Microfic
011975
011975

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

JUL _ 9 1996

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC [illegible]VIEWS

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: BCDMH. Review of Toxicology Data.
DP Barcode: D205506. Submission No.: S469965
Rereg Case No.: 3055 Case No.: 800364
P.C. Code No.: ~~006836~~ 006315 CB Tox. Chem. No.:114A

To: Kathleen Depukat/Tom Myers PM# 51
Reregistration Branch
Special Review and Reregistration Division (7508W)

From: Raymond K. Locke, Toxicologist Raymond K. Locke 5/6/96
Section 2, Toxicology Branch I
Health Effects Division (7509C)

Thru: Joycelyn E. Stewart, Ph.D., Section Head 6/20/96
Section 2, Toxicology Branch I
Health Effects Division (7509C) KB 7/2/96

Registrant: Lonza Inc.
Fair Lawn, NJ

Action Requested: Review toxicology data (MRID No.: 43290601) submitted to support reregistration of BCDMH and indicate whether these data meet the requirements for a multigeneration reproductive toxicity study in rats (guideline 83-4).

Conclusion: This study was adequately conducted and supports the reregistration of BCDMH.

In a two-generation reproduction study (MRID 43290601), groups of 28 male and 28 female F_0 and F_1 rats were administered 5,5-dimethylhydantoin (0, 2000, 6000, or 20000 ppm) in their diets for 10 weeks before mating and during mating, gestation, and lactation. Calculated doses were 136 and 127, 408 and 379, and 1396 and 1322 mg/kg/day, respectively, for F_0 and F_1 males (premating/postmating periods) and 176 and 158, 516 and 475, and 1775 and 1602 mg/kg/day, respectively, for F_0 and F_1 females (premating periods only).

There was no evidence of systemic toxicity in either F_0 or F_1 male and female rats. Therefore, **a LOEL for systemic toxicity cannot be established; the NOEL is >20,000 ppm.**

1/26

No effects were observed on indices of reproductive performance of F_0 or F_1 rats, litter sizes, pup viability, pup survival, or sex ratio. At 20000 ppm, a decrease in pup growth was indicated by statistically significant reductions in body weights (7-8%) and body weight gain (7-13%) of high-dose pups (male and female combined) from day 7 to 21 of lactation. **Therefore, the LOEL for reproductive toxicity is 20000 ppm and the corresponding NOEL is 6000 ppm.**

This study is classified as acceptable and it satisfies the guideline requirements for a multigeneration reproduction feeding study (83-4).

RATIONALE FOR DIFFERENCES FROM TESTING FACILITY'S CONCLUSIONS

The testing facility concluded that there were small increases in parental food consumption and body weight and slight decreases in offspring body weight observed in animals receiving DMH at 20000 ppm in the diet. The testing facility apparently did not consider the decreases in pup weight to be a reproductive effect and, based on these effects at 20000 ppm, concluded that the NOEL for parental animals and offspring was 6000 ppm, and that the NOEL for reproductive effects was at least 20000 ppm.

The EPA reviewer agrees with the contractor that the increases in food consumption (100-113% control) and body weight gain (43-157% control) were minimal (the higher values were sporadic and not time-related). Therefore, the NOEL for parental systemic toxicity is ≥ than 20000 ppm (HDT) and the LEL is > 20000 ppm. On the other hand, the decreases during days 7-14 of lactation in pup body weights (7-8% decrease) and body weight gain (7-13% decrease) in pups from high-dose (20000 ppm; HDT) parents represent significant reproductive toxicity. Therefore, the LEL for reproductive effects is 20000 ppm (HDT) and the NOEL is 6000 ppm.

DATA EVALUATION REPORT

5,5-DIMETHYLHYDANTOIN

STUDY TYPE: MULTIGENERATION REPRODUCTION - RAT (83-4)

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. 95-04

Primary Reviewer: K.A. Davidson, Ph.D., D.A.B.T.	Signature: Date:	K Davidson
Secondary Reviewers: C.S. Forsyth, Ph.D.	Signature: Date:	RWR for C.S. Forsyth 4-24-96
Robert H. Ross, M.S. Group Leader	Signature: Date:	Robert H. Ross 4-24-96
Quality Assurance: Susan Chang, M.S.	Signature: Date:	S S Chang 4-24-96

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Managed by Lockheed Martin Energy Research Corporation for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400

EPA Reviewer: Raymond K. Locke Raymond K. Locke Date: 5/6/96
Review Section II, Toxicology Branch I (7509C)
EPA Section Head:
M. Copley, D.V.M., D.A.B.T. Date: 6/26/97
Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Multigeneration Reproduction - Rat (83-4)

TOX. CHEM. NO: 114A

P.C. CODE: 006315

B.P. BAR CODE: D205506

MRID NO.: 43290601

TEST MATERIAL: 5,5-Dimethylhydantoin

SYNONYMS: Dantoin®, DMH

STUDY NUMBER: 91N0094

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

TESTING FACILITY: Bushy Run Research Center, 6702 Mellon Road, Export, PA 15632-8902

TITLE OF REPORT: Two for the premating period-Generation Reproduction Study in CD® Rats with 5,5-Dimethylhydantoin (DMH) Administered in the Diet

AUTHORS: T.L. Neeper-Bradley and M.F. Kubena

REPORT ISSUED: June 16, 1994 (study completion date)

EXECUTIVE SUMMARY: In a two-generation reproduction study (MRID 43290601), groups of 28 male and 28 female F_0 and F_1 rats were administered 5,5-dimethylhydantoin (0, 2000, 6000, or 20000 ppm) in their diets for 10 weeks before mating and during mating, gestation, and lactation. Calculated doses were 136 and 127, 408 and 379, and 1396 and 1322 mg/kg/day, respectively, for F_0 and F_1 males (premating/postmating periods) and 176 and 158, 516 and 475, and 1775 and 1602 mg/kg/day, respectively, for F_0 and F_1 females (premating periods only).

