	Thyroxine (T_4)
Creatinine phosphatase[c]	x A/G Ratio
x Lactic acid dehydrogenase	
τ-Glutamyl transpeptidase [GGPT]	

[a] Required for subchronic and chronic studies.
[b] Required only for organophosphates and carbamates.
[c] Required for chronic studies.

(1) Hematology

No effects of biological importance were noted throughout the study in the treated versus the control groups.

(2) Clinical Chemistry

Significantly ($p \leq 0.05$) higher total serum protein (4.12 gm/dl vs. 4.72 gm/dl) was noted in the 300 mg/kg females when compared to the concurrent control. This increase was not considered to be of any toxicological significance for no dose response was seen.

b. Urinalysis:

Urine samples were collected before commencement of treatment and immediately prior to sacrifice on day 20.

The following parameters marked with an (X) were examined. Parameters marked with an (*) were required by the guidelines.

X Appearance*
X Sediment*
X Protein*
X Total Bilirubin
X Glucose*
- Volume*
X ketones*
X Specific Gravity
X pH
X Blood (occult)*
X Sediment*

Urine sediment was examined microscopically for: epithelial cells, polymorphonuclear leukocytes, red blood cells, casts, crystals and other abnormal components.

(1) No effects of biological importance were noted throughout the study in the control versus the treated groups.

5. Sacrifice and Pathology:

All animals were euthanized with intravenous sodium pentobarbital anesthesia and necropsied for gross and microscopic pathology on day 21.

6. Gross Pathology

All animals were subjected to gross pathological examination at study termination. The external surface of the body, all orifices and the cranial, thoracic, abdominal and pelvic cavities and their contents were examined.

Except for a 1.5 cm x 1.0 cm scab observed in the middle of the treatment site in 1 male at 1000 mg/kg/day, no treatment related gross dermal lesions were seen.

7. Organ Weights

Fasting terminal body weights, selected absolute and relative organ weights and the organ/brain weight ratio of the liver, kidneys, gonads, adrenal glands and brain were evaluated.

At 1000 mg/kg/day, females showed a statistically significant decrease in the adrenal weight, the adrenal/body weight and the adrenal/brain weight ratios when compared to the respective concurrent controls (Table 3).

Table 3

Mean Adrenal Weight, Adrenal/Body Weight Ratio and Brain/Weight Ratio in Female Rabbits Treated Dermally with Cacodylic Acid for 21 Days[1]

Dose Weight (mg/kg/ day)	Adrenal Wgt (gm)	Adrenal/ Body Wgt (%)	Adrenal/ Brain Wgt (%)
0	0.2974	0.0114	3.6208
100	0.2385	0.0092	2.8633
300	0.2414	0.0092	3.0077
1000	0.2144*	0.0083*	2.4500**

[1]Adapted from original report, p. 56, 60, and 64.
* $p \leq 0.05$
** $p \leq 0.01$

Males at all dose levels showed a decrease in the absolute testes weight, the testes/body weight and testes/brain weight ratios when compared to the concurrent control value (Table 4).

Table 4

Mean Testes Weight, Testes/Body Weight Ratio and Testes/Brain Ratio in Rabbits Treated Dermally with Cacodylic Acid for 21 Days[1,2]

Dose mg/kg/ day	Testes Weight (gm)	Testes/ Body Wgt (%)	Testes/ Brain Wgt (%)
0	3.81	0.15	43.65
100	3.42	0.13	40.62
300	3.59	0.14	42.56
1000	3.09	0.12	35.30

[1]Adapted from original report, p. 55, 59, and 63.
[2]No statistical significance noted at any dose level.

Table 5

Mean Ovary Weight, Ovary/Body Weight Ratio and Brain/Weight Ratio in Rabbits Treated Dermally with Cacodylic Acid for 21 Days[1,2]

Dose (mg/kg/ day)	Organ Weight (gm)	Organ/ Body Wgt (%)	Organ/ Brain Wgt (%)
0	0.3520	0.0136	4.3548
100	0.3260	0.0126	3.8799
300	0.3000	0.0114	3.7948
1000	0.2540	0.0099	2.9208

[1]Adapted from original report, p. 57, 61, and 65.
[2]No statistical significance was noted.

As shown above in Table 5, the absolute ovary weight, ovary/body weight and ovary/brain weight ratios showed a dose related decrease compared to the concurrent control values. However, due to the lack of collaborative histopathological changes in this organ, the changes in the organ weight parameters were attributed to lower body weights rather than to treatment.

8. **Histopathology**

The checked (X) tissues from all control and high-dose rats were examined. In addition, the liver, kidneys, thyroid, stomach, testes/epididymides, 4 sections of the nasal turbinates and gross lesions were examined for all low- and mid-dose animals. evaluation.

