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

MAY - 1 9 1995

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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES
TOXIC SUBSTANCES

Subject: EPA ID # 006315: 5,5-Dimethylhydantoin (DMH)--Review of Carcinogenicity Study in Mice and Chronic Toxicity/Carcinogenicity Study in Rats (MRID No. 433977-01 and -02)

Tox. Chem. Number: 114A, 306, 309C, 366D, 568E
Submission Number: S475555
DP Barcode: D208529

From: Paul Chin, PhD Paul Chin 5/16/95
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. 5/16/95
Section Head
Section 2, Toxicology Branch I
Health Effects Division (7509C) 5/19/95

Registrant: Lonza Inc.

EXECUTIVE SUMMARY:

An carcinogenicity study in mice and a combined chronic toxicity/carcinogenicity in rats with dimethylhydantoin were reviewed by the Toxicology Branch I. These studies are **classified as core guideline** and **satisfy** the guideline requirements for carcinogenicity in mice (83-2) and combined chronic toxicity /carcinogenicity in rats (83-5), respectively.

[NOTE: 5,5-dimethylhydantoin is the organic moiety of 1-bromo-3-chloro-5,5-dimethylhydantoin. The Agency agreed that toxicology studies on the technical should be performed on the organic moiety.]

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

The following conclusions has been made regarding the toxicity of 5,5-dimethylhydantoin:

Carcinogenicity study in mice

DMH was administered in the diets of CD-1 mice at dietary concentrations of 0, 400, 1850 or 8500 ppm (target doses of 0, 100, 300 or 1000 mg/kg/day) for 78 weeks. Two control groups were used. At the highest dose tested, there was no evidence of carcinogenicity, nor were there compound related clinical signs, effects on mortality or on clinical pathology. In males at the highest dose level, there was a statistically significant (4 -9%) decrease in the mean absolute body weight and in mean body weight gain (19 and 28%, respectively, when compared to controls 1 and 2) which persisted from week 16 of the study till the study termination. Based on the decreases in body weight and body weight gain in males, the NOEL was 300 mg/kg and the LOEL was 1000 mg/kg. No signs of toxicity were reported in females.

Doses were adequate since the highest dose represents the limit dose.

The study satisfies the guideline requirements for a mouse carcinogenicity study as set forth in Subdivision F, Guideline 83-2.

Cor chronic toxicity/carcinogenicity in rats

In a chronic toxicity/carcinogenicity study, 5,5-dimethylhydantoin (DMH) was fed to 60 CD rats/sex/dose for 104 weeks at dietary levels of 0 (first control), 100, 300, 1000, or 0 (second control) mg/kg/day. These dosage levels correspond to dietary concentration of DMH over the course of the study that ranged between 1080-26794 ppm (males) and 945-20890 ppm (females).

In both sexes at the highest dose tested, there was no evidence of compound related clinical signs, mortality, food consumption, organ weight changes or on clinical pathology. At the highest dose level, statistically significant ($p<0.01$ for the first control and $p<0.05$ for the second control groups) treatment-related effects on body weight and body weight gain were observed in females only at weeks 90, 92, 94, and 96. During weeks 90 to 96 of the study, body weights were 14-15% lower than control 1 and 9% lower than control 2 and the body weight gain were 23-24% lower than the control 1 and 16% lower than control 2. There were statistically significant increased incidences of hyperplasia of submandibular lymph nodes [5/19 (26%) vs 0/31 to 1/33 (0-3%) of both controls] at week 104 ($p<0.05$ and $p<0.01$ for controls 1 and 2, respectively) in the high dose males only. This increased incidence was considered to be related to the administration of the DMH.

The NOEL for systemic toxicity was 300 mg/kg/day and the LEL was 1000 mg/kg/day based on the decreases in body weight and body

weight gain in females and hyperplasia of submandibular lymph nodes in males.

There was no increases in tumor incidences for the DMH treated groups when compared to the control group.

Dosing was adequate since the highest dose represents the limit dose.

The study is classified as **Core Guideline and satisfies** the guideline requirement for a chronic toxicity/carcinogenicity study (83-5) in rats.

REQUESTED ACTION:
The Reregistration Division requested that the Toxicology Branch review the above studies with DMH.

C:\wp51\DMH\onco-mice/chr-ono-rat 5/10/95

Reviewed by: [illegible] /15/95
Section II [illegible] Branch [illegible] (H[illegible])
Secondary Reviewer: [illegible]celyn E. Stewart, Ph.D. 5/11/95
Section II, [illegible] Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity - Mouse

GUIDELINE #: 83-2

TOX. CHEM. #: 114A

MRID #: 433977-01

TEST MATERIAL: 5,5- Dimethylhydantoin (99.8%)

SYNONYMS: DMH

STUDY NUMBERS: 91N0112

SPONSOR: Lonza Inc.
Fair Lawn, N.J.

TESTING FACILITY: Bushy Run Research Center
Union Carbide Corporation
Export, Pa.

TITLE OF REPORT: Chronic Dietary Oncogenicity Study with 5,5-Dimethylhydantoin (DMH)

AUTHORS: Hermansky and Loughran

REPORT ISSUED: August 31, 1994

EXECUTIVE SUMMARY:

DMH was administered in the diets of CD-1 mice at dietary concentrations of 0, 400, 1850 or 8500 ppm (target doses of 0, 100, 300 or 1000 mg/kg/day) for 78 weeks. At the highest dose tested, there was no evidence of carcinogenicity, nor were there compound related clinical signs, effects on mortality or on clinical pathology. In males at the highest dose level, there was a statistically significant (4 -9%) decrease in the mean absolute body weight and in mean body weight gain (19 and 28%, respectively, when compared to controls 1 and 2) which persisted from week 16 of the study. Based on the decreases in body weight and body weight gain in males, the NOEL was 300 mg/kg and the LOEL was 1000 mg/kg. No signs of toxicity were reported in females.

Doses were adequate since the highest dose represents the limit dose.

The study satisfies the guideline requirements for a mouse oncogenicity study as set forth in Subdivision F, Guideline 83-2.

MATERIALS:

The test substance was 5,5-dimethylhydantoin (DMH). lot number NO432543. The test substance was a white crystalline solid that was 99.8% pure. Male and female CD-1 mice were the test animals. At their arrival from Charles River Laboratories in Portage, Michigan, the animals were 31 days old.

