CASWELL FILE

10-27-89

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DATA EVALUATION REPORT II

STUDY TYPE: Developmental Toxicity - rat TOX CHEM NO. 585

ACCESSION NUMBER: 408731-01 MRID NO:

TEST MATERIAL: MRD-87-072

SYNONYMS: Aquatreat DN-30, "approximately 30% Nabam"

STUDY NUMBER(S): 207234

SPONSOR: ALCO Chemical Corporation
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TITLE OF REPORT: Teratogenicity Study in Rats of MRD-87-072 (Aquatreat DN-30, approximately 30% Nabam)

AUTHOR: Mitala, J. J.

REPORT ISSUED: July 1, 1988

CLASSIFICATION: Core Supplementary Data

CONCLUSIONS:

1. The test material (MRD-87-072) was administered by oral intubation to groups of 22-24 mated female Crl:CD (Sprague-Dawley) rats at target dose levels of 0 (vehicle only), 5, 50 or 500 mg/kg/day on days 6-15 of gestation. MRD-87-072 is identified as containing approximately 30% Nabam, so that the

target dosages in terms of Nabam would have been 0, 1.5, 15 and 150 mg/kg/day. However, on some occasions low analytical values (about 50% of the target concentrations) of Nabam in the dose were found. Therefore, for regulatory purposes, we can assume that the animals actually received half the target dose levels (equivalent to 0. 0.75, 7.5 and 75 mg/kg/day Nabam).

2. The maternal NOEL was then 7.5 mg/kg, and the LEL (significantly reduced mean body weight gains during the dosing period, significantly reduced mean food consumption day 6-9, significantly increased mean thyroid weight) was 75 mg/kg.

3. It is noteworthy that some of the variations observed were suggestive of ETU effects. Specifically, these were the following:

   - A significantly elevated incidence of slightly dilated cerebral ventricles in the low dose group (8/163) relative to the controls (1/143). The p (Fisher's Exact Test) = 0.0289. However, the incidences for this observation at the mid and high dose groups (1/153 and 3/149) were not significantly elevated relative to controls.

   - At the highest dose level, there were significantly increased incidences in some incompletely ossified cranial bones, including interparietal (59/161, compared with 35/143 in the controls, with p = 0.0148), squamosal (7/161, compared to 1/143 in controls, with p = 0.048) and supraoccipital (51/161, compared to 29/143 in controls, with p = 0.01655). The incidence of incomplete squamosal ossification was also significantly elevated in the mid-dose group (8/151, compared to 1/143 for the controls, with p = 0.02207), and the incidence of incomplete ossification of the supraoccipital was elevated in the low-dose group (49/162, compared to 29/143 in the control group with p = 0.03104).

   However, there was no evident grouping or clustering of these findings at any one dose level.

4. There was a significantly increased incidence of kinky tail at the high dose level (13/316 fetuses, as compared with 0/286 controls). All 13 fetuses with this finding were in one litter, and it is doubtful that this was an effect of the test compound. Since some or all of the increased incidences of incompletely ossified cranial bones indicated above may been an effect of the test material, it seems appropriate to set the developmental toxicity NOEL for this study at 7.5 mg/kg, and the LEL at 75 mg/kg.

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5. The study is currently classified as core supplementary data. At the least, the following additional data should be supplied before we could consider upgrading this classification:

   - Individual fetal variation findings, indicating which fetuses showed specific variations.

   - The date on which dosage was initiated for each female.

A. MATERIALS:

1. Test compound: MRD-87-072, identified as Aquatreat DN-30, containing approximately 30% Nabam, lot no. #1612. Received January 15, 1988. Described as a pale yellow liquid with a specific gravity of 1.15. This was stored at room temperature under a nitrogen atmosphere, with protection from light. The test material was administered in distilled water purged with nitrogen.

2. Test animals: Species: rat; strain: Crl:CD (Sprague-Dawley) from Charles River Breeding Laboratories. These were held in acclimation for at least 13 days. "Animals were examined by a veterinarian during the acclimation period. Only those animals deemed to be in good physical condition were utilized for this study."