Digestive System	Respiratory System
x Salivary glands[a] x Esophagus[a] x Stomach x Duodenum[a] x Jejunum[a] x Cecum[a] x Colon[a] x Ileum[a] x Rectum[a] x Liver[ac] x Pancreas[a] Gall bladder[ab] Neurological System x Brain[ac] x Pituitary[a] x Peripheral nerve[ab] x Spinal cord (3 levels)[ab] x Eyes (optical nerve)[ab] Glandular System x Adrenals[a] Lacrimal glands[b] x Parathyroids[ad] x Thyroids[ad]	x Trachea[a] x Lung[a] Pharynx[a] Larynx[a] x Head with nasal turbinate Nose[a] Cardiovascular/Hemo.System x Aorta (thoracic)[a] x Heart[a] x Bone marrow[a] x Lymph nodes[a] x Spleen[a] x Thymus[a] Urinogenital System x Kidneys[ac] x Urinary bladder[a] x Testes[ac] x Epididymides x Prostate x Seminal vesicles x Uterus[a] x Ovaries[ac] Others x Skin x Mammary glands x All gross lesions and masses x Skeletal muscle[a]

a. Required for subchronic and chronic studies.
b. In subchronic studies examined only if indicated by toxicity or target organ involvement.
c. Organ weights required in subchronic and chronic studies.
d. Organ weights required for nonrodent studies.
e. Required for chronic inhalation study.

Histopathological changes observed in the testes are summarized in Table 6. Hypospermia was seen in 3/5 males at 1000 mg/kg/day compared to 1/5 males in the controls. Tubular hypoplasia was increased at 1000 mg/kg [4/5] compared to 0/5 in the controls. Incidences of other testicular lesions were comparable between treated and control groups.

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Table 6

Lesion Incidence in the Testes[1,2]

	Dose Level (mg/kg/day)			
	0	100	300	1,000
No. Organs Examined	5	5	5	5
Testes				
Aspermia	0	0	0	1
Hypospermia	1	1	1	3
Normal Spermatogenesis				
Bilateral	4	4	4	1
Unilateral	0	0	1	0
Tubular Hypoplasia, Bilateral, Diffuse	0	1	0	3
Tubular Hypoplasia, Bilateral, Multifocal	0	0	0	1

[1]Adapted from original report, p. 503.
[2]No statistical analysis performed.

Other incidental findings noted in both control and treated groups were similar to those seen in rabbits.

The ovaries in the treated groups were considered to be normal versus the control groups.

The differential red blood cell smear data from the terminally sacrificed animals revealed a small incidence of hypochromasia, anisocytosis (considerable variation in cell size) and poikilocytosis (variation in the shape of the red blood cell) in both males and females throughout all treated and control groups. These differences were not considered to be related to the administration of the test article for they occurred at all dose levels. [The reviewer notes that the raw data for the above observations were not found in the study].

D. DISCUSSION:

At the 1000 mg/kg dose, dermal irritation was noted in 1/5 male animals versus 0/5 control animals as evidenced by a scab in the middle of the treatment area as seen at the gross necropsy. Histologically, this lesion was characterized as epidermal hyperplasia, focal epidermatitis and hyperkeratosis. No signs of dermal irritation were seen in the other animals during the 21 day study.

A decrease in the rate of body weight gain of female animals was noted at 1000 mg/kg/day throughout the study. Male animals showed an increase in the rate of weight gain compared to the concurrent controls.

In the 1000 mg/kg/day animals, Cacodylic acid produced a decrease in the absolute testes weight, the testes/body weight and testes/brain weight ratios. Sperm production in the males was affected. Histologically, hypospermia and tubular hypoplasia were seen in 4/5 animals. Both effects occurred at the 1000 mg/kg/day. The prostate in all animals was considered normal; 1/5 males at 1000 mg/kg/day showed diffuse hypoplasia in the seminal vesicles.

Although the authors attribute the weight change in the testes to the fact that immature animals might have been used, the reviewer notes that most of the animals showing this affect was concentrated in the 1000 mg/kg/day group. If the animals were properly randomized, this variability should have been equally distributed throughout the various dose levels.

The ovaries also showed a decrease in the absolute weight, the ovary/body weight and ovary/brain weight ratios. Since pathological alterations were not seen in the treated groups the decreases were attributed to lower body weights rather than to treatment. The reviewer notes that the weight of the ovaries would not have been affected by the maturity of the animals.

The changes observed in the adrenal weights in females at 1000 mg/kg/day were not considered to be treatment related due to the lack of any histological changes in the organ.

E. CONCLUSIONS:

Cacodylic Acid was applied dermally under an occlusive bandage to 5 rabbits/sex/group at doses of 0 (Control), 100, 300 and 1000 mg/kg once daily, five days per week for 6 hours for a period of 21 days.

Cacodylic Acid did not elicit any effects on the rabbit skin. At 1000 mg/kg/day the test substance caused a decrease in the rate of body weight gain in females and testicular weight decreases and histopathological alterations in the testes in the males.

Under conditions of this study, the following NOEL and LOEL are established. In males, the LOEL is based on the decrease in testicular weights and histopathological lesions. In females, the LOEL is based on the decrease in body weight gain.

Dermal Irritation:	NOEL:	1000 mg/kg/day
	LOEL:	not achieved
Systemic Toxicity:	NOEL:	300 mg/kg/day
	LOEL:	1000 mg/kg/day

F. CORE CLASSIFICATION: - Minimum

This study satisfies the data requirement 82-2 for a repeated dose dermal toxicity in rabbits and is acceptable for regulatory purposes.