METHODS:

Upon arrival at the test site, 10 male and 10 female mice were subjected to a pretest health screening that consisted of a viral screen, a test for parasites, gross and histopathology on selected tissues. Mice not selected for pretest were housed 2 to a cage for the first week. Ater the first week the animals were individually housed. The environmental temperature ranged from 66 to 77 degrees F, and the relative humidity was maintained at 40 to 70 %. Animals were on a 12 hour light/dark cycle and food and water were available ad libitum.

The acclimation period for animals selected to be in the study was approximately 3 weeks, at which time, animals were weighed three times, clinical examinations were conducted weekly and cage side observations and mortality checks were conducted twice daily. Assignment to one of the 5 designated treatment groups was by the use of a random procedure.

The following treatment groups were used in the study:

Group	DMH Dose (mg/kg/day)	Animals M	Animals F
Control 1	0	60	60
Low	100	60	60
Intermediate	300	60	60
High	1000	60	60
Control 2	0	60	60

The mice received these dietary concentrations for 78 weeks, with the exception of the 10 animals in the high dose and both control groups that were selected for clinical pathology examinations at 12 months. Fresh diets were prepared each week. Some females in the mid and high dose groups were inadvertently administered diets that were prepared for males during week 51 to 52. During thsi interval, the test substance consumption values were not calculated for the affected groups.

Diets were prepared by adding DMH to ground rodent chow. The mixtore was milled in a ball mill and then mixed with a Hobart mixer to provide for distribution of the chemical in the diet. The dose levels selected for this study were based on the results

of a 28 day range finding study in the same strain of animals. In this study, the test material was administered at dietary concentrations of 0, 1000, 3500, or 7000 ppm for 28 days. There were no treatment related effects at the highest dose tested and DMH did not appear to affect the palatability of the feed. The 700 ppm dose is equivalent to a limit dose of 1000 mg/kg and this was selected as the highest dose for testing in the oncogenicity study. The low and intermediate doses were selected based on a half log scale below the high dose. Two control groups were used to aid in the identification of false positive effects. Controls were not combined because of the independent manner in which the data were collected.

Homogeneity, Stability and Concentration

The concentration of the test material in the diet was analyzed using high performance liquid chromatography (HPLC). The stability of diets containing concentrations of 400 and 8500 ppm were analyzed prior to the start of the study. Stability was also determined for diets stored under room temperature in feeders on the day of preparation and after days 7 and 14 in open and closed containers and after day 21 in a closed container.

The homogeneity of the DMH in 400, 1850 and 8500 ppm diets was evaluated prior to the start of the study. Diets were verified for DMH for the first four weeks of the study and every fourth week, thereafter.

Animal Observations:

Animals were observed for mortality twice daily. Clinical observations and palpations were performed once each week and body weight and food consumption were collected weekly for the first 14 weeks and every other week, thereafter.

Blood was collected for clinical pathology (hematology) at 12 and at 18 months. Ten animals per sex from the high dose and control groups were evaluated at month 12 and 10 animals per sex per group from all dose groups were evaluated at 18 months. The following parameters were measured or calculated:

Hematocrit	Hemoglogin
Erythrocyte count	MCV
MCH	MCHC
Total leukocytes	Differential Leukocyte count
Platelet	

At the end of the study (78 weeks), animals were anesthetized with methoxyflurane and exsanguinated by severing the brachial vessels. Body weights were obtained on the day of sacrifice and the following tissues were collected and retained in 10% neutral buffered formalin (NBF). Weighed organs are designated by (xx). All tissue from the high dose and control groups were examined histologically. Lungs, liver, kidneys and all gross lesions were examined microscopically in the low and intermediate groups.

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

STATISTICS:

Levene's test, ANOVA, and t-tests were used to compare data from the three groups. Non-parametric data were analyzed using Kruskall- Wallis and Mann- Whitney U tests. Mortality and mean time to first palpable mass were analyzed by life-table analyses. Incidence data were compared using the Fisher's Exact Test. Significance was at $p < 0.05$.

QUALITY ASSURANCE:

A statement of Quality Assurance dated 8/31/94 and a statement of compliance with Good Laboratory Practices, dated 8/25/94 were included in the submission. In the GLP statement it is noted that the study director had no knowledge of the procedures used for analysis of water contaminants.

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RESULTS:

Stability, Homogeneity and Concentration
Stability of the test material was confirmed for 14 days when the feed was stored at room temperature in roof top feeders and for 21 days when stored in closed polyethylene containers. The chemical was found to be uniformly distributed in the diet and verification of the concentration demonstrated that the material was present in the test diet throughout the study. Analytical values ranged from 90 to 109% of nominal. (See Table I).

Mortality

There were no compound related effects on mortality. The survival rate for males was 72, 78, 75, 77 and 73% and for females the survival rate was 80, 83, 82, 78 and 73% for control-I, low, mid, high and control-II groups, respectively. The mean survival in males was 511, 536, 518 543 and 528 days and for females the mean survival was 541, 538, 541, 536 and 529 days.

Food Consumption

There were no compound related effects on food consumption in males or females. Statistically significant decreases and occassional increases in food consumption occured in both sexes, but was sporadic and generally not dose -related.

Body Weight and Body Weight Gain

Statistically significant ($p< 0.01$; $p< 0.05$) decreases were reported for high dose males for body weight and for body weight gain. The body weight was 4 to 9% lower for high dose males when compared to both of the control groups. The body weight gain in high dose males for the entire study was 19% lower than the first control group and 28% lower than the second control group. Similar effects were not reported in females. The decreases in body weight and in body weight gain were first observed at week 16 and persisted until the termination of the study.

Clinical Observations

There were no compound related effects on clinical observations or on hematology in either sex at the evaluation intervals. Frequently observed clinical observations across all groups of males included anal ulcers, alopecia and ocular opacities which involved either one or both eyes. In females, ocular opacities, alopecia and skin ulcerations were reported with the greatest frequency; however, there was no dose-related increase in the incidence of this lesion.

Gross and Microscopic Observations

In low dose females, there was a significant increase ($p< 0.05$) in the incidence of hydronephrosis. This finding was not

considered to be related to the administration of the test material because there was no dose response. In high dose females, there was a statistically significant increase (5 for both control groups) in the incidence of amyloidosis of the heart and, to a lesser degree, in the ileum and jejunum. The historical prevalence of systemic amyloidosis in CD-1 mice is high; this finding is not considered to be related to the administration of DMH. In high dose males, there was a statistically significant increase in the incidence of hepatocellular necrosis when compared to the second control group, but not the first. Amyloidosis was also prevalent in males; however the incidence was not associated with the dose of DMH that was administered and there was no statistical significance. In low dose males, there was an increase in the incidence of renal mineralization in males that were sacrificed at week 79. This lesion was not treatment related.