B. STUDY DESIGN:

1. Dosage selection: No information is given as to how these particular dose levels were selected.

2. Breeding of Rats:

   From p. 12: "Virgin female rats (approximately 60 days of age) were paired (1:1) with males (approximately 60 days of age) for mating. Females were confirmed to have mated by observation of a copulatory plug in the vagina or by observation of sperm in a vaginal rinse (day 0)."

3. Animal assignment:

   From p. 12: "Confirmed mated females were assigned to groups randomly, using a random numbers table, and until each group contained at least 20 gravid rats."

   From p. 17: "To assure compliance with EPA Guideline 83-3, which recommends at least 20 pregnant rats at each dose level, it was necessary to add animals to the 5, 50, and 500 mg/kg dose groups." It is not certain when the additional animals were added, but the dosing was carried out over a six-week period. The following dose levels and

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schedule were used (from p. 12):

Group No.	Number of mated rats	target Dose Level[a] mg/kg/day	Dosing Schedule (Gestation Days)
I (control)	22	0[b]	6-15
II (low dose)	23	10	6-15
III (mid dose)	24	100	6-15
IV (high)	23	200	6-15

[a]For MRD-87-072, identified as containing approximately 30% Nabam.
[b]Received 5 ml/kg vehicle (distilled water purged with nitrogen).

4. Dosage:

"Females assigned to groups were dosed by oral intubation daily on days 6-15 of gestation... The dose was based on day 6G body weight. The dosing volume was 5 mL/kg for all groups. Nitrogen-blanketed test material was mixed with the vehicle immediately prior to dosing on each day. During the dosing, in an effort to minimize any potential degradation, the test material solution was maintained under a continuous flow of nitrogen until drawn into the dosing syringe."

5. Statistical Analysis of Data:

Refer to appended pages 1 and 2.

6. There is a signed and dated Quality Assurance Statement on page 6 of the report, with dates of inspections. There is a signed and dated GLP Compliance Statement on page 3 of the report (this is signed by the study director).

C. METHODS AND RESULTS:

1. Chemical Analyses of Dosing Solutions:

Since the rats were receiving 5 mL/kg, the target values for the concentrations of the MRD-87-072 were the following (mean analytical values are from Table III, p. 80).

Group and Dose Level (mg/kg)	Concentration of MRD-87-072 (ug/ml) Target	Mean analysis
II 5.0	1000	730
III 50.0	10000	8028
IV 500.0	100000	87537

Refer to appended page 3 for the results of the dose solution concentration analyses.

2. Maternal Toxicity:

From p. 12: "Physical examinations of mated females were performed on days 0, 6, 9, 12, 16 and 20G. Animals were also examined for viability once in the morning and once in the afternoon. Any extreme or unusual conditions were noted."

Results:

From p. 19: "During this study, one gravid dam in the Control Group died, apparently as a result of an intubation error. Alopecia was noted in animals of each experimental group, and was judged not to be treatment related."

Maternal body weights:

"Mated females were weighed on days 0, 6, 9, 12, 16 and 20G."

Results:

No significant mean body weight differences between groups were observed at any of these weighings. It is, however, noteworthy that the mean body weight for the control females was somewhat less than those of the other groups at day 0 (controls: 227.0 gm; 5 mg/kg: 240.3; 50 mg/kg: 237.6; 500 mg/kg: 234.4). This may have been related to the addition of animals (possibly older and heavier) to the 5, 50 and 500 mg/kg dose groups (refer to the statment on p. 17 of the report).

Mean body weight gains were significantly lower in the 500 mg/kg dose group with respect to controls for the periods of days 6-9, 12-16, and 0-20. The following is from Table 2, p. 27:

	Mean body weight gain (gm)						
Group No.	Days 0-6	Days 6-9	Days 9-12	Days 12-16	Days 16-20	Days 0-20	Days 6-20
I (control)	31.0	8.0	18.0	28.1	65.2	150.0	43.8
II (low dose)	34.5	11.1	16.9	27.4	68.0	158.0	40.0
III (mid dose)	33.6	6.8	16.0	26.8	67.7	150.8	36.5
IV (high)	32.5	3.3*	15.6	21.1*	63.6	136.1*	25.0**

*Significantly different from control value with $p \leq 0.05$.
**Significantly different from control value with $p \leq 0.01$.