4

There was no evidence of systemic toxicity in either F_0 or F_1 male and female rats. Therefore, **a LOEL for systemic toxicity cannot be established; the NOEL is >20,000 ppm.**

No effects were observed on indices of reproductive performance of F_0 or F_1 rats, litter sizes, pup viability, pup survival, or sex ratio. At 20000 ppm, a decrease in pup growth was indicated by statistically significant reductions in body weights (7-8%) and body weight gain (7-13%) of high-dose pups (male and female combined) from day 7 to 21 of lactation. **Therefore, the LOEL for reproductive toxicity is 20,000 ppm and the corresponding NOEL is 6000 ppm.**

This study is classified as acceptable and it satisfies the guideline requirements for a multigeneration reproduction feeding study (83-4). Deficiencies in this study include, (1) a 2- to 5-week break in feeding of test diet to F_1 offspring and (2) no data on pups dying during lactation. The break in feeding the test diet to F_1 offspring is a serious deficiency, which would warrant an "unacceptable" classification except the study showed very low reproductive toxicity (small decreases in pup growth) at doses exceeding 1000 mg/kg/day.

Special Review Criteria (40 CFR 154.7) None

I. MATERIALS AND METHODS

A. MATERIAL

1. Test material: 5,5-Dimethylhydantoin

   Description: white, crystalline powder
   Lot/Batch No.: NO432543
   Purity: 99.8% a.i.
   Stability of compound: stable
   CAVES No.: 77-71-4
   Structure: not available

2. Vehicle and/or positive control

   The test material was administered in the feed; no other vehicle was used. A positive control was not included in the study.

3. Test animals

   Species: rat
   Strain: outbred albino CD®
   Age and weight at start of study: 6 weeks old; 199.0 to 199.5 g (F_0 males); 162.3 to 163.1 g (F_0 females)

Source: Charles River Laboratories, Portage, MI
Housing: 2/cage for the first week, then 1/cage except during cohabitation and lactation. Animals were housed in stainless steel wire mesh cages, except from gestation day (gd) 20 through lactation, when they were housed in plastic shoe box cages.
Environmental conditions:
Temperature: 66-77°F
Humidity: 40-70%
Air Changes: not reported
Photoperiod: 12 hours light/12 hours dark
Acclimation period: ~14 days

4. Diet preparation and analysis

Diet was prepared weekly by mixing milled crystalline DMH with ground rodent feed for 1 h to prepare a homogeneous concentrated mixture. Appropriate dilutions were prepared by adding the concentrated mixture or the next highest concentration to rodent feed and mixing in a Hobart mixer for 15 min. The dietary preparations were stored at room temperature. Homogeneity (2000, 6000, and 20,000 ppm) of the dietary preparation was determined by measuring concentrations taken from the top, middle, and bottom of the mixture. Stability (2000 and 20,000 ppm) of the dietary preparations was determined after storage of samples at room temperature in open glass or closed polyethylene containers for 7, 14, and 21 days (closed container only). Samples of treated food were analyzed weekly for verification of concentration during the first 4 weeks of the study and every 4 weeks thereafter.

Results -

a. Homogeneity analysis - All samples were within ±10% of the nominal and measured concentrations.

b. Stability analysis - Samples stored in the open glass container up to 14 days or the closed container up to 21 days were within ±10% of the concentration measured on day 0.

c. Concentration analysis - All measured concentrations were within ±10% of the nominal concentrations.

5. Diet

Animals received Rodent Chow® #5002 (Purina Mills, Inc.) and tap water *ad libitum*.

B. PROCEDURES AND STUDY DESIGN

1. Animal assignment

F_0 animals were randomly assigned to test groups as seen in Table 1 based on body weight; only animals with body weights within ±20% of the population mean were assigned to a group. F_1 parents were selected from each F_1 litter at 28 days postpartum using a computer-generated randomization scheme.

TABLE 1. Animal Assignment

Dose Group	Conc. in diet[a] (ppm)	No. of Animals per Group[b]	
		Male	Female
0 (Control)	0.00	28	28
1 (Low)	2000	28	28
2 (Intermediate))	6000	28	28
3 (High)	20,000	28	28

Data taken from page 13, MRID No. 432906-01 .
[a]Diets were administered from the beginning of the study until the animals were sacrificed
[b]The same number of animals were picked from the F_1 litters as parents for the F_2 generation.

Starting at 6 weeks of age, F_0 animals were fed the DMH diets during the 10-week premating period and the 21-day mating, gestation, and lactation periods. Selected 28-day old F_1 offspring were given the same diets as their corresponding F_0 parents starting at 5 to 8 weeks of age and continuing through the 10-week premating period and the 21-day mating gestation, and lactation periods. The F_2 offspring were exposed to DMH indirectly during gestation, indirectly and directly during lactation, and in the diet for 1 week after weaning. Control animals received basal diet without the DMH supplement.