In males and females, a non-significant increase in lung adenomas was observed at both the low and mid dose levels when compared to the first group of controls, but not when compared to the second group of controls. When lung adenomas and lung carcinomas were combined, the numbers were slightly higher than the combined incidence for the first control group. These findings are believed to be incidental because there was no associated dose response, there was no statistical significance and there was no difference when a comparison is made to the second control group.

There were no other reported compound related statistically significant increases in any gross lesion in either male or female test groups. In addition, there were no compound related neoplastic or non-neoplastic lesions that were reported in either sex.

DISCUSSION:

Based on the results of this study conducted in CD-1 mice, DMH was not associated with carcinogenicity when administered at dietary levels up to the limit dose of 1000 mg/kg. Additionally, there were no compound related clinical signs, effects on mortality or effects on clinical, gross or microscopic pathology. In high dose males, there was a statistically significant decrease (4 - 9%) in mean absolute body weight and in mean body weight gain (19% lower than control-I and 28% lower than control II). The reported decreases in body weight and in body weight gain were first observed at week 16 of the study and persisted up to termination of the study. No similar changes in body weight or body weight gain were reported in females.

Based on the decreases in body weight and body weight gain reported in high dose males, the NOEL for systemic toxicity was 300 mg/kg and the LOEL was 1000 mg/kg (limit dose).

The study satisfies the guideline requirements for a Mouse Carcinogenicity study as outlined in Subdivision F, 83-2.

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TABLE I
Homogeneity, Stability and Concentration

Homogeneity

Measured range in concentration

Sampling Area	Dose (ppm) 400	1850	8500
Top	422 - 437	1827 - 1829	7803 - 8655
% Nominal	105.5 - 109.2	98.8 - 98.9	91.8 - 101.8
Middle	393 - 435	1662 - 1778	8088 - 8656
% Nominal	98.2 - 108.8	89.8 - 96.1	95.2 - 101.8
Bottom	407 - 435	1640 - 1799	8591 - 9075
% Nominal	101.8 - 108.8	88.6 - 97.2	101.1 - 106.8
Mean	420	1756	8434
% Nominal	105	94.9	99.2

Stability

Dose (ppm)	Day	Storage Condition	Concentration Measured	% Nominal
400	0	-	420	105.0
	7	open	375	93.8
		closed	404	101.1
	14	open	394	98.6
		closed	387	96.7
	21	closed	402	100.6
8500	0	-	8434	99.2
	7	open	8080	95.0
		closed	8018	94.3
	14	open	7815	91.9
		closed	7874	92.6
	21	closed	7782	91.6

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TABLE II
Body Weight (g) - Males

Week	Dose (mg/kg) 0	100	300	1000	C
0	28.5	28.4	28.4	28.3	28.2
1	29.9	29.8	29.5	29.6	29.8
2	30.8	30.9	30.6	30.4	30.8
3	31.8	31.7	31.4	31.6	32.0
4	32.5	32.4	32.2	32.1	32.8
8	34.5	34 3	33.9	33.7	34.8
12	35.8	36.1	35.4	35.2	36.2
16	36.9	36.8	36.6	35.7ad	37.3
20	37.5	38.0	37.3	35.9bd	37.5
24	38.3	38.2	38.1	36.8bd	38.6
28	38.5	38.4	38.1	37.1bd	38.9
32	38.9	38.9	38.4	37.2bd	39.0
40	39.8	39.8	39.3	37.8bd	40.1
48	40.0	40.1	39.5	37.9bd	40.5
56	39.8	40.1	39.1c	38.0ad	40.8
64	39.9	40.5	40.0	38.2d	41.3
72	40.0	40.8	40.2	38.0ad	41.0
78	40.4	41.3	40.2	37.8bd	41.4

Table extracted from data reported in Table 4 of the submission.
a = different from first control group ($p < 0.05$)
b = different from first control group ($p < 0.01$)
c = different from second control group ($p < 0.05$)
d = different from second control group ($p < 0.01$)

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TABLE III
Body Weight Gain (g)
Males

Week	Dose (mg/kg) 0	100	300	1000	0
0 - 1	1.4	1.3d	1.1ad	1.3d	1.7a
0 - 4	4.0	4.0d	3.8d	3.3	4.6b
0 - 8	6.1	5.9	5.5ad	5.4bd	6.3
0 - 16	8.4	8.4c	8.3c	7.5bd	9.1a
0 - 24	9.8	9.7	9.8	8.5bd	10.7
0 - 32	10.4	10.5	10.1	9.0bd	11.2
0 - 48	11.5	11.7	11.2	9.7bd	12.3
0 - 64	11.4	12.1	11.8	10.0	13.1a
0 - 78	11.9	13.0	11.8	9.6bd	13.3

Data taken from table 5 of the submission.
a = different from first control group ($p < 0.05$)
b = different from first control group ($p < 0.01$)
c = different from second control group ($p < 0.05$)
d = different from second control group ($p < 0.01$)

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Table IV
Neoplastic Lesions

Females Organ/tumor type	Dose (ppm) 0	450	1850	8500	0
LIVER number examined	60	60	60	60	60
Hepatocellular adenoma	2	1	2	0	3
Carcinoma	0	1	0	0	1
Histiocytic Cell sarcoma	0	0	1	1	0
Hemangiosarcoma	0	2	1	3	1
PITUITARY number examined	59	2	-	59	60
Adenoma	1	1	-	0	0
MAMMARY number examined	55	3	2	56	54
Adenocarcinoma	1	1	0	1	0
SPLEEN number examined	60	25	19	60	60
Hemangiosarcoma	2	2	1	0	2
UTERUS number examined	59	38	37	60	60
Glandular polyp	0	2	0	0	0
Stromal polyp	0	3	1	0	0
Leiomyosarcoma	1	0	1	1	0
LUNGS number examined	60	60	60	60	60
Adenoma	2	6	8	1	6
Carcinoma	1	3	0	5	3

Table extracted from data in Table 19.

