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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Methanearsonic Acid: Review of Multigeneration Toxicity Study/Rat

FROM: Steven L. Malish, Ph.D., Toxicologist S.L. Malish 12/19/94
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and Marcia van Gemert 12/20/94

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Tox Branch II; HED (7509C)

Task Identifications: Submission: S462413 DP Barcode: D201426
P.C. Code: 013803 Caswell No.: 295

ACTION REQUESTED: Review of [§ 83-4] Multigeneration Toxicity Study/Rats [MRID 431783-01]

Regulatory Criteria: This Multigeneration Toxicity Study/Rats [MRID 431783-01] does not fulfil the criteria for a 6a2 submission. The reproductive NOEL [17.8 mg/kg/day (300 ppm)] is greater than 100 times the current ADI/RfD [0.01 mg/kg/day = 1 mg/kg/day], and not "less than 100 times the current ADI" as stated in the document.

A Data Evaluation Report for the above referenced study is attached. A Summary is provided below.

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[S 83-4] Multigeneration Toxicity Study/Rats

In a two generation study, Methanearsonic Acid [>99.44%] was administered in the feed at concentration levels of 0, 100, 300 and 1000 ppm [equivalent, respectively, to 0, 5.8, 17.8, and 63.5 mg/kg/day for males and 7.5, 22.5, and 77.6 mg/kg/day for females to 30 Sprague-Dawley rats/sex/group in the $F_0$ and $F_1$ generations. Animals were mated for 21 days in a ratio of one to one after receiving the test compound for 14 weeks. Mated females continued to receive the test compound throughout the ensuing gestation and lactation periods. All parental animals were continued on treatment until sacrifice soon after weaning of the last litters. Evaluated parameters included body weight, clinical observations, food consumption, mating, pregnancy and fertility indices, gestation, parturition and mean litter data, prostate gland weight, prostate to body weight ratio, testes weight, testes to body weight ratio, sperm assessment parameters [testicular spermatid count, cauda epididymal sperm count and mean sperm mortality], and histopathology.

No adverse effect of treatment occurred at 100 ppm in the evaluated $F_0$ and $F_1$ parental generation parameters.

$F_0$ parental males at the 300 ppm dietary level, showed a suggestion of an adverse effect as evidenced by a reduction [≈9%] in mean body weight gain over the entire treatment interval correlated with a suggestion of an increase [≈6%] in food consumption during the post-mating period. Analyses of the premating period parameters showed a decrease in the efficiency of food utilization of ≈12%.

At the 1000 ppm dietary levels, treatment effects over the entire treatment interval included reduction in mean body weight [≈8%] for the $F_1$ parental males; reduced mean body weight gain [≈9%] for the $F_0$ and $F_1$ parental males which correlated with an increased food consumption in these males [≈15%]. Analyses of the premating period parameters showed a treatment related decrease in the efficiency of food utilization in the $F_0$ generation males of ≈15% and $F_1$ males of ≈19%.

Evidence of a reproductive toxicity effect was noted at 1000 ppm for both parental generations ($F_0$ and $F_1$) where decreased pregnancy rates and male fertility rates, and decreased weights of the prostate gland and testes and prostate to body weight ratio occurred.

The neonatal parameters for the $F_1$ and $F_2$ generations were not remarkable compared to the respective control at any dietary level.

The NOEL [parental/systemic toxicity] = For males: 100 ppm (≈5.8 mg/kg/day); LOEL = 300 ppm (≈17.8 mg/kg/day) based on decreased body weight gain and efficiency of food utilisation in both

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parental generations. For females, a systemic NOEL/LOEL was not determined. The NOEL [reproductive toxicity] = 300 ppm (=17.8 mg/kg/day); LOEL = 1000 ppm (=63.5 mg/kg/day) based on lower pregnancy rates and lower male fertility rates and decreased prostate and testes weights in both generations.

The study is classified as Core Guideline and satisfies the [§ 83-4] guideline requirement for a reproduction study in rats.

PRIMARY REVIEWER: Steven L. Malish, Ph.D., Toxicologist S.L. Malish 12/1
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Section III, Toxicology Branch II

**DATA EVALUATION REPORT**

**MULTIGENERATION TOXICITY STUDY**

STUDY TYPE: Multigeneration Toxicity/Rats GUIDELINE: § 83-4

IDENTIFICATION: Submission: S462413 DP Barcode: D201426
MRID No.: 431783-01 Caswell No.: 295
P.C. Code: 013803

TEST MATERIAL: Methanearsonic Acid

REGISTRANT: MAA Research Task Force Three
Dr. Elizabeth Owens
ISK Biotech, Inc.
Mentor, OH 44061