Maternal food consumption:

"Food consumption by mated females was calculated for days 0-6G, 6-9G, 9-12G, 12-16G, and 16-20G based on feed jar weights measured on corresponding days."

Results:

Food consumption tended to be somewhat lower in the 500 mg/kg dose group with respect to controls, but this was statistically significant only for the period from days 6-9. From Table 3, p. 28:

	Mean food consumption (gm)				
Group No.	Days 0-6	Days 6-9	Days 9-12	Days 12-16	Days 16-20
I (control)	145.2	72.5	75.4	104.8	117.0
II (low dose)	158.6*	81.0**	81.7*	108.8	118.8
III (mid dose)	157.2*	74.3	77.2	107.7	119.4
IV (high)	151.7	64.7*	74.1	98.3	117.3

*Significantly different from control value with $p \leq 0.05$.
**Significantly different from control value with $p \leq 0.01$.

3. Gross Pathological Observations:

From p. 13: "Mated females were sacrificed by CO_2 inhalation on day 20G. A gross necropsy was performed, and abnormal tissues found were saved in 10% neutral buffered formalin but were not examined histologically... The thyroid and attached trachea were excised from mated females in the control and high dose groups, and placed in 10% neutral buffered formalin. After fixation of these tissues, each thyroid was removed from the trachea, weighed, and examined histologically."

Results:

From p. 19: "At necropsy, dilated renal pelvis was noted in both control and compound-treated animals, and was judged not to be treatment related." With the exception of the mean thyroid weights, all other findings were incidental and occurred without indication of a dose-related pattern.

The mean thyroid weight in the 500 mg/kg group was significantly elevated from that of the controls. From Table 9 (p. 36):

Group No.	No. of Animals	Mean thyroid weight (gm)	and S.D.
I (control)	21	0.0194	0.0044
IV (high)	22	0.0215*	0.0041

*Significantly different from control value with $p \leq 0.05$.

4. Cesarean Section Observations:

Following sacrifice on day 20, intact uteri with ovaries attached were weighed and corpora lutea were counted. The uterine contents were examined for the number of implanta-

tion sites, early and late resorptions, live and dead fetuses. "Fetuses were individually identified, examined externally, weighed, and sexed."

The following is from information on pages 19, Appendix A (pages 37-45), and Appendix D (p. 54-57):

	0 mg/kg	5 mg/kg	50 mg/kg	500 mg/kg
#Animals Assigned	22	23	24	23
#Pregnant Animals	21	21	20	21
Pregnancy Rate (%)	95.5	91.3	83.3	91.3
Maternal Wastage				
#Died	1	0	0	0
#Died/Pregnant	1	0	0	0
Total Corpora Lutea	350	374	335	365
Corpora Lutea/Dam	16.67	17.81	16.75	17.38
Total Implantations	314	342	315	325[a]
Implantations/Dam	15.0	16.3	15.8	15.5
Total Live Fetuses	285[b]	324	304	316
Live Fetuses/Dam	14.3	15.4	15.2	15.0
Total Resorptions	12	18	11	10
Early	12	18	10	10
Late	0	0	1	0

[a]Two placentas fused together at one implantation site (#410418 - refer to p. 57).

[b]One dead fetus present for #410104; 16 implantations for #410116 (which died on day 8) are not included in this total.

The values calculated by this reviewer for corpora lutea/dam, agree with the values given in the study report in Table 4 (p. 29).

5. Developmental Toxicity Examinations:

From p. 13: "Fetuses were individually identified, examined externally, and weighed. Approximately one-half of the fetuses of each litter were decapitated. These heads were preserved in Bouins solution and subsequently sectioned and examined by Wilson's technique... The viscera of all decapitated fetuses were examined by the Staples technique... The remaining fetuses were killed by CO_2 inhalation, then eviscerated. All fetuses were processed for skeletal staining with Alizarin Red S. Examination of the skeletons for malformations and variations was conducted on the fetal specimens that were not decapitated."

