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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: ID. No.: 006836-00115, 90 Day Subchronic Oral Study in Rats with Dimethylhydantoin

Tox. Chem. No.: 114A
Project No.: 1-2456
Record No. : S403060

FROM: Melba S. Morrow, D.V.M. MSM 12/4/91
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CONCLUSIONS:
Under the conditions of the study, when dimethylhydantoin was administered to Crl:CDBR rats at doses of 0, 100, 300 and 1000 mg/kg for 90 days, the NOEL was 300 mg/kg and the LOEL was 1000 mg/kg based on statistically significant decreases in mean absolute and relative liver weights, decreases in mean body weight up to day 63 and decreases in food consumption in high dose males.

The study satisfies the the criteria set forth in the Subdivision F Guidelines 82-1 for a subchronic oral study and is classified as core guideline.

A copy of the DER is attached for your reference.

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Reviewed by: Melba S. Morrow, D.V.M. MSM 12/4/91 008972
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Joycelyn E. Stewart, Ph.D.
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DATA EVALUATION REPORT

STUDY TYPE: Subchronic 90 day Oral toxicity

GUIDELINE #: 82-1

TOX. CHEM. #: 114A

MRID #: 420092-01

TEST MATERIAL: Dimethylhydantoin

SYNONYMS: DMH, 5,5-dimethylhydantoin, Dantobrom

STUDY NUMBERS: 169070

SPONSOR: Lonza, Inc.
Fairlawn, N.J.

TEST FACILITY: Exxon Biomedical Sciences, Inc.
East Millstone, N.J.

TITLE OF REPORT: A 90 Day Subchronic Oral Toxicity Study in Rats with DMH

AUTHORS: T.M. Federici

REPORT ISSUED: July 25, 1991

CONCLUSIONS: Under the conditions of the study, when DMH was administered to Crl:CDBR rats at doses of 0, 100, 300 and 1000 mg/kg for 90 days, the NOEL was 300 mg/kg and the LOEL was 1000 mg/kg based on statistically significant decreases in mean absolute and relative liver weights, decreased mean body weights at day 63 and decreased mean food consumption in males.

CLASSIFICATION: Guideline
TOX. CATERGORY: N/A

MATERIALS: DMH, 99.5% purity, batch number F9421299, a white crystalline material was the test compound. Crl:CDBR (Sprague Dawley) rats were the test species. Animals were obtained from Charles River laboratories and were approximately 7 weeks of age at the start of the study. Males weighed 204.5 to 270.3 grams and females weighed 161.7 to 214.1 grams.

METHODS: After a 14 day acclimation period, animals were assigned to treatment groups and housed in an environment that allowed for a 12 hour light/dark cycle, temperature between 68 to 76 ° F and a relative humidity of 40 to 60%. Animals were assigned to the following treatment groups:

Group #	Target Dose (mg/kg)	Actual Dose (mg/kg)	Males	Females
1	0	0	15	15
2	100	102	15	15
3	300	303	15	15
4	1000	1019	15	15

The test material was mixed in reverse osmosis water to ensure a volume of 10 mL/kg. Test material was administered by gavage for five days a week for a total of 13 weeks. The amount of test material was recalculated weekly to allow for changes in body weight.

Homogeneity testing was conducted on samples from the 100 mg/kg and the 1000 mg/kg groups. Triple aliquots were analyzed and good uniformity was demonstrated at the levels tested. Samples of the test material in reverse osmosis water were diluted and analyzed by High Pressure Liquid Chromatography to determine the concentration of the test compound. Concentration verification analyses were within 9% of the target value. Determination of stability was performed in a previously conducted range finding study. The compound was found to be stable for up to 14 days upon refrigeration and upon being kept at room temperature.

Animals were observed twice daily for morbidity and mortality. Weekly observations were made for clinical signs of toxicity. Body weights were measured prior to the start of the study, on the first day of dosing and weekly thereafter. Body weights were also recorded at the time of sacrifice or at the time of death. Food consumption was measured weekly.

Ophthalmoscopic examinations were conducted prior to the start of treatment and during the final week of the study. Clinical chemistries were assessed for 10 animals/sex/group at terminal sacrifice. Blood samples were collected after methoxyflurane anesthesia from the abdominal aorta. The following parameters were assessed.

x Hematocrit (HCT)
x Hemaglobin (HGB)
x Leukocyte count (WBC)
x Erythrocyte count (RBC)
x Platelet count
x Leukocyte differential
Mean corpuscular hemaglobin
Mean corpuscular hemaglobin concentration
Mean corpuscular volume
x Reticulocytes

Blood clotting measurements:
Thromboplastin time
Clotting time
Prothrombin time

Electrolytes:
x Calcium
x Chlorine
Magnesium
x Phosphorus
x Potassium
x Sodium

Enzymes:
x Creatinine phophokinase
x Alkaline phosphatase
Lactic dehydrogenase
x SGPT
x SGOT
x GGT
Glutamate dehydrogenase
Cholinesterase

Other Serum Chemistry Values:
x Albumen
x Blood creatinine
x BUN
Cholesterol
x Globulin
x Glucose
x Total Bilirubin
x Total protein
Triglycerides
Serum protein electrophoresis

A full gross necropsy was performed on all animals at the end of the thirteen week period. Animals were terminated by exsanguination while they were under methoxyflurane anesthesia. Brain, liver, kidneys, adrenals and testes /ovaries were weighed. Tissue were preserved in 10% neutral buffered formalin, fixed and stained with hematoxylin and eosin and submitted for histopathological examination.