TESTING LABORATORY: Pharmaco LSR, Inc.
Mettlers Road
East Millstone, NJ 08875

TITLE OF REPORT: A Two-Generation Reproduction Study in Rats with Methanearsonic Acid (MAA)

STUDY IDENTIFICATION: 91-36668

AUTHOR: R. E. Schroeder REPORT DATE: 3/17/94

EXECUTIVE SUMMARY:

In a two generation study, Methanearsonic Acid [>99.44%] was administered in the feed at concentration levels of 0, 100, 300 and 1000 ppm [equivalent, respectively, to 0, 5.8, 17.3, and 63.5 mg/kg/day for males and 7.5, 22.5, and 77.6 mg/kg/day for females to 30 Sprague-Dawley rats/sex/group in the $F_0$ and $F_1$ generations. Animals were mated for 21 days in a ratio of one to one after



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receiving the test compound for 14 weeks. Mated females continued to receive the test compound throughout the ensuing gestation and lactation periods. All parental animals were continued on treatment until sacrifice soon after weaning of the last litters.

Evaluated parameters included body weight, clinical observations, food consumption, mating, pregnancy and fertility indices, gestation, parturition and mean litter data, prostate gland weight, prostate to body weight ratio, testes weight, testes to body weight ratio, sperm assessment parameters [testicular spermatid count, cauda epididymal sperm count and mean sperm mortality], and histopathology.

No adverse effect of treatment occurred at 100 ppm in the evaluated $F_0$ and $F_1$ parental generation parameters.

$F_0$ parental males at the 300 ppm dietary level, showed a suggestion of an adverse effect as evidenced by a reduction [≈9%] in mean body weight gain over the entire treatment interval correlated with a suggestion of an increase [≈6%] in food consumption during the post-mating period. Analyses of the premating period parameters showed a decrease in the efficiency of food utilization of ≈12%.

At the 1000 ppm dietary levels, treatment effects over the entire treatment interval included reduction in mean body weight [≈8%] for the $F_1$ parental males; reduced mean body weight gain [≈9%] for the $F_0$ and $F_1$ parental males which correlated with an increased food consumption in these males [≈15%]. Analyses of the premating period parameters showed a treatment related decrease in the efficiency of food utilization in the $F_0$ generation males of ≈15% and $F_1$ males of ≈19%.

Evidence of a reproductive toxicity effect was noted at 1000 ppm for both parental generations ($F_0$ and $F_1$) where decreased pregnancy rates and male fertility rates, and decreased weights of the prostate gland and testes and prostate to body weight ratio occurred.

The neonatal parameters for the $F_1$ and $F_2$ generations were not remarkable compared to the respective control at any dietary level.

The NOEL [parental/systemic toxicity] = for males: 100 ppm (≈5.8 mg/kg/day); LOEL = 300 ppm (≈17.8 mg/kg/day) based on decreased body weight gain and efficiency of food utilization in both parental generations. For females, a systemic NOEL/LOEL was not determined. The NOEL [reproductive toxicity] = 300 ppm (≈17.8 mg/kg/day); LOEL = 1000 ppm (≈63.5 mg/kg/day) based on lower pregnancy rates and lower male fertility rates and decreased prostate and testes weights in both generations.

The study is classified as Core Guideline and satisfies the [§ 83-4] guideline requirement for a reproduction study in rats.

I. OBJECTIVE:

The objective of this study was to assess the long term effects of Methanearsonic Acid administered via the dietary route through two generations and to determine if the test material produced abnormalities in parental activities from mating through lactation or in growth and development of offspring from conception through maturity.

II. MATERIALS AND METHODS:

A. Test Material

| | |
|---|---|
| Identity: | Methanearsonic Acid (MAA) |
| Batch No.: | 003040[illegible] |
| Purity: | >99.44% a.i. |
| Description: | White crystalline powder |
| Storage: | 60-83° F. in a fiber container with a desiccant |

Vehicle: Test substance administered in the diet.

B. Test Animals

| | |
|---|---|
| Species/Sex: | Albino Rats (Outbred) |
| Strain: | [CD®-Crl: CD®(SD) BR] |
| Source: | Charles River Laboratories, Portage, MI 49081 |
| Age: | 59 days [$F_0$ generation at study start] |
| Identification: | Metal ear tag |
| Acclimation: | ≈4 weeks |
| Housing: | Individually in suspended stainless steel cage |
| Food: | Purina Certified Rodent Chow® Brand Diet #5002 (meal) ad libitum |
| Water: | Tap water ad libitum |
| Environment: | Temperature - 19 to 25°C; Humidity - 28 to 75%; Air Changes - NA; Light/Dark Cycle: 12 hours light/12 hours dark |

C. Diet Preparation and Analysis

The test substance was administered at a constant concentration (ppm) in the diet. Fresh diets were prepared at 3 week intervals. Animals were provided with fresh feed at least weekly during the study.