2. Dose selection rationale

Doses were selected by the sponsor based on a 90-day gavage study in rats (MRID No. 42009201), a 14-day palatability study, and the interim results from a chronic feeding study (BRRC Project No. 91N0113). According to

the study authors a dose of 1000 mg/kg/day (duration not stated) was well-tolerated by the adult rats.

3. Mating procedure

After treatment for 10 weeks, each F_0 female was randomly mated with one male of the same dose group for 7 days. If mating did not occur, the female was mated with another unmated male (same dose group) for successive 7-day periods until mating occurred or for a total of 21 days, whichever came first. Evidence of mating was determined by examining the area under the cages twice each day for a copulation plug or by taking vaginal smears once a day for evidence of sperm. After mating occurred, males and females were housed separately. The day a copulation plug or a sperm-positive smear was observed was designated as gestation day (gd) 0. For females that showed no evidence of mating, the last day of cohabitation was designated as gd 0.

Offspring from F_1 litters were randomly selected at 28 days of age to produce the F_2 litters; after the 10-week premating period, males and females were mated using the protocol as described for F_0 parents. Brother-sister matings were avoided when possible.

C. METHODS

1. Observation schedule

a. Parental animals - During the premating period, F_0 males and females were examined twice daily for mortality and moribundity, once daily for clinical signs of toxicity, and once a week for detailed clinical evaluation. Mated F_0 females were examined three times a day starting on gd 20 for production of litters. All F_0 animals were weighed weekly during the premating period; mated females were weighed on gd 0, 6, 15, and 20; and dams with litters were weighed on postnatal days 0, 7, 14, and 21. Food consumption of F_0 animals was measured once weekly during the premating period and at 3- to 4-day intervals during gestation (gd 0-20) and on lactation days 0 to 14.

F_1 males and females selected to produce the F_2 generation were examined as described for F_0 animals.

b. Reproductive performance - The following reproductive indices, as described by the study authors, were calculated for parental animals:

Female mating index = (No. plug- or sperm-positive females/Total no. females paired) x 100

Male mating index = (No. males impregnating females/Total no. males paired) x 100

Female fecundity index = (No. pregnant females/Total no. plug- or sperm-positive females) x 100

Male fecundity index = (No. males siring litters/Total no. of males impregnating females) x 100

Female fertility index = (No. females pregnant/Total no. females paired) x 100

Male fertility index = (No. males siring litters/Total no. males paired) x 100

Gestation index - (No. females with live litters/No. females pregnant) x 100

c. Litter observations - Litter observations were made from birth to weaning (Table 2). Initial examination of litters took place as soon as possible after birth (postnatal day 0); the number of viable and stillborn pups was recorded. Litters were examined twice daily for mortality, and survival indices were calculated on days 0, 4, 7, 14, 21 and 28. Pup were sexed on the day of birth and individually weighed and sexed on postnatal days 1, 4, 7, 14, 21, and 28. All pups were examined externally throughout lactation. Culled pups were examined for external abnormalities, killed, and discarded. Pups dying before weaning were necropsied to determine a possible cause of death.

The following litter indices were calculated:

Live birth index = (No. live pups born/No. live + dead pups born) x 100

4-Day survival or viability index = (No. live pups at day 4 (precull)/No. pups born alive) x 100

Lactation index = (No. live pups at day 21/No. pups alive at day 4 (postcull) x 100

TABLE 2. F_1/F_2 Litter Observations						
Observations	Time of Observation (Lactation Day)					
	Birth	Day 4	Day 7	Day 14	Day 21	Day 28
No. of viable or live pups	X	X	X	X	X	X
No. of stillborn pups	X					
Individual pup weight	X	X	X	X	X	X
External alterations	X	X	X	X	X	X
Sex of each pup (m/f)	X	X	X	X	X	X
Clinical signs						

2. Postmortem Studies

a. Sacrifice - F_0 and F_1 parents were killed by exsanguination following anesthetizing with methoxyflurane.

b. Necropsy -

1) Parental animals - All surviving parental F_0 and F_1 females were sacrificed after their litters were weaned; F_1 males were killed after delivery of their pups. All the animals were subjected to gross and histopathological examinations as presented in Table 3.

TABLE 3. Pathologic examination of parental animals		
Animals Examined	Macroscopic	Microscopic
Found dead	X	X
Unscheduled sacrifice		
Scheduled sacrifice	X	X

2) Offspring - Ten F_1 and ten F_2 offspring per sex in each control and dose group were randomly selected at 28 days of age and necropsied at 42-44 days of age. Ten additional F_2 male pups in each control and dose group were necropsied at 51 days of age. The offspring were subjected to *post mortem* examinations as presented in Table 4. After reviewing the necropsy data, the remaining F_1 pups were examined externally, killed, and discarded.

TABLE 4. Pathologic examination of offspring		
Animals Examined	Macroscopic	Microscopic
Found dead	X	
Scheduled sacrifice	X	

3) Necropsy observations of F_0, F_1, and F_2 animals - Gross necropsy consisted of detailed external and internal examinations. The following tissues (X) from F_0 and F_1 male and female parental animals in the control and high-dose groups and all F_1 and F_2 pups selected for necropsy were prepared for microscopic examination. The testes, epididymides, and organs with gross lesions from low- and intermediate-dose males that did not sire litters were also examined microscopically. The uteri of females failing to produce a litter were stained with potassium ferricyanide for detection of implantation sites. No tissues were weighed.