"Fetal observations were interpreted to be MALFORMATIONS based on their perceived potential to affect survival, growth, development, functional competance, or external appearance. VARIATIONS include alterations believed to represent retardations in development, transitory alterations, or permanent alterations not believed to adversely affect survival, growth, development, function, longevity, or external appearance."

Mean fetal body weights:

The following are the mean fetal body weights by sex as presented in Appendix E, Table 5 (p. 30) of the study report:

	Dose Level 0 mg/kg	5 mg/kg	50 mg/kg	500 mg/kg
Mean Fetal wt (gm) - males	3.42	3.51	3.44	3.44
Mean Fetal wt (gm) - females	3.26	3.29	3.25	3.35

According to the text (p. 21): "There were no statistically significant differences in fetal body weight among the experimental groups, for either male or female fetuses."

Malformations:

From p. 21: "Incidences of individual types of malformations were not statistically significantly different among the experimental groups, based on the litter as the experimental group. Using the fetus as the experimental unit, the only incidence of individual types of malformations that was found to be statistically significantly different from controls was that of vestigial or kinky tail in the 500 mg/kg group. Review of the other malformations... indicated that these occurred sporadically and/or were unique to individual fetuses, and therefore were considered not to be treatment-related."

"A statistically significant increase was noted at the 500 mg/kg dose level in...total number of malformed live fetuses/total live fetuses and total number of malformed live fetuses/total implantations, when compared to the control group. However, it should be recognized that 13 of the 14 malformed fetuses were of the same litter, and, for these 13 fetuses, kinky tail was the only malformation observed."

Fetus no. 5 from litter no. 410205 (dose level: 5 mg/kg) is reported (p. 66) as having "apparent partial acrania; head consists only of dorsal cranium, no snout or facial features present, no ear bulges present... Both frontals

and both nasals fused together into one mass; no right mandible present; no palate bones present; vomer, both palatines, presphenoid not present; no premaxillae, maxillae, malars or squamosal processes present; left mandible incompletely ossified (entire 3/4 front not present); both squamous bones incompletely ossified and misshapen; basisphenoid misshapen and fused to basiocci-pital." A number of visceral anomalies were also present in this fetus.

The following incidences of malformations are reported (Table 6, p. 31 and Table 7, p. 32):

FETAL INCIDENCES

	0 mg/kg	5 mg/kg	50 mg/kg	500 mg/kg
EXTERNAL				
Acrania	0/286	1/324	0/304	0/316
No anal opening	0/286	1/324	0/304	0/316
Vestigial or kinky tail	0/286	1/324	0/304	13/316**
VISCERAL				
No spleen or spleen half normal size	1/143	1/163	0/153	0/155
No esophagus	0/143	1/163	0/153	0/155
Stomach situs inversus	1/143	0/163	1/153	0/155
SKELETAL				
Thoracic ribs angular and/or knobby and/or wavy	3/143	0/162	0/151	0/161
Lumbar vertebrae extra transverse process	0/143	0/162	0/151	1/161
TOTAL FETAL INCIDENCE	3/285	2/324	1/304	14/316**

**Statistically significant with respect to the control incidence at $p \leq 0.01$.

The following litter incidences are reported (when more than one fetus was affected in any one dose group):

	0 mg/kg	5 mg/kg	50 mg/kg	500 mg/kg
EXTERNAL				
Vestigial or kinky tail	0/20	1/21	0/20	1/21
SKELETAL				
Thoracic ribs angular and/or knobby and/or wavy	1/20	0/21	0/20	0/21
TOTAL LITTER INCIDENCE	2/20	2/21	1/20	2/21

Variations:

The following fetal incidences (litter incidences given in parentheses) of variations are among those reported (Table 8, pp. 33-34):