Table IV
Neoplastic Lesions

Males Organ/tumor type	Dose (ppm) 0	450	1850	8500	0
LIVER					
number examined	60	59	59	60	60
Hepatocellular adenoma	3	3	5	10	11
Adenoma w/carcin in situ	0	0	0	1	1
Carcinoma	0	1	1	4	3
Histiocytic Cell sarcoma	0	0	0	1	0
Hemangiosarcoma	0	1	3	0	1
PITUITARY					
number examined	60	0	1	60	59
Adenoma	0	-	1	0	0
ADRENAL					
number examined	60	0	2	60	59
Adenoma	5	-	0	3	3
Carcinoma	0	-	0	0	1
Lymphosarcoma	1	-	0	0	1
TESTES					
number examined	60	6	4	60	60
Interstitial Cell adenoma	0	0	1	1	1
Hemangiosarcoma	0	1	0	0	0
LUNGS					
number examined	60	60	60	60	60
Adenoma	5	9	9	6	9
Carcinoma	3	5	2	3	3

Table extracted from data in Table 16.

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TABLE V
Mean Organ Weights (g)

Dose (ppm)	0	450	1850	8500	0
Females					
Final body wt	36.0	34.9	35.4	36.0	36.3
Liver	1.936	2.025	1.968	2.282a	2.103
Kidneys	0.511	0.520	0.509	0.517	0.503
Heart	0.182	0.180	0.183	0.185	0.181
Spleen	0.143	0.182	0.167	0.214	0.176
Brain	0.518	0.505	0.512	0.513	0.511
Males					
Final body wt	41.0	41.3	40.3	38.8ad	41.9
Liver	2.405	2.219	2.236	2.326a	2.283
Kidneys	0.791	0.805	0.772	0.761	0.753
Heart	0.237	0.219b	0.225a	0.226	0.216b
Spleen	0.156	0.116	0.126	0.125	0.137
Brain	0.502	0.508	0.510	0.494	0.502
Testes	0.213	0.214	0.222	0.210	0.223

a = Significantly different from first control ($p < 0.05$)
b = Significantly different from first control ($p < 0.01$)
c = significantly different from second control ($p < 0.05$)
d = significantly different from second control ($p < 0.01$)

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Primary Reviewer: Paul Chin, Ph.D. Paul Chin 5/10/95
Section 2, Tox. Branch 1 (7509C)
Secondary Reviewer: Joycelyn Stewart, Ph.D., Section Head
Section 2, Tox. Branch 1 (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Chronic Oral Feeding/Carcinogenicity Study - Rat (83-5)

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

P.C. CODE: 006315

MRID NO.: 433977-02

TEST MATERIAL: Dantoin

SYNONYMS: DMH; dimethylhydantoin, 5,5-Dimethylhydantoin

LABORATORY PROJECT NO.: 91N0113

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

TESTING FACILITY:
Bushy Run Research Center, Union Carbide Corp. , 5702 Mellon Road, Export, PA 15632

TITLE OF REPORT:
Chronic Dietary Toxicity/Oncogenicity Study with 5,5-Dimethylhydantoin (DMH) in Rats

AUTHORS: S. J. Hermansky and C. L. Benson

REPORT ISSUED: August 31, 1994

EXECUTIVE SUMMARY:

In a chronic toxicity/carcinogenicity study, 5,5-dimethylhydantoin (DMH) was fed to 60 CD rats/sex/dose for 104 weeks at dietary levels of C (first control), 100, 300, 1000, or 0 (second control) mg/kg/day. These dosage levels correspond to dietary concentration of DMH over the course of the study that ranged 1080-26794 ppm (males) and 945-20890 ppm (females). [NOTE: 5,5-dimethylhydantoin is the organic moiety of 1-bromo-3-chloro-5,5-dimethylhydantoin. The Agency agreed that toxicology studies on the technical should be performed on the organic moiety.]

In both sexes of the highest dose tested, there was no evidence of compound related clinical signs, mortality, food consumption and organ weights, or on clinical pathology. At the highest dose level, statistically significant ($p<0.01$ for the first control

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and $p<0.05$ for the second control groups) treatment-related effects on body weight and body weight gain were observed in females only at weeks 90, 92, 94, and 96. During weeks 90 to 96 of the study, body weights were 14-15% lower than control 1 and 9% lower than control 2 and the body weight gain were 23-24% lower than the control 1 and 16% lower than control 2. There were statistically significant increased incidences of hyperplasia of submandibular lymph nodes [5/19 (26%) vs 0/31 to 1/33 (0-3%) of both controls] at week 104 ($p<0.05$ and $p<0.01$ for controls 1 and 2, respectively) in the high dose males only. This increased incidence was considered to be related to the administration of the DMH.

The NOEL for systemic toxicity was 300 mg/kg/day and the LEL was 1000 mg/kg/day based on the decreases in body weight and body weight gain in females and hyperplasia of submandibular lymph nodes in males.

There was no increases in tumor incidences for the DMH treated groups when compared to the control group.

Dosing was adequate since the highest dose represents the limit dose.

The study is classified as Core Guideline and satisfies the guideline requirement for a chronic toxicity/carcinogenicity study (83-5) in rats.

A. MATERIALS

1. Test Material: 5,5-dimethylhydantoin
   Description: white crystalline solid
   Lot/code #: NO43254/40-683
   Purity: 99.8%
   Stability of compound: not reported
   CAS number: 77-71-4

2. Vehicle: diet

3. Test animals

   Species: Rat
   Strain: CD® Sprague-Dawley
   Age: 33 days old
   Weight: The body weight range on the day of first treatment was 224.3 to 282.8 g for male groups and 147.6 to 198.0 g for female groups.
   Source: Charles River Lab, Inc., Portage, MI
   Housing: Housed 1 animal/wire-mesh cage
   Environmental conditions:
   Temperature: 66 to 77°F

Humidity: 40 to 70%
Air changes: 10/hour
Photoperiod: Fluorescent lighting was provided 12 hours/day
Acclimation period: 3 weeks

B. STUDY DESIGN

1. Animal assignment

Animals were assigned to 3 treatment groups and 2 control groups using a computer weight stratified randomization procedure. At the time of group assignment, only animals with body weights within +/- 20% of the population mean for each sex were included. The following table summarizes the organization of the study.