Adults	Pups		Adults	Pups	
X	X	Ovaries	X	X	Epididymides
X	X	Uterus	X	X	Prostate
X	X	Vagina	X	X	Seminal vesicle
X	X	Gross Lesions	X	X	Testes

D. STATISTICAL ANALYSIS

The statistical unit of comparison was the adult male, the pregnant dam, or the litter. Pairwise analysis of continuous data was performed by using Levene's test for equality, analysis of variance (ANOVA), and t-tests. The t-test was used when the results from ANOVA were significant, and the pooled t-test was used when Levene's test showed similar variances and ANOVA was significant. If Levene's test showed heterogenous variances, then ANOVA was used for unequal variances followed by a variance t-test. Nonparametric data were analyzed using the Kruskal-Wallis test followed by Mann-Whitney U test when appropriate. Quantal (incidence) data were analyzed using the Fisher exact test. Statistical significance was indicated by a p-value$\leq$0.05 (two-tailed).

E. Signed and dated GLP, Quality Assurance, Confidentiality, and Flagging statements were provided.

II. RESULTS

A. PARENTAL TOXICITY

1. Mortality and clinical signs

No F_0 male or female rats died during the study. No statistically significant increases occurred in the incidence or frequency of clinical signs of toxicity in F_0 adults. Alopecia was observed on the front legs of six F_0 females receiving 20,000 ppm of the test material, compared with one control.

One F_1 adult male in the 20,000-ppm group died after the mating period due to an unknown cause. One F_1 female in the 6000-ppm group was sacrificed after the lactation period because of ulceration in the inguinal region. Neither death is considered to be due to treatment with DMH. Alopecia was present on both front paws of eight high-dose F_1 males compared with one control. No other clinical signs of toxicity attributable to the test material were observed in the F_1 male or female parents.

2. Body weight and food consumption

a. Premating period - Selected data for body weights, body weight gain, and food consumption are presented in Table 5. Adult F_0 males receiving 20,000 ppm of DMH weighed slightly more than corresponding controls from week 2 to 10 of the premating period (up to 106%), during the mating period (107%), and during the postmating period (106 or 107% week); statistical significance was achieved during the mating ($p<0.05$, weeks 12 and 13) and postmating periods ($p<0.05$ or 0.01). Weight gain in high-dose adult F_0 males was 9% more than that of controls during the premating period; statistical significance ($p<0.01$) was achieved during a few weekly intervals. Food consumption in high-dose adult F_0 males was significantly elevated (up to 113%, $p<0.01$) during the entire treatment period, suggesting that the increases in body weights and body weight gain were due to the increased food consumption. Statistically significant increases in food consumption in F_0 males receiving 2000 are not considered to be treatment-related, because the increases were sporadic and no significant increases occurred at 6000 ppm.

Except for some sporadic statistically significant effects, body weights and body weight gain were similar in treated and control adult F_0 female during the premating period, but food consumption of high-dose females was significantly elevated (up to 109%, $p<0.01$) between premating weeks 1 and 8.

In adult F_1 male and female rats receiving DMH during the premating period, no treatment-related effects were noted on body weights or overall body weight gain. Food consumption was consistently elevated during the premating period (up to 107%, N.S. most weekly intervals) and the post-mating period (110%, $p<0.01$) in the high-dose males.

b. Gestation and lactation period - Adult body weights and food consumption at selected times during gestation and lactation are presented in Table 6. Feeding of DMH to F_0 and F_1 dams had no effect on mean body weights or weight gain during gestation, or body weights during lactation. Overall body weight gain during lactation was increased in high-dose F_0 (213%, $p<0.01$) and F_1 dams (122%, N.S.) due to less weight loss during days 14 to 21 compared with that of control animals. Food consumption was similar in control and treated groups except for statistically significant increases (108-110%, $p<0.01$) in high-dose F_0 dams during gd 14-20 and F_1 dams during gd 0 to 4.

TABLE 5. Mean body weights, body weight gain, and food consumption during the premating or postmating (males only) periods in rats fed DMH for two generations