	0 mg/kg	5 mg/kg	50 mg/kg	500 mg/kg
VISCERAL				
Dilated Ureter	10/143 (8/20)	7/163 (6/21)	9/153 (6/20)	19/155 (10/21)
Slightly dilated cerebral ventricles	1/143 (1/20)	8/163* (6/21)	1/153 (1/20)	3/149 (3/20)[b]
SKELETAL-CRANIUM				
Interparietal incompletely ossified	35/143 (13/20)	41/162 (17/21)	36/151 (14/20)	59/161* (13/21)
Malar incompletely ossified	2/143 (1/20)	3/162 (3/21)	2/151 (2/20)	6/161 (2/21)
Maxilla incompletely ossified	1/143 (1/20)	0/162 (0/21)	1/151 (1/20)	3/161 (1/21)
Squamosal incompletely ossified	1/143 (1/20)	3/162 (3/21)	8/151* (5/20)	7/161[a] (6/21)
Supraoccipital incompletely ossified	29/143 (11/20)	49/162* (15/21)	34/151 (14/20)	51/161* (11/21)
SKELETAL-VERTEBRAE				
Cervical vertebrae transverse processes vascular foramen not evident	17/143 (9/20)	31/162 (13/21)	27/151 (12/20)	35/161* (18/21)**

*Reported as statistically significant with respect to the control incidence at $p \leq 0.05$.

**Reported as statistically significant with respect to the control incidence at $p \leq 0.01$.

[a]Not reported as statistically significant with respect to the control incidence, but $p \leq 0.05$ by Fisher's Exact Test.

[b]Reported as 3/20 (see p. 33); presumably should be 3/21.

The report does not include information specifying which of the individual fetuses showed variations.

D. DISCUSSION:

The authors of this report conclude (p. 8-9) that the maternal NOAEL was 50 mg/kg and the maternal LEL (significantly reduced

mean body weight gains during the dosing period; significantly reduced mean food consumption during days 6-9) was 500 mg/kg. The authors also state (p. 9) that: "If the increased fetal incidence of kinky tail at 500 mg/kg is an effect of the test material, the developmental NOAEL is also 50 mg/kg. Because the litter incidence of this relatively common, genetically-mediated malformation was not significantly different from the concurrent control incidence, and the fetuses with this malformation were littermates, it is also possible that these observations were unrelated to the test material. In the latter case, the developmental NOAEL is greater than 500 mg/kg. Thus, possible adverse effects on development occurred only at a dosage that was clearly toxic to the dam, indicating that the test material does not present a unique hazard to development."

What is noteworthy is that some of the variation findings which occurred are suggestive of ETU effects. Specifically, these findings included the following:

1. A significantly elevated incidence of slightly dilated cerebral ventricles in the 5 mg/kg dose group (8/163) relative to the controls (1/143). The p (by Fisher's Exact Test) = 0.0289. However, the incidences for this finding at 50 and 500 mg/kg (1/153 and 3/149) were not significantly elevated.

2. At 500 mg/kg, there were significantly increased incidences in a number of incompletely unossified cranial bones, including interparietal (59/161, compared with 35/143 in the controls, with p = 0.0148), squamosal (7/161, compared with 1/143 in the controls, with p = 0.048) and supraoccipital (51/161, compared to 29/143 in the controls, with p = 0.01655). The incidence of the squamosal variation was also significantly elevated at 50 mg/kg (8/151, compared with 1/143 for controls, with p = 0.02207), and the incidence of the incomplete ossification of the supraoccipital was elevated at 5 mg/kg (49/162 as compared to the control 29/143, with p = 0.03104).

However, there was no evident "grouping" or "clustering" of these findings at any one dose level.

According to the 10-14-82 PD4 on the ethylene bisdithiocarbamates: "The chemistry of the EBDC's is complicated by their instability and their propensity to form polymers." It is not known whether more concentrated solutions of Nabam would be more stable with respect to formation of ETU than would be less concentrated solutions. It is also quite

possible that, by maintaining (and manipulating) the test material under a nitrogen atmosphere, its tendency to degrade to ETU under storage conditions was considerably reduced.

Within the study itself, on some occasions low analytical values (about 50% of the target concentrations) of Nabam were found in the dosing solutions. Therefore, for regulatory purposes, we can assume that the animals actually received half the target dose levels of the 30% Nabam solution (which would then be equivalent to 0.75, 7.5 and 75 mg/kg of Nabam).

The study is currently classified as core supplementary data. At the least, the following additional data should be supplied before we would consider upgrading this classification:

1. Individual fetal variation findings, indicating which fetuses showed specific variations.

2. The date on which dosage was initiated for each female.

Withheld

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