The following CHECKED (x) tissues were collected for histological examination on 10 animals/sex/dose for high dose and controls. Gross lesions, tissue masses, liver, lung and kidneys were collected from 10 animals/sex/dose for the mid and low dose groups. Weighed organs are designated by (xx). Values required under Subdivision F Guidelines are designated by (*).

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Digestive system	Cardiovasc./Hemat.	Neurologic
Tongue	x Aorta	xx Brain *
x Salivary glands *	x Heart *	x Periph. nerves *
x Esophagus *	Bone marrow *	x Spinal cord
x Stomach *	x Lymph nodes*	
x Duodenum *	x Spleen *	
x Jejunum *	x Thymus *	Glandular
x Ileum *		x Parathyroids
x Cecum *		xx Adrenals *
x Colon *	Urogenital	x Thyroid *
Rectum *	xx Kidneys *	x Pituitary *
	x Urinary bladder *	x Mammary
xx Liver *	xx Testes *	
Gall bladder	x Epididymides	Other
x Pancreas *	x Prostate	x Bone (femur)
	x Seminal vesicle	x Skin
Respiratory	xx Ovaries	x Skel. muscle
x Trachea	x Uterus *	x All gross lesions
x Lung *	Vagina	
Nose		
Pharynx		
Larynx		

QUALITY ASSURANCE: A statement of quality assurance dated 7/23/91 was included in the submission along with a statement of compliance with good laboratory practices dated 7/24/91.

STATISTICAL ANALYSIS: Statistical analysis of hematology, serum chemistry, organ weights, organ:body weight ratios, organ:brain weight ratios , body weights and food consumption were analysed using Bartlett's Test to determine variance. Parametric methods were used if variances were equal. These included one way ANOVA followed by Dunnett's.

Kruskall- Wallis was used for nonparametric procedures followed by Dunn's summed rank. Jonckheere's test for monotonic trend was also used. Significance was at $p \leq 0.05$ and $p \leq 0.01$ for all tests except Bartlett's. Significance for Bartlett's equal variance was $p \leq 0.01$.

RESULTS:

Mortality/Clinical Observations

No treatment related effects were reported for clinical signs of toxicity. There was no effect on mortality in either sex. One control male was euthanized on day 41 because of a broken snout and one female in the 300 mg/kg group was found dead. The cause

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of death in this animal was associated with inappropriate dosing technique.

Body Weight:

In high dose males, mean body weight was depressed when compared to controls; however, the observed decreases in body weight were not considered to be significant. The highest reported difference between high dose and control groups was 8%. In females, mean body weights were comparable to or slightly higher than for controls.

Food Consumption:

A decrease in food consumption was observed in high dose males during most of the study and in low dose males during the last 2 to 3 weeks. In females, food consumption was comparable to controls throughout the 90 day study. (See Table I for male food consumption data).

Clinical Chemistry, Hematology

No treatment related effects were observed on hematology or serum chemistry values.

Organ Weights, Gross Pathology

Mean absolute and relative liver weights were decreased for high dose males when compared to controls. It is believed that the observed decreases in liver weight are the result of decreases in body weights for animals in this group and are probably associated with the test material.

In high dose females, mean absolute and relative liver weights were increased. Mean absolute kidney weights were also increased in these high dose females. The observed increases in organ weights in this group of animals are believed to be associated with increases in body weight.

At necropsy several observations were made; however, none could be associated with the administration of the test material. These included gas in the gastrointestinal tract, emaciation, small senimal vesicles, liquid in stomach, distended uterus, large ovaries, liver thickening, lung discoloration and distended cecum.

Histopathology:

None of the observed histological lesions were associated with the administration of the test compound. (See Table II for the distribution of the most frequently occurring lesions).

DISCUSSION:

Based on the results, the NOEL for systemic toxicity was 300 mg/kg/day and the LOEL was 1000 mg/kg/day based on decreases in absolute and relative liver weights, slight decreases in mean body weight and decreases in food consumption observed at several intervals throughout the study in male rats.

The study meets the guideline criteria for a 90 day subchronic oral study.

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TABLE I
Mean Weekly Food Consumption[a]

Males (g)	Concentration (mg/kg/day)			
Week	0	100	300	1000
1	186.0	183.3	178.5	172.4*
2	194.7	194.9	185.5	177.2*
3	199.5	197.3	192.3	184.5*
4	199.0	200.6	190.3	187.7
5	200.8	196.5	196.0	184.1*
7	213.3	213.0	196.7	185.1*
9	202.2	202.6	197.2	183.0**
11	204.9	184.4	197.6	195.3
13	204.3	206.5	202.2	196.1

a = extracted from Table 3 of study report
* = $p \leq 0.05$
** = $p \leq 0.01$

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TABLE II
Histopathology Lesions*

Males

Organ/Lesion	Dose(mg/kg) 0	100	300	1000
Liver Mononuclear cell infiltration	8/11	6/10	5/10	7/10
Lymph Node Hyperplasia	6/11	0/10	0/10	3/10
Kidney Degenerative cortical tubules	1/11	2/10	2/10	2/10

Females

Liver Mononuclear cell infiltration	4/10	5/10	6/10	5/10
Liver Hyperplasia	4/10	0/10	0/10	4/10
Kidney Degenerative cortical tubules	1/10	1/10	2/10	0/10

* = These are a few selected lesions. There was no treatment related histopathology in the 90 day study.