Observation	Control	2000 ppm	6000 ppm	20,000 ppm
F_0 Generation - Males				
Body weight (g) - Week 0	199.5	199.0 (100)[a]	199.3 (100)	199.2 (100)
Body weight (g) - Week 5	438.7	448.2 (102)	440.7 (100)	460.2 (105)
Body weight (g) - Week 10	544.1	556.8 (102)	545.5 (100)	575.4 (106)
Body weight (g) - Week 15	591.2	607.0 (103)	588.1 (99)	635.0** (107)
Body weight (g) - Week 19	631.3	648.0 (103)	622.4 (99)	672.1* (106)
Weight gain (g)[b] - Week 0-10	344.6	357.8 (104)	346.2 (100)	376.2 (109)
Weight gain (g)[b] - Week 10-19	87.2	91.2 (105)	76.9 (88)	96.7 (111)
Food consumption (g/rat/day)[c] - Week 1-10	29.9	30.9 (103)	30.2 (101)	32.5** (109)[d]
Food consumption (g/rat/day)[c] - Week 10-19	28.5	30.1 (106)	29.3 (103)	31.5 **(111)[d]
F_0 Generation - Females				
Body weight (g) -Week 0	162.9	163.1 (100)	163.0 (100)	162.3 (100)
Body weight (g) -Week 5	253.4	252.7 (100)	254.9 (101)	257.4 ((102)
Body weight (g) - Week 10	293.8	291.9 (99)	298.1 (101)	298.6 (102)
Weight gain (g)[b] - Week 0-10	130.9	128.8 (98)	135.1 (103)	136.3 (104)
Food consumption (g/rat/day)[d] - Week 1-10	20.2	20.9 (103)	20.7 (102)	21.6 (107)[e]
F_1 Generation - Males				
Body weight (g) - Week 0	307.8	300.6 (98)	307.0 (100)	306.8 (100)
Body weight (g) -Week 5	501.4	500.4 (100)	503.2 (100)	499.0 (100)
Body weight (g) - Week 10	596.3	600.5 (101)	599.0 (100)	594.0 (100)
Body weight (g) -Week 15	626.9	644.7 (103)	642.5 (102)	640.0 (102)
Weight gain (g)[b] - Week 0-10	288.5	299.9 (104)	292 (101)	287.2 (100)
Weight gain (g)[b] - Week 10-15	30.6	44.2 (144)	43.5 (142)	46 (150)
Food consumption (g/rat/day)[c] - Week 1-10	31.5	32.2 (102)	32.0 (102)	33.2 (105)
Food consumption (g/rat/day)[c] - Week 13-16	30.3	31.6 (104)	31.9 (105)	33.3** (110)

TABLE 5. Continued

Observation	Control	2000 ppm	6000 ppm	20,000 ppm
F_1 Generation - Females				
Body weight (g) - Week 0	207.0	207.0 (100)	205.2 (99)	206.0 (100)
Body weight (g) - Week 5	288.0	293.9 (102)	280.5 (97)	279.7 (97)
Body weight (g) - Week 10	319.0	326.8 (102)	308.8 (97)	307.4 (96)
Weight gain (g)[b] - Week 0-10	112	119.8 (107)	103.6 (93)	101.4 (90)
Food consumption (g/rat/day)[c] - Week 1-10	20.9	22.0 (105)	20.9 (100)	21.4 (102%)

Data taken from Tables 4,6, 8, 9, 10, 11, 23, 25, 27, 28, 29, and 30; pages 35, 36, 38, 40-45, 63, 65 and 67-72; MRID No. 432906-01.
[a]The numbers in parentheses are the percents of the control values.
[b]Calculated by the reviewer from using body weights.
[c]Average of the weekly mean values.
[d]Values for the individual weekly intervals from week 1 to 19 were statistically significant, $p<0.01$.
[e]Values for the individual weekly intervals from week 1 to 8 were statistically significant, $p<0.01$
*$p<0.05$, **$p<0.01$

TABLE 6. Selected mean body weights, body weight gain, and food consumption values for pregnant and nursing rats fed DMH for two generations

Observation/Gestation day	Treatment Group			
	Control	2000 ppm	6000 ppm	20,000 ppm
F_0 Generation - F_1 Litter				
Mean body weight (g)				
Day 0 of gestation	293.47	293.66 (100)[a]	299.17 (102)	299.03 (102)
Day 20 of gestation	440.92	436.28 (99)	442.29 (100)	448.96 (102)
Day 0 of lactation	335.30	335.0 (100)	333.81 (100)	333.45 (99)
Day 21 of lactation	349.63	349.40 (100)	352.82 (101)	363.91 (104)
Mean body weight gain (g)				
Day 0-20 of gestation	147.45	142.63 (97%)	143.13 (97%)	149.93 (102%)
Day 0-21 of lactation	14.33	14.40 (100%)	19.21 (134%)	30.46** (213%)
Mean food consumption (g/rat/day)				
Day 0-20 of gestation[b]	25.97	26.41 (102%)	26.48 (102%)	27.71 (108%)[c]
Day 0-14 of lactation[b]	47.18	47.25 (100%)	46.05 (98%)	48.04 (102%)
F_1 Generation - F_2 Litter				
Mean body weight (g)				
Day 0 of gestation	318.17	318.26 (100)	308.35 (97)	311.76 (98)
Day 20 of gestation	453.70	455.82 (100)	457.83 (101)	456.64 (101)
Day 0 of lactation	349.62	354.14 (102)	349.64 (100)	354.41 (102)
Day 21 of lactation	358.72	362.91 (101)	356.07 (99)	368.39 (103)
Mean body weight gain (g)				
Day 0-20 of gestation	135.53	137.56 (101)	149.48 (110)	144.88 (107)
Day 0-21 of lactation	11.43	8.76 (77)	6.43 (56)	13.98 (122)
Mean food consumption (g/rat/day)				
Day 0-20 of gestation[b]	25.86	25.95 (100)	26.93 (104)	27.65 (107)[c]
Day 0-14 of lactation[b]	47.85	45.03 (94)	47.86 (100)	47.03 (98)

Data taken from Tables 13, 14, 15, 16, 32, 33, 34, and 35; pages 47-50 and 74-77; MRID No. 432906-01.
[a]Percent of control value.
[b]Average of weekly mean values calculated by the reviewer
[c]$p<0.01$ for the following intervals: gd 14-17 and 17-20 (F_0) and gd 0-4 (F_1).
*$p<0.05$, **$p<0.01$

16

3. Test substance intake

Compound consumption (calculated by the study authors and assumed to be based on food consumption data, body weights, and nominal dietary concentrations) was calculated as mg/kg/day and reported for premating period for adult F_0 and F_1 females and for the premating and postmating periods for the corresponding males. These data are summarized in Table 7. The high dose for both generations of male and female rats exceeded the limit dose of 1000 mg/kg/day.

