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MAY 12 1995

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

MEMORANDUM

Subject: EPA ID # 006315: 5,5-Dimethylhydantoin (DMH)-  
-Review of 90-Day Subchronic Dermal Toxicity  
Study in Rats (MRID No. 431739-01)

Tox. Chem. Number: 114A; 306,  
309C

Submission Number: S462520

DP Barcode: D201469

PC Code: 006315

From: Paul Chin, PhD *Paul Chin 4/25/95*  
Section 2  
Toxicology Branch I  
Health Effects Division (7509C)

To: Tom Myers, PM 51  
Reregistration Branch  
Special Review and Reregistration Division (7508W)

Thru: Joycelyn Stewart, Ph.D. *J 4/25/95 - KB 5/4/95*  
Section Head  
Section 2, Toxicology Branch I  
Health Effects Division (7509C)

Registrant: Lonza Inc.

Conclusions:

Subchronic Dermal Toxicity Study in rats with dimethylhydantoin was reviewed by the Toxicology Branch I. This study is classified as core minimum and satisfies the guideline requirement (82-3) for 90-day subchronic dermal toxicity study in rats.

[The Data Evaluation Reports are appended to this memorandum.]

The following conclusion has been made regarding the toxicity of dimethylhydantoin:

EXECUTIVE SUMMARY: Male and female CD<sup>0</sup> rats were treated with 5, 5-dimethylhydantoin (DMH) by dermal occlusion at doses of 0, 39, 130, or 390 mg/kg/day for 6 hours/day, 5 days/week for 13 weeks. No statistically significant differences in group body weight



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means or cumulative body weight gains occurred for any treated group of either sex as compared to controls. No treatment-related mortality, skin irritation, or clinical signs of toxicity were seen in any rats. DMH did not cause any alterations in hematology, clinical chemistry, or gross or microscopic pathology of male or female rats. Males receiving 39 ppm or 390 ppm had significant decreases in absolute adrenal gland weight (12.5% and 14.3 %, respectively;  $p < 0.05$ ) and adrenal gland to brain weight ratio (15.2%;  $p < 0.01$  and 13.6%;  $p < 0.05$ , respectively). Females given 130 ppm also had a significant decrease in adrenal gland to body weight ratio (11.1%;  $p < 0.05$ ). However, these differences were slight, not dose-responsive, and not correlated to any microscopic lesions.

Under conditions of this study, the NOEL is 390 mg/kg/day; a LOEL was not identified. However, DMH levels for this study were set at the maximum water solubility (13%) and the maximum volume which could be administered in the test system (3 ml/kg/day). Therefore, higher doses would not be possible. Also, according to information from the sponsor, the doses used in this study exceed normally, expected human exposure conditions.

REQUESTED ACTION:

The Reregistration Division requested that the Toxicology Branch review the above study with DMH. C:\wp51\DMH\subchr-derm 4/24/95

# DATA EVALUATION REPORT

5, 5-DIMETHYLHYDANTOIN (DMH)

Study Type: SUBCHRONIC DERMAL - RAT (82-3)

Prepared for

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

Prepared by

Chemical Hazard Evaluation Group  
Biomedical and Environmental Information Analysis Section  
Health Sciences Research Division  
Oak Ridge National Laboratory\*  
Oak Ridge, TN 37831  
Task Order No. 94-17

Primary Reviewer:

Carol S. Forsyth, Ph.D.

Signature: \_\_\_\_\_

Date: \_\_\_\_\_

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Robert H. Ross, M.S., Group Leader

Signature: \_\_\_\_\_

Date: \_\_\_\_\_

Quality Assurance:

Susan Chang, M.S.