Test Group	DMH Target Dosage (mg/kg/day)	No. of Rats Male	Female
Control 1	0	60	60
Low dose	100	60	60
Mid dose	300	60	60
High dose	1000	60	60
Control 2	0	60	60

A rationale for dose selection was based on the results of a 14-day dietary dose range-finding study in the same strain of rats. In this study, CD rats were exposed to DMH in the diet at concentrations of 0, 7000, 14000, or 20000 ppm (corresponding to 571, 1157, and 1663 mg/kg/day in males and 596, 1160, and 1717 mg/kg/day in females) for 14 days. There were no treatment-related effects observed based on parameters measured which included food consumption, body weights and body weight gains, and gross pathology. Based on this data, the limit dose of 1000 mg/kg/day was selected as the target dosage level for the high dose group and the target dosage levels for the mid and low dose groups were selected on a half log scale below the high dose.

2. Diet preparation

The test diets were prepared weekly by appropriate dilutions of a concentrated premix containing DMH in a Hobart mixer for 15 minutes. Prepared diets were stored in closed polyethylene containers at room temperature until fed to the animals. The concentration of DMH was determined for all

prepared diets every fourth week.

3. Animals received ground rodent feed for 104 weeks and water *ad libitum*.
4. Statistics:

The following procedures were utilized:
"The data for quantitative continuous variables were intercompared for the 3 treatment groups and each of the control groups by use of Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. The t-tests were used when the F value from the ANOVA was significant. When Levene's test indicated similar variances, and the ANOVA was significant, a pooled t-test was used for pairwise comparisons. When Levene's test indicated heterogeneous variances, all groups were compared by an ANOVA for unequal variances followed, when appropriate, by a separate variance t-test for pairwise comparisons.
Nonparametric data were statistically evaluated using the Kruskal-Wallis test followed by the Mann-Whitney U test when appropriate. Mortality and mean time to first palpable mass data were analyzed by life-table analyses. Incidence data were compared using the Fisher's Exact Test. Additional statistical tumor analyses were performed on the combined incidence of mammary gland adenomas, fibroadenomas, and carcinomas for all female rats in the control and high dose groups using computer software developed by the National Toxicology Program (Haseman, 1984 and Peto et al., 1980)."
Two untreated control groups were included in this study. "The purpose of including two control groups in this study was to collect data that would provide information regarding the range of normal or control values for the parameters evaluated in this study."

5. A GLP statement was provided.
   A QA statement was not provided. QA unit inspection summary was provided in p 89 of the study report.

C. METHODS AND RESULTS

1. Diet analysis for stability, homogeneity, and concentration verification
The stability and homogeneity of test compound in the diets were analyzed. The stability of DMH in 950 and 34000 ppm diets was tested following storage at room temperature after preparation (day 0), 8, 14, and 21 days. The homogeneity of DMH in 950, 1052, 6500, and 34000 ppm diets was analyzed. The concentration of DMH in 1080-26794 ppm (males) and 945-20890 ppm (females) diets over the study period was analyzed. These

concentrations correspond to dietary levels of 100-1000 mg/kg/day.

Results: Concentration of test material in the prepared test diets material ranged from 89.5 to 109.8% of the nominal concentration over the study period. Homogeneity analyses revealed mean concentrations ranged from 97.6 to 108.2% of target values; stability analyses after 14 and 21 days of storage, revealed concentrations ranged from 95.6 to 105.8% and 97.1 to 101.5% of day 0 values, respectively.

2. Observations
Animals were inspected twice daily for signs of toxicity and mortality.

Results - There were no signs of toxicity indicative of response to treatment except for the following findings (Table 1). Increased incidence of urine stains were observed in the high dose males. In all treated females, increased incidence of urine stains and increased incidences of unkempt were observed. The high incidences of these findings in the treated animals are not attributed to treatment with DMH because these findings are frequently found in animals prior to death.

There were no statistically significant difference in the incidence of palpable masses between the treated and the control groups.

TABLE 1. SELECTED CLINICAL OBSERVATIONS (Urine stains and unkempt) IN RATS FED DMH FOR 2 YEARS

Finding	Males				
	Target dosage level (mg/kg/day)				
	0	100	300	1000	0
Urine stains	6 (a)	14	13	22	12
	Females				
Urine stains	13	22	21	22	12
Unkempt	13	28	24	22	13

a Numbers represent the number of animals exhibiting the finding at least once during the study.

Source: Table 3 (p 25 and p 41) of the study report.

Mortality - Table 2 summarizes mortality (percent survival) and mean survival time. At termination, survival rates in male groups ranged from 32 to 55% and in female groups from 47 to 68%. Survival rates were lowest for males (32%) and females (42%) of the high dose

groups. However, the study report stated that survival rates of animals from high dose groups were similar to historical control ranges in the same strain of rats obtained from three previous studies conducted at the same laboratory. [The historical control ranges for the percent survival reported by the registrant for males and females were 33-63% and 50-62%, respectively. However, the investigators did not supply the historical control data for the mean percent survival].

In males, the difference in mean survival time between the high dose animals (626 days) and control groups (668 and 659 days for controls 1 and 2, respectively) was statistically significant. In females, the mean survival time from the low (676 days), mid (663 days), and high dose groups (647 days) was statistically significantly different from the second control group (703 days) only. However, the study report stated that mean survival time of both sexes of animals from the high dose groups were similar to historical control ranges obtained from three previous studies. [The historical control ranges for the mean survival time reported by the registrant for male and female rats were 532-692 days and 527-681 days, respectively. The investigators did not supply the historical control data for the mean survival time.]

TABLE 2. MORTALITY (PERCENT SURVIVAL) AND MEAN SURVIVAL TIME IN RATS FED DMH FOR 2 YEARS

	Males				
	Target dosage level (mg/kg/day)				
	0	100	300	1000	0
Total no. of animals	60	60	60	60	60
No. sacrificed at termination (% survival)	33 (55)	26 (43)	34 (57)	19 (32)	31 (52)
Mean survival time (days)	668	662	688	626 (c,d)	659
	Females				
Total no. of animals	60	60	60	60	60
No. sacrificed at termination (% survival)	36 (60)	28 (47)	31 (52)	25 (42)	41 (68)
Mean survival time (days)	669	676 (c)	663 (c)	647 (d)	703

a Significantly different from the first control group ($p<0.05$) using the Generalized Savage and/or the Generalized Wilcoxon test statistic.
c Significantly different from the second control group ($p<0.05$) using the Generalized Savage and/or the Generalized Wilcoxon test statistic.
d Significantly different from the second control group ($p<0.01$) using the Generalized Savage and/or the Generalized Wilcoxon test statistic.
Source: Extracted from Table 1, p 20 of 1977, of study report

3. Body weight

Animals were weighed weekly for the first 14 weeks of the study and every other week thereafter. Group mean body weights were calculated weekly and body weight gain data were derived.