TABLE 7. Test substance (mg/kg/day) intake in rats fed DMH during the premating/postmating period

Week	Male			Female		
	2000 ppm	6000 ppm	20,000 ppm	2000 ppm	6000 ppm	20,000 ppm
F_0 Generation						
Range of weekly means[a]	91.61-239.36	276.04-721.69	908.17-2431.11	142.26-228.19	415.48-670.30	1402.80-2244.64
Grand mean[b]	155.06	463.02	1602.64	175.92	515.53	1774.75
Grand mean[c]	136.10	407.74	1395.77	NA	NA	NA
F_1 Generation						
Range of weekly means[a]	97.30-187.86	295.22-553.89	1038.89-1926.54	130.38-197.94	462.86-560.00	1393.44-1963.39
Grand mean[b]	135.58	402.10	1404.62	158.13	475.13	1601.54
Grand mean[c]	127.06	379.17	1321.89	NA	NA	NA

Data taken from Tables 5, 7, 24, and 26; pages 37, 39, 64, and 66; MRID No. 432906-01.
[a]Range of weekly values for premating and postmating periods for males, but excluding mating period (weeks 0-16), premating period for females.
[b]Average of weekly values (weeks 0-10)
[c]Average of weekly values for the premating and postmating periods, but excluding mating period (weeks 0-16)

4. Reproductive performance

Results for the parental (F_0 and F_1) animals are summarized in Tables 8a,b. No treatment-related effects were observed in either generation.

5. Necropsy results

a. Organ weights - Organs were not weighed in this study and are not required for multigeneration reproduction studies (83-4).

b. Pathology -

1) Macroscopic examination - No gross lesions attributable to feeding of DMH were observed in adult male or female rats (F_0 and F_1).

2) Microscopic examination - No microscopic lesions attributable to feeding of DMH were observed in adult male or female rats (F_0 and F_1).

KB

TABLE 8a. Reproductive performance in F_0 rats fed DMH

Observation	Treatment Groups			
	Control	2000 ppm	6000 ppm	20,000 ppm
Males				
No. at start of study	28	28	28	28
No. paired with females	28	28	28	28
No. impregnating females[a]	28	28	27	27
No. siring litters[b]	26	27	23	25
Females				
No. at start of study	28	28	28	28
No. paired with males	28	28	28	28
No. of plug-or sperm positive	28	28	28[c]	28[c]
No. pregnant	26	27	24	25
No. with live litters	26	27	24	25
Indices				
Mating index-males	100	100	96.4	96.4
Mating index-females	100	100	100	100
Fecundity index-males	92.9	96.4	85.2	92.6
Fecundity index-females	92.9	94.4	85.7	89.3
Fertility index-males	92.9	86.4	82.1	89.3
Fertility index-females	92.9	96.4	85.7	89.3
Mean gestation length (days)	22.0 ± 0.04[d]	22.0 ± 0.3	21.9 ± 0.5	22.3 ± 0.4

Data taken from Table 12, page 46, MRID No. 432906-01.
[a]Produced plug- or sperm-positive females.
[b]Determined by delivery of litter or positive uterine staining for implantation sites.
[c]Copulation plug or sperm missed in one female; these values were reported by study author.
[d]Mean ± standard deviation

TABLE 8b. Reproductive performance in adult F_1 rats fed DMH

Observation	Treatment Groups			
	Control	2000 ppm	6000 ppm	20,000 ppm
Males				
No. at start of study	28	28	28	28
No. paired with females	28	28	28	28
No. impregnating females[a]	27	27	28	27
No. siring litters[b]	21	23	25	23
Females				
No. at start of study	28	28	28	28
No. paired with males	28	28	28	28
No. of plug- or sperm positive	27[c]	27	28	27
No. pregnant	23	23	25	23
No. with live litters	22	23	25	23
Reproductive indices (%)				
Mating index - males	96.4	96.4	100	96.4
Mating index - females	96.4	96.4	100	96.4
Fecundity index - males	77.8	85.2	89.3	85.2
Fecundity index - females	85.2	85.2	89.3	85.2
Fertility index - males	75.0	82.1	86.3	82.1
Fertility index - females	82.1	82.1	89.3	82.1
Mean gestation length (days)	22.1 ± 0.6[d]	22.1 ± 0.3	22.1 ± 0.4	22.1 ± 0.5

Data taken from Table 31, page 73, MRID No. 432906-01.
[a]Produced plug- or sperm-positive females.
[b]Determined by delivery of litter or positive uterine staining for implantation sites.
[c]Copulation plug or sperm missed in one female.
[d]Mean ± standard deviation

20

B. OFFSPRING TOXICITY

1. Viability and clinical signs

The data concerning viability and mean litter sizes are summarized in Tables 9a, b. Mean litter sizes, viability, survival, and sex ratios were similar in treatment groups and controls. One F_1 litter from the 6000-ppm group had only three male pups and no females pups at birth, all were dead by day 9 postpartum (p.p.). All the pups from two F_2 litters (15 pups in one litter and 2 in the other) in the control group were stillborn, cannibalized, or sacrificed on the day of birth.