Signature: \_\_\_\_\_

Date: \_\_\_\_\_

## Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

\*Managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under Contract No.  
DE-AC05-84OR21400

[DMH] Subchronic Dermal Study (82-3)

EPA Reviewer: E. Budd, M.A. \_\_\_\_\_

Date: \_\_\_\_\_

Review Section III, Toxicology Branch I (7509C)

EPA Section Head: M. Copley, D.V.M. \_\_\_\_\_

Date: \_\_\_\_\_

Review Section IV, Toxicology Branch I (7509C)

## DATA EVALUATION REPORT

STUDY TYPE: 90-Day Subchronic Dermal - Rat (82-3)

TOX. CHEM. NO.: 114A, 306, 309C, 366D, 568E

P.C.CODE.: 006315

MRID NO.: 431739-01

TEST MATERIAL: 5,5 - Dimethylhydantoin (DMH)

SYNONYMS: none given

STUDY NUMBER: 92N1016

SPONSOR: Lonza Inc., 17-17 Route 208, Fair Lawn, NJ 07410

TESTING FACILITY: Bushy Run Research Center (BRRC), Union Carbide Chemicals and Plastics Company Inc., 6702 Mellon Road, Export, PA 15632-8902

TITLE OF REPORT: Ninety-Day Dermal Toxicity Study with 5,5-Dimethylhydantoin (DMH) in CD<sup>0</sup> Rats

AUTHOR(S): J.S. Chun and K.A. Loughran

REPORT ISSUED: March 10, 1994 (study completion date)

EXECUTIVE SUMMARY: Male and female CD<sup>0</sup> rats were treated with 5, 5-dimethylhydantoin (DMH) by dermal occlusion at doses of 0, 39, 130, or 390 mg/kg/day for 6 hours/day, 5 days/week for 13 weeks. No statistically significant differences in group body weight means or cumulative body weight gains occurred for any treated group of either sex as compared to controls. No treatment-related mortality, skin irritation, or clinical signs of toxicity were seen in any rats. DMH did not cause any alterations in hematology, clinical chemistry, or gross or microscopic pathology of male or female rats. Males receiving 39 ppm or 390 ppm had significant decreases in absolute adrenal gland weight (12.5% and 14.3 %, respectively;  $p < 0.05$ ) and adrenal gland to brain weight ratio (15.2%;  $p < 0.01$  and 13.6%;  $p < 0.05$ , respectively). Females given 130 ppm also had a significant decrease in adrenal gland to body weight ratio (11.1%;  $p < 0.05$ ). However, these differences were slight, not dose-responsive, and not correlated to any microscopic lesions.

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Under conditions of this study, the NOEL is 390 mg/kg/day; a LOEL was not identified. However, DMH levels for this study were set at the maximum water solubility (13%) and the maximum volume which could be administered in the test system (3 ml/kg/day). Therefore, higher doses would not be possible. Also, according to information from the sponsor, the doses used in this study exceed normally, expected human exposure conditions.

This study is classified as core-minimum and satisfies the guideline requirements for a 90-day subchronic dermal study (82-3) in rats.

Special Review Criteria (40 CFR 154.7) None

## A. MATERIALS

### 1. Test material: 5,5-Dimethylhydantoin (DMH); Dantoin

Description: white, crystalline powder

Lot/Batch No.: NO432543

Purity: >99.8 %

Stability of compound: not available (on file with sponsor)

CAS No.: 77-71-4

Structure: not available

### 2. Vehicle and/or positive control

Milli-Q<sup>®</sup> filtered water was used as the diluent and negative control. No positive control was used.

### 3. Test animals

Species: Rat

Strain: CD<sup>®</sup>

Age and weight at study initiation: 7.5 weeks; males: 236.3 - 313.6 g; females: 159.1 - 215.2 g.

Source: Charles River Laboratories, Portage, MI.

Housing: Rats were housed individually in stainless steel hanging wire cages.

Environmental conditions:

Temperature: 66-77°F

Humidity: 40-70%

Air changes: at least 10 changes/hour

Photoperiod: 12 hour light/dark cycle

Acclimation period: 3 weeks

## B. STUDY DESIGN

### 1. Animal assignment

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All animals used in the study met physical and weight gain criteria for this age and strain of rat. Also, any animals that did not adapt to the pretreatment wrapping procedure were not used in the study. Rats were assigned to treatment groups using a stratified randomization procedure based on body weight. Study design is presented in Table 1.

An additional 10 rats/sex were selected upon receipt from the supplier for a pretest health screen consisting of a viral screen, examinations for fecal parasites, clinical pathology evaluation, gross necropsy examinations, and histopathology of selected tissues.