Results - Table 3 presents mean body weights and weight gains at selected intervals. There were no differences between control and treated males with respect to body weights and body weight gain.

In high dose females, statistically significant ($p<0.01$ for the first control group and $p<0.05$ for the second control group) treatment-related effects on body weight and body weight gain were observed at weeks 90, 92, 94, and 96. In these animals, at weeks 90 and 94, body weights were 14-15% lower than the first control group and 9% lower than the second control group and body weight gain were 23-24% lower than the first control group and 16% lower than the second control group.

In mid dose females, statistically significant ($p<0.05$ for the first control group only) treatment-related effects on body weight and body weight gain were observed at weeks 90, 92, 94 and 96. In these animals, body weights and body weight gain were 10-11% and 16-17% lower than the first control group, respectively. In low dose females, statistically significant ($p<0.05$ for the first control group only) effects on body weight were observed at week 90 only and effects on body weight gain were observed at weeks 90, 94 and 96. In these animals, at both weeks 90 and 94, body weights and body weight gain were 8% and 13% lower than the first control group, respectively. The decrease in body weights in the mid and low dose females was considered a transient effect of treatment due to the lack of a consistent pattern, small magnitude of the changes (8-11% lower than the first control group and 4-6% lower than the second control group), and was observed only during 90 to 96 weeks of the study.

4. Food consumption and compound intake

The quantity of food consumed were measured weekly for the first 14 weeks of the study and every other week thereafter. Intake of DMH was calculated from the nominal dietary concentrations, food consumption, and mean weekly body weights.

Results -

a. Food consumption - Overall, there were no differences between control and treated males and females with

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respect to food consumption.

b. Compound consumption - Mean compound consumption for the 100, 300, and 1000 mg/kg/day groups ranged 88.9-112.2,

TABLE 3.

MEAN BODY WEIGHTS AND CUMULATIVE BODY WEIGHT GAINS (% change relative to untreated first control group) IN RATS FED DMH FOR 2 YEARS

Week	Males				
	Target dosage level (mg/kg/day)				
	0	100	300	1000	0
Body Weight (g)					
0	254.5	254.6	253.7	254.3	253.5
26	575.2	571.6	574.1	583.1	569.3
52	646.7	645.0	645.8	656.2	640.6
78	673.7	684.1	706.0	681.2	679.4
104	588.0	611.7	585 2	595.1	599.4
Body weight gain (g)					
0-26	320.7	317.2	320.4	328.8	315.9
0-52	392.2	391.1	392.1	402.2	386.8
0-78	419.5	431,0	452.2	426.9	425.3
0-96	402.3	393.7	385.2	377.1	402.1
0-100	375.4	372.9	372.1	341.7	400.7
0-104	331.7	357.6	332.8	341.8	346.6

a= Significantly different from the first control group ($p<0.05$)

b= Significantly different from the first control group ($p<0.01$)

c= Significantly different from the second control group ($p<0.05$)

#= % change relative to untreated first control group

Source: Extracted from Tables 5-8 of study report

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TABLE 3 (continued)

MEAN BODY WEIGHTS AND CUMULATIVE BODY WEIGHT GAINS (% change relative to untreated first control group) IN RATS FED DMH FOR 2 YEARS

Week	Females				
	Target dosage level (mg/kg/day)				
	0	100	300	1000	0
Body weight (g)					
0	169.5	169.2	168.9	169.9	168.1
26	296.8	297.8	294.6	295.0	299.9
52	353.5	355.6	355.8	352.2	358.7
78	426.5	412.6	397.2	395.1	416.4
90	455.5	417.6 (a) (-8)#	409.3 (a) (-10)	389.2 (b,c) (-15)	427.8
94	452.0	415.7 (-8)	403.1 (a) (-11)	389.6 (b,c) (-14)	428.6
104	433.5	404.5	389.8	396.0	426.2
Body weight gain (g)					
0-26	127.3	128.6	125.7	125.1	131.8
0-52	189.8	191.4	191.2	185.2	193.0
0-78	256.6	243.9	227.9	224.5	248.0
0-90	285.9	248.4 (a) (-13)	241.2 (a) (-16)	218.0 (b,c) (-24)	259.8
0-94	282.4	245.9 (a) (-13)	235.0 (a) (-17)	217.8 (b,c) (-23)	260.3
0-104	263.8	236.3	221.4	224.2	257.4

a= Significantly different from the first control group ($p<0.05$)

b= Significantly different from the first control group ($p<0.01$)

c= Significantly different from the second control group ($p<0.05$)

#= % change relative to untreated first control group

Source: Extracted from Tables 5-8 of study report

265.3-342.5, and 896.9-1132.4 mg/kg/day for males and 90.9-117.6, 269.0-347.7, and 908.8-1147.9 mg/kg/day for females, respectively.

c. Food efficiency - There were no differences between control and treated males and females with respect to food efficiency.

5. Ophthalmoscopic examinations

Eyes were examined for all rats at 0 and 104 weeks using indirect ophthalmoscopy and slit lamp biomicroscopy following dilation of eyes with MYDRIACYL 1% Ophthalmic Solution.

Results - No treatment related ophthalmic findings were observed.

6. Clinical Pathology

Blood was collected by orbital sinus puncture from 15 animals/sex in the control and high-dose groups at 0, 26, 52, 78 and 104 weeks. Animals were fasted 16-18 hours before bleeding. The CHECKED (X) parameters were examined.

a. Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
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)
(Clotting time)
(Prothrombin time)
X Leukocyte differential count*
X Mean corpuscular HGB (MCH)

* Required for subchronic and chronic studies

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Results - No compound related changes in hematology parameters were observed. A slight decrease in segmented neutrophils was seen in mid dose males at week 104, but the decreases occurred only in the mid dose group and no dose-relationship was apparent.

b. Clinical Chemistry

Electrolytes	Other
X Calcium*	X Albumin*
X Chloride*	X Albumin/globulin ratio
Magnesium	X Creatinine*
X Phosphate*	X Urea nitrogen*
X Potassium*	X Globulins
X Sodium*	X Total protein*
	X Indir. bilir.
Enzymes	X Glucose*
	X Total bilirubin
X Alkaline phosphatase (ALP)	X Dir. bilirubin
Cholinesterase	X Cholesterol*
X Creatinine phosphokinase	
Lactic acid dehydrogenase	
X Serum alanine aminotransferase (also SGPT)*	
X Serum aspartate aminotransferase (also SGOT)*	
X Gamma glutamyl transferase (GGT)	
Glutamate dehydrogenase	

* Required for subchronic and chronic studies

Results - No effects on any of the parameters measured were observed. As can be seen in Table 4, inorganic phosphorus was increased (11%) ($p < 0.01$) in the high dose males at week 52 and returned to normal at week 78. Therefore, this transient change was not considered to be treatment related. Inorganic phosphorus was increased (11%) ($p<0.05$) in the high dose females at week 104. However, this change was not considered to be related to the treatment because the increase was contributed by 2 high dose females. Cholesterol was increased (38%) ($p<0.05$) in the mid dose females at week 104, however, this increase was not considered to be treatment related because there was no dose relationship.