TABLE 9a. Viability and clinical observations of F_1 offspring during lactation

Observation/study time	Control	2000 ppm	6000 ppm	20,000 ppm
Total no. of litters	26	27	24	25
Total no. of pups born	389	397	370	396
Total no. born alive	389	394	367	394
Total no. stillborn	0.00	3	3	2
Mean litter size - day 0	15.0	14.7	15.4	15.8
Mean no. live pups per litter (total pups alive)				
Day 0	15.0 (389)	14.6 (394)	15.3 (367)	15.8 (394)
Day 4 (precull)	14.7 (383)	14.2 (383)	14.9 (357)	15.5 (388)
Day 4 (post cull)	8.0 (208)	7.9 (212)	7.8 (186)	8.0 (200)
Day 21	7.8 (203)	7.6 (205)	7.6 (175)	7.9 (197)
No. litters weaned	26	27	23	25
Survival indices				
Live birth index (%)	100	99.3	99.2	99.5
Viability index (%)	98.5	97.4	96.3	98.7
Lactation index (%)	97.6	96.8	91.1	98.5
Sex ratio (% males)-day 0	51.1	49.9	53.9	46.4
Sex ratio (% males)-day 4 (precull)	51.7	50.3	54.2	46.1

Data taken from Tables 17, 19, and 20; pages 51, 56, and 57; MRID No. 432906-01.

21

TABLE 9b Viability and clinical observations of F_2 offspring during lactation

Observation/study time	Control	2000 ppm	6000 ppm	20,000 ppm
Total no. of litters	22	23	25	23
Total no. of pups born	307	320	364	329
Total no. born alive	302	314	364	326
Total no. stillborn	5	6	0.00	3
Mean litter size - day 0	14.0	13.9	14.6	14.3
Mean no. live pups per litter (total no. of pups alive)				
Day 0	13.7 (302)	13.7 (314)	14.6 (364)	14.2 (326)
Day 4 (precull)	14.1 (283)	13.3 (307)	13.8 (344)	13.7 (316)
Day 4 (post cull)	7.8 (155)	7.6 (175)	8.0 (200)	7.7 (178)
Day 21	7.4 (148)	7.1 (163)	7.6 (189)	7.5 (172)
No. litters weaned	20	23	25	23
Survival indices				
Live birth index (%)	98.5	98.3	100	99.3
Viability index (%)	89.4	96.7	95.2	96.9
Lactation index (%)	95.6	93.5	94.5	96.7
Sex ratio (% males)-day 0	43.7	51.3	48.9	51.6
Sex ratio (% males)-day 4 (precull)	42.8	50.4	49.6	51.5

Data taken from Tables 36, 38, and 39; pages 78, 83, and 84; MRID No. 432906-01.

2. Body weight

Selected group mean body weights and body weight gain for F_1 and F_2 litters are summarized in Table 10. Pup weight and weight gain in the low- and intermediate--dose groups were similar to those of controls. In the high-dose group, pup weights and weight gain were similar to control on lactation days 1, 4 and 7 for both generations. The mean weights of F_1 pups at day 14 and 21 p.p. were significantly lower (8% on both days, $p<0.01$) at the high dose compared with the controls; this trend continued to day 28 p.p. (data not presented in Table 10) at which time the pup weight was 5% ($p<0.05$) less than that of the control

22

TABLE 10. Group mean body weight and body weight gain

Observation/study time	Control	2000 ppm	6000 ppm	20,000 ppm
F_1 Litters				
Mean pup weight per litter (g)				
Day 1	7.17	7.22	6.99	7.00
Day 4 (precull)	10.34	10.62	10.14	10.01
Day 4 (postcull)	10.33	10.63	10.09	10.06
Day 7	16.85	17.73	16.45	16.32
Day 14	35.57	36.09	35.55	32.65**
Day 21	57.09	57.97	56.69	52.69**
Mean pup weight gain per litter (g)				
Day 1-4	3.17	3.40	3.15	3.01
Day 4-7	6.51	7.11	6.31	6.31
Day 7-14	18.72	18.36	18.87	16.33**
Day 14-21	21.52	21.87	21.14	20.04
Day 1-21[a]	49.92	50.75	49.7	45.69
F_2 Litters				
Mean pup weight per litter (g)				
Day 1	7.39	7.49	7.54	7.36
Day 4 (precull)	10.65	11.06	10.95	10.81
Day 4 (postcull)	10.66	11.08	11.03	10.81
Day 7	18.14	18.26	18.40	18.07
Day 14	38.27	38.59	37.89	36.55
Day 21	58.66	60.15	57.77	55.24**
Mean pup weight gain per litter (g)				
Day 1-4	3.26	3.57	3.41	3.44
Day 4-7	7.49	7.20	7.44	7.26
Day 7-14	20.13	20.33	19.50	18.48**
Day 14-21	20.40	21.56	19.88	18.69**
Day 1-21	51.27	52.66	50.23	47.88

Data taken from Tables 18 and 37, pages 53-55 and 80-82, MRID No. 432906-01.
[a]Calculated by the reviewer: day 1-21 weight gain.
*p<0.05, **p<0.01

23

group. Weight gain was significantly decreased (13%, $p<0.01$) between days 7 and 14 p.p. in F_1 high-dose litters. On day 14 and 21 p.p., the weights of female and male pups separately were also significantly lower ($p<0.01$) than the corresponding control weights (data not presented in Table 10). The weight of pups in high-dose F_2 litters were significantly lower only at day 21 p.p. (6%, $p<0.05$). Calculated weight gain of the F_2 pups showed significant decreases at the day 7-14 (8%, $p<0.01$) and the 14-21 intervals (8%, $p<0.05$).