TABLE 1: STUDY DESIGN				
Dose Group	No. of Animals		Dose (mg/kg/day)	Volume of Dose <sup>a</sup> (ml/kg/day)
	Male	Female		
Control <sup>b</sup>	15	15	0	3.0
Low	15	15	39	0.3
Mid	15	15	130	1.0
High	15	15	390	3.0

Data taken from page 10, MRID No. 431739-01.

<sup>a</sup>A concentration of 13% (w/w) DMH in Milli-Q<sup>®</sup> filtered water was used for all treatment groups.

<sup>b</sup>Control animals were treated with Milli-Q<sup>®</sup> filtered water.

Dose selection rationale: In a preliminary study (included as Appendix 13 in MRID No. 431739-01), the testing laboratory treated 5 male CD<sup>®</sup> rats dermally 6 hours/day for 5 days with 2 ml/kg of 5 or 10% aqueous solutions or with 25 or 50% aqueous mixtures of DMH. These correspond to doses of 100 to 1000 mg/kg/day. No toxicity or skin irritation was seen in any animal at any dose level. Homogeneous mixtures were not achieved above the saturation of DMH in water (13%) due to clumping and were difficult to apply evenly. Information from the sponsor to the testing laboratory indicated that concentrations above the saturation point would not simulate human exposure conditions. Therefore, DMH levels for the 90-day study were chosen based on the results of the 5-day preliminary study, the maximum water solubility (13%), and the maximum volume which could be administered in the test system (3 ml/kg/day).

2. Test substance preparation and analysis

The dosing solution was prepared by diluting DMH with the appropriate amount of Milli-Q® water to make a 13% solution. A fresh dosing solution was prepared weekly and stored at room temperature in a glass Erlenmeyer flask.

**Results –**

- a. Homogeneity analysis – The homogeneity of the dosing solution was 13.16, 13.06, and 13.13% for top, middle, and bottom regions, respectively. The mean of the measured concentrations of DMH in the 13% solution was 100.9% of nominal.
- b. Stability analysis – The 13% dosing solution was found to be stable at room temperature for 7 and 14 days. The mean measured concentration of triplicate samples ranged from 103.9 to 104.4% of nominal.
- c. Concentration analysis – Duplicate samples of the 13% dosing solutions for Weeks 1, 2, 3, 4, 8, and 13 were analyzed for DMH. Absence of test material was confirmed in the control solution. DMH concentrations ranged from 12.89 to 13.82% which are 99.2 and 106.3% of nominal, respectively. These data are listed in Table A-1 in the Appendix.

3. Dose Application

Prior to study initiation, the fur on the back of each rat was clipped with a veterinary clipper from the scapular region to just above the rump. Animals were clipped as needed during the study and on each Friday afternoon. The dosing solution was applied directly to the back at each application, the application site was covered with a nonsterile gauze pad, the rat was wrapped with VETRAP® Bandaging Tape, and the bandage secured with Elastikon® Elastic Tape. The dosing site remained occluded 6 hours/day during which the rats were deprived of food. After removal of the wraps, the skin was rinsed with water and blotted with a dry cloth. All animals were dosed 5 days/week, Monday through Friday, for 13 weeks.

4. Diet

Ground Purina® Certified Rodent Chow®, except during daily exposure periods, and water were available *ad libitum*.

5. Statistics

The data for quantitative continuous variables were intercompared for the 3 treatment groups and the control group by use of Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. Nonparametric data were evaluated using the Kruskal-Wallis test followed by the Mann-Whitney U-test when appropriate. The level of significance for all tests was set at  $p < 0.05$  (two-tailed).

6. Signed and dated GLP and quality assurance statements were present.

## C. METHODS AND RESULTS

### 1. Observations

All rats were observed twice daily for mortality and signs of toxicity. Detailed physical examinations, with special attention paid to the skin of the dose site, were conducted at each weekly weighing interval and prior to sacrifice.