Table 4. Selected Clinical Chemistry Values

	Males				
	Target dosage level (mg/kg/day)				
	0	100	300	1000	0
Inorganic Phosphorus					
Week 52	55	55	55	60 (b,d)	55
Week 78	50	51	53	53	63
	Females				
Inorganic phosphorus					
Week 104	52	53	49	57 (a,c)	51
Cholesterol					
Week 104	0.97	1.14	1.34 (a,c)	1.08	0.94

a Significantly different from the first control group ($p<0.05$)
b Significantly different from the first control group ($p<0.01$)
c Significantly different from the second control group ($p<0.05$)
d Significantly different from the second control group ($p<0.01$)

Source: Extracted from Table 11, p 135, 139, and 145 of study report

7. Urinalysis

Urine was collected from unfasted rats at 25, 51, 77 and 103 weeks. The CHECKED (X) parameters were examined.

X Appearance	X Sediment (microscopic)	
X Bilirubin		
X Volume	X Protein	X Blood
X Specific gravity	X Glucose	Urobilinogen
X pH	X Ketones	Nitrate
X Bile salts/bile pigments		

Results - No treatment-related effects on any urinary parameters were observed. There was increased urine volume and decreased specific gravity in high dose males at week 77 only. These findings were not considered to be treatment related because these parameters were within the normal range at other measurement intervals.

8. Sacrifice and Pathology
All animals that died or were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected from all rats for histological examination. The (XX) organs, in addition, were weighed at termination. All tissues from the high dose and control groups were processed histologically and examined microscopically. In addition, the lungs, liver, kidneys, pituitary gland and all gross lesions for all rats and mammary glands of female rats in the low and mid dose groups were examined.

	Digestive system		Respiratory		Urogenital
x	Salivary system *	x	Trachea *	xx	Kidney * +
x	Esophagus *	x	Lung *	x	Urinary bladder *
x	Stomach *			xx	testes * +
x	Duodenum *			x	epididymides
x	Jejunum *		Cardiovascular/ Hemat.	x	Prostate
x	Ileum *	x	Aorta *	x	Seminal vesicles
x	Cecum *	xx	Heart *	xx	Ovaries * +
x	Colon *	x	Bone Marrow *	x	Uterus *
x	Rectum *	x	Lymph nodes *	x	Vagina
xx	Liver * +	xx	Spleen *		
x	Pancreas *	x	Thymus *		
	Neurologic		Glandular		Other
x	Peripheral nerve *	xx	Adrenals *	x	Bone *
x	Spinal cords *	x	Mammary gland *	x	Muscle (skeletal) *
x	Pituitary *	x	Parathyroid *	x	Skin *
x	Eyes *	x	Thyroids	x	All gross lesions and tissue masses *
xx	Brain * +		Harderian gland		

* Required for subchronic and chronic studies.
\+ Organ weight required for subchronic and chronic studies.

Results -

a. Organ weight (Table 5) - No biologically important changes in organ weight were observed when treated groups were compared with controls.
The relative liver weight as percent of body weight in mid dose and high dose females was significantly increased when compared to controls. However, the increased

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relative liver weight in these animals were not considered to be treatment related because the relative liver weight increase was due to slight decrease in body weights in these groups. The biological significance of the increased relative liver weight was not supported by findings from gross pathology and histopathology.

In low dose males, both the absolute spleen weight and the spleen weight as percent of brain weight was significantly increased. However, this finding was not considered to be related to treatment because increased mean spleen weight for this group was caused by a single animal which had a large splenic neoplasm.

Table 5. SELECTED ORGAN WEIGHTS (g) AT WEEK 104

	Males				
	Target dosage level (mg/kg/day)				
	0	100	300	1000	0
Spleen					
Absolute wt.	1.054	1.368 (a,c)	0.918	0.992	1.007
Relative wt. as % of body wt.	0.190	0.230	0.164	0.175	0.177
Relative wt. as % of brain wt.	30.225	30.191 (c)	30.458 (c)	31.418 (c)	38.806 (a)
	Females				
Liver					
Absolute wt.	10.332	10.557	10.893	10.773	10.369
Relative wt. as % of body wt.	2.589	2.803	3.025 (b,d)	2.963 (a,c)	2.652

a Significantly different from the first control group ($p<0.05$)
b Significantly different from the first control group ($p<0.01$)
c Significantly different from the second control group ($p<0.05$)
d Significantly different from the second control group ($p<0.01$)
Source: Extracted from Table 5, p 175-180 of 1977, of study report

b. Gross pathology - No treatment related changes in the incidence of findings was observed.

c. Microscopic pathology -

1) Non-neoplastic -
Table 6 summarizes nonneoplastic pathology in rats evaluated

for relationship with DMH administration. In high dose males, there were statistically significant increased incidences of hyperplasia of submandibular lymph nodes at week 104 ($p<0.05$-control 1 and $p<0.01$-control 2) and when data were combined ($p<0.01$ for controls 1 and 2) from animals sacrificed at week 104, animals that died or were sacrificed moribund. The study report stated that the lesion was attributed to random biologic variation due to the low incidence of the lesion. However, this increased incidence of hyperplasia of submandibular lymph nodes [5/19 (26%)] - high dose males vs 0/31 to 1/33 (0-3%)-controls 1 and 2] was considered to be related to the administration of the DMH. In addition, in these high dose males, there were statistically significant increased incidences of hepatocellular necrosis of the liver ($p<0.05$ for controls 1 and 2) at week 104. This necrosis was not considered to be related to the administration of the test material because there was no dose response relationship. In addition, the incidence of lesion was low and the microscopic grade of this necrosis was between mild and medium.