3. Necropsy results

a. Organ weight - Organs were not weighed in this study and are not required for multigeneration reproduction studies (83-4).

b. Pathology -

1) Macroscopic examination - There were no gross lesions attributable to DMH in F_1 or F_2 weanling (ten per sex per dose group in each generation) sacrificed at 6 weeks of age. The most notable lesion was the dilated pelvis observed in five low-dose F_1 male weanlings compared with one control and three each in the intermediate- and high-dose groups. The same lesion was observed in five high-dose F_2 weanlings compared with four each in the intermediate- and low-dose groups and two in the control group.

2) Microscopic examination - Tissues from F_1 and F_2 weanlings were not examined microscopically.

III. DISCUSSION

Groups of 28 male and 28 female rats (F_0 and F_1 generations) were fed DMH in their diets at concentrations of 0, 2000, 6000, or 20,000 ppm for 10 weeks before mating and during mating, gestation, and lactation (until weaning of their respective litters). There was a 2- to 5-week delay between weaning and feeding DMH to F_1 offspring selected to parent the second generation.

A. SYSTEMIC TOXICITY

Feeding of DMH produced no treatment-related effects on mortality, clinical signs of toxicity, gross lesions, or microscopic lesions in F_0 or F_1 adults of either sex. A

higher incidence of alopecia was observed in F_0 females and F_1 males receiving the high dose. This effect was not seen in F_0 males or F_1 females and is probably not treatment-related. Body weights and body weight gain were significantly increased in F_0 males fed 20,000 ppm of the test material during the mating and postmating periods. The increased body weight is not considered to be a toxic response, because food consumption was also significantly increased during the same period. There were no treatment-related effects on body weight or weight gain in adult F_0 or F_1 females during the premating period. Body weights and body weight gain in F_0 and F_1 females during gestation and lactation were similar to their corresponding controls except for a significantly elevated body weight gain (213%) during lactation of F_0 females receiving 20,000 of DMH. There was a nonsignificant increase (122%) in body weight gain during lactation of the F_1 females receiving 20,000 of DMH. The increased body weight gain is not considered to be a toxic effect. The NOEL for systemic toxicity is >20,000 ppm (1322 and 1602 mg/kg/day, respectively) for male and female rats based on the absence of toxicity at any dose. The lack of toxicity at doses greater than the limit dose of 1000 mg/kg/day, suggests that the test material has low systemic toxicity. These values are based on the test substance intake for the premating/postmating periods for males and premating periods only for females.

B. REPRODUCTIVE TOXICITY

The indices (mating, fertility, and fecundity) of reproductive performance in F_0 and F_1 animals were similar to those of their corresponding controls. Endpoints evaluated for offspring toxicity showed no effects on litter sizes, pup viability, pup survival, or sex ratio. Growth of F_1 and F_2 pups was reduced as indicated by lower body weights (males and females combined) (7 or 8%) and body weight gain (7 to 13%) between day 7 of lactation and weaning compared with the controls. Body weights were still significantly lower on day 28 in F_1 and F_2 female weanlings compared with controls. The decrease did not carry over to the older animals, because body weights of F_1 adults were similar to their corresponding controls. The effects on the growth of F_1 and F_2 pups is attributed to a reproductive mechanism because the test material had no toxic effect on the growth of mature animals. However, there was a 2- to 5-week period during which the rats did not receive the test material. The lowest-observed-effect level (LOEL) is 20,000 ppm (1322 and 1602 mg/kg/day,

during lactation; the corresponding NOEL is 6000 ppm (408 and 475 mg/kg/day, respectively). These values are based on the test substance intake for the premating/postmating periods for males and premating periods only for females.

C. RATIONALE FOR DIFFERENCES FROM TESTING FACILITY'S CONCLUSIONS

The testing facility concluded that there were small increases in parental food consumption and body weight and slight decreases in offspring body weight observed in animals receiving DMH at 20000 ppm in the diet. The testing facility apparently did not consider the decreases in pup weight to be a reproductive effect and, based on these effects at 20000 ppm, concluded that the NOEL for parental animals and offspring was 6000 ppm, and that the NOEL for reproductive effects was at least 20000 ppm.

The EPA reviewer agrees with the contractor that the increases in food consumption (100-113% control) and body weight gain (43-157% control) were minimal (the higher values were sporadic and not time-related). Therefore, the NOEL for parental systemic toxicity is ≥ than 20000 ppm (HDT) and the LEL is > 20000 ppm. On the other hand, the decreases during days 7-14 of lactation in pup body weights (7-8% decrease) and body weight gain (7-13% decrease) in pups from high-dose (20000 ppm; HDT) parents represent significant reproductive toxicity. Therefore, the LEL for reproductive effects is 20000 ppm (HDT) and the NOEL is 6000 ppm.

D. STUDY DEFICIENCIES

There was a 2- to 5-week period between weaning and premating treatment in which the F_1 offspring were not fed the test diet.

Data (external description or necropsy) were not presented on pups dying during lactation.

Data were not presented to verify litter origin of the F_1 mating pairs.