**Results** – No treatment-related mortality, skin irritation, or clinical signs of toxicity were seen in any rats. Sporadic clinical observations included alopecia, mild eye and nose discharge, skin excoriation, and overgrown incisors. One control male rat was found dead on day 17 and 1 low-dose male rat was sacrificed moribund on day 39. All other animals survived to scheduled sacrifice. Tremors, cold extremities, and pallor were observed for the two rats that died early. The low-dose rat also had urine stains on its abdomen. Death of this animal was not considered treatment-related due to lack of a dose-response.

### 2. Body weight

Animals were weighed prior to dose initiation (Week 0) and weekly thereafter. Fasting body weights were taken immediately before sacrifice.

**Results** – No statistically significant differences in group body weight means or cumulative body weight gains occurred for any treated group as compared to controls. High-dose males had consistently lower mean body weights than controls but the differences were not significant. This trend was not apparent in females.

### 3. Food consumption

Food consumption for each animal was measured weekly.

#### **Results** –

- a. Food consumption – No treatment-related effects on food consumption occurred for any group of either sex. A statistically significant ( $p < 0.01$ ) increase was measured for 130 mg/kg/day females at Week 7 - 8, but this was considered sporadic and not dose-related.
- b. Food efficiency – Feed efficiency ( $\{\text{body weight gain [kg]}/\text{food consumption [kg per unit time]}\} \times 100$ ) values were not calculated by the study authors but were derived from the body weight gain and food consumption data. The overall mean food efficiency value was slightly lower for high dose males as compared to controls. However, there were no significant differences in overall food efficiency means of any group of either sex (Table 2).

TABLE 2: OVERALL MEAN FOOD EFFICIENCY (WEEKS 1-13)		
Dose Group (mg/kg/day)	Males	Females
0	8.3	4.4
39	8.4	4.2
130	8.7	4.7
390	7.9	4.4

Data derived from Tables 3, 5, 7, and 8, pp. 21, 24, 27, and 28, respectively, MRID No. 431739-01.

4. Ophthalmoscopic examination

The eyes of all rats were examined before study initiation and during Week 13 using indirect ophthalmoscopy and biomicroscopy. Tropicamide (1%) was used to dilate the pupils.

**Results** – All ophthalmoscopic findings were considered incidental to treatment with DMH. The most common pre- and post-treatment anomaly was the presence of corneal crystals. Tear film disruption observed in the rats at the pretest examination was attributed to stress from sham wrapping of the animals prior to study initiation.

5. Blood was collected for hematology and clinical analysis prior to sacrifice from all surviving animals. Rats were fasted overnight prior to blood collection. Animals were anesthetized with methoxyflurane and blood was collected via retroorbital sinus puncture. The CHECKED (X) parameters were examined.

a. Hematology.

X	Hematocrit(HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
Blood clotting measurements			
(Thromboplastin time)			
(Clottng time)			
(Prothrombin time)			

\*Required for subchronic studies.

[DMH] Subchronic Dermal Study (82-3)

**Results** – No treatment-related effects on any hematology parameters were seen for any group of either sex. High-dose males had a slight but significantly ( $p < 0.05$ ) increased lymphocyte count but the mean was within the historical control range established for males of this age and strain of rat. There was also a dose-responsive increase in eosinophils for males but none of the values were statistically significantly different from controls.

**b. Clinical chemistry**

<u>X</u>		<u>X</u>	
	Electrolytes		Other
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorus*		Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
		X	Total serum protein (TP)*
	Enzymes		Triglycerides
X	Alkaline phosphatase (ALK)		Serum protein electrophoresis
	Cholinesterase (ChE)		
	Creatine phosphokinase*		
	Lactic acid dehydrogenase (LDH)*		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

\* Required for subchronic studies.

**Results** – No treatment-related effects on clinical chemistry parameters were observed for any dose group of either sex. Slight but significant ( $p < 0.05$ ) increases in alanine aminotransferase occurred in low- and mid-dose females, however, the biological significance of this change is unclear.

**6. Urinalysis**

Urinalysis was not performed on any rats on this study.