In females, there were statistically significant increased incidences of cholangitis of the liver in the low and mid dose groups, skeletal muscle atrophy in the high dose group, interstitial pneumonitis in the low dose group, and mammary gland mastitis in the low and high dose groups. All of these findings in females were considered to be attributed to random biological variation found in aging animals because there was no relationship of incidence with dose level.

2) Neoplastic -

Table 7 summarizes neoplastic pathology in rats evaluated for relationship with DMH administration.

For males receiving 0, 100, 300, 1000 or 0 mg/kg/day, the incidence of pituitary adenoma was 27/36, 25/28 ($p<0.05$ for control 2), 31/31 ($p<0.01$ for both controls 1 and 2), 20/25 and 26/41, respectively. The incidence of benign mammary tumors in females was 2/36, 7/28 ($p<0.05$ for control 1), 5/31, 6/25 and 3/41 in groups receiving 0, 100, 300, 1000 or 0 mg/kg/day, respectively. These findings were not considered to be related to the administration of the test material because there was no dose response relationship. An additional peer review was performed on the neoplastic changes in the mammary gland as well as the degree of secretory activity of the mammary gland for all female rats in the study. Both the primary and peer review pathologists concluded that there was no evidence to relate a variation in mammary tumor frequency to DMH treatment for any mammary tumor type.

Tumors at other sites occurred at a normal incidence for rats of this strain and no compound-related increases were observed.

D. DISCUSSION

Based on the results of this chronic toxicity/carcinogenicity study in Sprague Dawley rats, DMH at dietary levels up to the limit dose of 1000 mg/kg/day, there was no increases in tumor incidences for the DMH treated groups when compared to the control group. There was no evidence of compound related clinical signs, mortality, food consumption and organ weights, or on clinical pathology. In high dose females, there was a statistically significant ($p<0.01$ for control 1 and $p<0.05$ for control 2) treatment-related effects on body weight and body weight gain at weeks 90, 92, 94, and 96. During weeks 90 to 96 of the study, body weights were 14-15% lower than control 1 and 9% lower than control 2 and the body weight gain were 23-24% lower than the control 1 and 16% lower than control 2. In addition, there were statistically significant increased incidences of hyperplasia of submandibular lymph nodes [5/19 (26%) vs 0/31 to 1/33 (0-3%) of both controls] at week 104 ($p<0.05$ and $p<0.01$ for controls 1 and 2, respectively) in the high dose males only.

The NOEL for systemic toxicity was 300 mg/kg/day and the LEL was 1000 mg/kg/day (limit dose) based on the decreases in body weight and body weight gain in high dose females and hyperplasia of submandibular lymph nodes in high dose males.

The survival rates for males (32% vs 52-55% for controls 1 and 2) and females (42% vs 60-68% for controls 1 and 2) in the high dose groups were slightly lower than the concurrent controls but they were not statistically significantly different from the controls. In addition, the survival rates in these animals were slightly lower than the historical control ranges (33-63% for males and 47-68% for females). Although there was a decrease in survival in both sexes of the high dose rats, the decrease was not statistically significant when compared to the concurrent controls or with the historical controls. Therefore, it does not appear that administration of DMH affected survival.

The study is classified as **Core Guideline and satisfies** the guideline requirement for a chronic toxicity/carcinogenicity study (83-5) in rats.

TABLE 6. NONNEOPLASTIC HISTOLOGICAL FINDINGS (tissues affected/tissues examined) IN RATS FED DMH FOR 2 YEARS

		Males				
		Target dosage level (mg/kg/day)				
		0	100	300	1000	0
Liver	Hepatocellular necrosis					
	Sacrificed at week 104	0/33	1/26	2/34	3/19 (a,c)	0/31
Lymph	Hyperplasia of submandibular nodes					
	Sacrificed at week 104	1/33	0/6	0/4	5/19 (a,d)	0/31
	All animals on study (e)	1/59	0/14	1/10	11/60 (b,d)	1/60
		Females				
Liver	Cholangitis					
	Sacrificed at week 104	0/36	16/28 (a,c)	16/31 (a,c)	11/25	10/41
	All animals on study (e)	10/60	21/60 (a,c)	18/60	12/60	10/60
Skeletal muscle	Atrophy					
	Sacrificed at week 104	12/36	2/2	2/2	16/25 (a,c)	15/41
	All animals on study (e)	18/60	6/6	7/9	32/60 (a,c)	20/60
Lungs	Interstitial pneumonitis					
	Sacrificed at week 104	1/36	15/28 (b,d)	3/31	0/25	3/41
	All animals on study (e)	2/60	18/60 (b,d)	8/60	2/60	4/60
Mammary gland	Mastitis Sacrificed at week 104	2/36	7/28 (a)	5/30	9/25 (b,c)	5/41

a=Significantly different from the first control group ($p<0.05$)
b=Significantly different from the first control group ($p<0.01$)
c=Significantly different from the second control group ($p<0.05$)
d=Significantly different from the second control group ($p<0.01$)

e=All animals include animals sacrificed at week 104, found dead and sacrificed moribund.
Source: Extracted from Tables 13, 15, 17, and 19 of study report

TABLE 7. NEOPLASTIC HISTOLOGICAL FINDINGS (tissues affected/tissues examined) IN RATS FED DMH FOR 2 YEARS

		Females				
		Target dosage level (mg/kg/day)				
		0	100	300	1000	0
Pituitary	Adenoma					
	Sacrificed at week 104	27/36	25/28 (c)	31/31 (b,d)	20/25	26/41
	All animals on study (e)	41/60	53/60 (a,c)	54/60 (b,d)	44/60	41/60
	Carcinoma					
	Sacrificed at week 104	3/36	1/28	0/31	1/25	1/41
	All animals on study (e)	6/60	4/60	6/60	5/60	4/60
Mammary gland	Adenoma	2/36	7/28 (a)	5/31	6/25	3/41
	Fibroadenoma	9/36	8/28	10/31	7/25	9/41
	Adenocarcinoma Sacrificed at week 104	4/36	1/28	5/31	7/25	6/41
	All combined	15/36	16/28	20/31	20/25	18/41

a=Significantly different from the first control group ($p<0.05$)
b=Significantly different from the first control group ($p<0.01$)
c=Significantly different from the second control group ($p<0.05$)
d=Significantly different from the second control group ($p<0.01$)

e=All animals include animals sacrificed at week 104, found dead and sacrificed moribund.

Source: Extracted from Tables 17, 19, and 20 of study report