**7. Sacrifice and pathology**

One control group male was found dead on day 17 and one low-dose male was sacrificed moribund on day 39. All other animals survived to scheduled sacrifice. Rats were anesthetized with methoxyflurane and killed by exsanguination. Gross pathological examinations were conducted and the CHECKED (X) tissues were

collected for histological examination. The (XX) organs, in addition, were weighed.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta*	XX	Brain**
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen	X	eye (optic n.)*
X	Jejunum*	X	Thymus*		Glandular
X	Ileum*		Urogenital	XX	Adrenal gland*
X	Cecum*	XX	Kidneys**	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes**	X	Parathyroids***
XX	Liver**	X	Epididymides	X	Thyroids***
	Gall Bladder*	X	Prostate		Other
X	Pancreas*	X	Seminal vesicle	X	Bone*
	Respiratory	XX	Ovaries**	X	Skeletal muscle*
X	Trachea*	X	Uterus*	X	Skin*
X	Lung*			X	All gross lesions and masses*
	Nose				
	Pharynx				
	Larynx				

\* Required for subchronic and chronic studies.

+ Organ weight required in subchronic and chronic studies.

\*\*Organ weight required for non-rodent studies.

### Results -

- a. Organ weight - There were no treatment-related changes in absolute organ weights or organ weight ratios of any group of either sex. Low- and high-dose males had significant decreases in absolute adrenal gland weight (12.5% and 14.3%, respectively;  $p < 0.05$ ) and the adrenal to brain weight ratio (15.2%;  $p < 0.01$  and 13.6%;  $p < 0.05$ , respectively). Mid-dose females also had a significant decrease in the adrenal to body weight ratio (11.1%;  $p < 0.05$ ). However, these differences were slight, not dose-responsive, and not correlated to any microscopic lesions.
- b. Gross pathology - No DMH-related abnormalities were observed on the skin of any treated rat of either sex. Gross lesions observed at necropsy such as color

[DMH] Subchronic Dermal Study (82-3)

changes of the liver, lungs, and thymic region and lymph node abnormalities were spurious and not dose-related.

c. Microscopic pathology -

1) Non-neoplastic - Mild, multifocal epidermitis was observed in the treated skin of one low dose male. No other microscopic abnormalities occurred in the skin of treated rats of either sex. Mid-dose females had a significantly ( $p < 0.05$ ) increased incidence of lymph node hyperplasia but lymph node abnormalities were observed in treated and control rats of both sexes. Lesions of the liver and kidney were observed for both sexes but were not dose related. Males also had inflammation and mineral deposits in the prostate, but this occurred in controls as well as treated animals. The two male rats that died early had evidence of a urogenital infection.

2) Neoplastic - No neoplastic lesions were observed in any rat of either sex.

D. DISCUSSION

Male and female CD<sup>\*</sup> rats were treated dermally with 0, 39, 130, or 390 mg/kg/day of 5, 5-dimethylhydantoin (DMH) 6 hours/day, 5 days/week for 13 weeks. No statistically significant differences in group body weight means or cumulative body weight gains occurred for any treated group as compared to controls. The trend toward lower mean body weights in the high-dose males as compared to controls did not occur in the preliminary dose-finding study nor was this trend apparent in females. Due to the decreased weight gain of the high-dose males, this group had a slight, although not significant, decrease in food efficiency. Dermal treatment with DMH did not cause any alterations in hematology, clinical chemistry, or gross or microscopic pathology of male or female rats. Decreased adrenal weight of the low- and high-dose males is probably not a result of DMH application since the effect was mild, not clearly dose-responsive, and did not occur in females.

Under conditions of this study, the NOEL exceeds 390 mg/kg/day; a LOEL was not identified. However, DMH levels for this study were set at the maximum water solubility (13%) and the maximum volume which could be administered in the test system (3 ml/kg/day). Therefore, higher doses would not be possible. Also, according to information from the sponsor, the doses used in this study exceed normally expected human exposure conditions.

E. STUDY DEFICIENCIES

Clinical chemistry parameters not measured include lactic acid dehydrogenase, creatine phosphokinase, cholesterol, and magnesium. Failure to collect these data does not hinder interpretation, however, since no gross or microscopic lesions were observed to indicate that these parameters would have been affected.

[DMH] Subchronic Dermal Study (82-3)

The authors should have calculated body weight gain as a function of food consumption in order to determine whether the decreased weight gain in high-dose males was treatment-related.