D. Analytical Analyses

(a) Homogeneity

Mock batches of diets at the low and high concentrations were evaluated to determine homogeneity. Three (3) randomly drawn diet samples were taken from each mix at each of 3 levels (top, middle, bottom) in the mixer and analyzed by gas chromatography (GC) using a nitrogen phosphorus detector (NPD) for quantatation of MAA as the methylthioglycolate derivative. A confirmatory quantitative analysis was also performed using atomic absorption spectrophotometry (AAS).

(b) Stability Analysis

A 21 day ambient storage stability assessment was performed. These analyses were performed at the low (100 ppm) and high (4000 ppm) concentration diets.

(c) Analytical Confirmation of Concentration Level:

Two diet samples were collected for each test diet at preparation throughout the study.

Analyses to confirm concentration levels of diets intended for use on study were performed on the first 5 mixes (study Weeks 1-9) and subsequently for every fourth mix (Study Weeks 16, 22, 24, 26, 34, 42 and 47) for the remainder of the study.

III. STUDY DESIGN:

A. Duration of Treatment

$F_0$ Generation: $F_0$ generation animals received the appropriate treated diets for 14 weeks prior to initiating of mating and treatment continued until sacrifice.

$F_1$ Generation: $F_1$ generation animals received the appropriate treated diets for 14 weeks prior to the initiation of mating and treatment continued until sacrifice. $F_1$ pups consumed the diet at the dietary level of the dam late in lactation and selected $F_1$ animals continued to consume diets at these concentration levels during the post-weaning period through to the initiation of the pre-mating period.

B. Mating Procedure ($F_0$ and $F_1$)

Animals were mated in a ratio of 1 to 1 until observation of a copulatory plug and/or sperm in a vaginal smear or for a maximum of 7 days. Females not mated after the initial 7 day period were randomly distributed to a different male within the same treatment group until evidence of mating was observed or for 7 additional days. The same procedure was repeated for a third. 7 day mating procedure for unmated females. The day on which a plug or sperm in a lavage sample was detected was considered to be gestation Day 0.

Once mated, females were removed from the mating unit and housed individually for the remainder of gestation. The report did not indicate whether sibling matings were avoided.

C. Selection of $F_1$ Parental Generation

At weaning of each litter on Day 21 of lactation, two pups/sex/litter were randomly chosen to become a pool of animals from which the $F_1$ parental generation was selected (30/sex/group). These pups received diets at the same dietary level as their parents. The excess pups were culled so that each litter was represented in the parental generation by at least one pup of each sex.

D. Animal Assignment

$F_0$ animals were randomly assigned to test groups based on body weight. $F_1$ animals were assigned as described above.

Animal Assignments

| Group | Dietary Levels | Conc. (ppm) | Animals/Group | |
|---|---|---|---|---|
| | | | ♂ | ♀ |
| 1 | Control | 0 | 30 | 30 |
| 2 | Low | 100 | 30 | 30 |
| 3 | Mid | 300 | 30 | 30 |
| 4 | High | 1000 | 30 | 30 |

E. Observations

(a) Parental Animals

Animals were checked twice daily for mortality and gross signs of toxicologic or pharmacologic effects. Detailed physical examina-

tions for signs of local or systemic toxicity, pharmacological effects and palpation for tissue masses were performed pre-test (study weeks -2, -1 and 0) then weekly, thereafter, for the $F_0$ generation until terminal sacrifice. For the $F_1$ generation, these examinations initiated at the formal start of the pre-mating period and then weekly thereafter until terminal sacrifice.

After Day 20 of gestation, dams were observed twice daily for signs of parturition. The day on which all pups were delivered was designated Day 0 of lactation.

Body weight and food consumption were recorded weekly during pre-mating for both males and females in the $F_0$ and $F_1$ parental generations. In addition, for females, body weight was determined on Gestation Days (GDs) 0, 7, 14, and 20 and Lactation Days 0, 4, 7, 14, and 21. Food consumption was not recorded during the mating or lactation periods but was recorded on GDs 0-7, 7-14, and 14-20.

(b) Offspring

Litters were observed as soon as possible after delivery (Day 0 of lactation) for the number of live and dead pups and pup abnormalities. Thereafter, litters were observed twice daily (morning, afternoon) through to Day 21 of lactation.

Pups were counted on Days 0, 4 (pre- and post-cull), 7, 14, and 21 of lactation for live, dead and missing individuals and external sex determination was performed. Individual live pup body weights were recorded on Days 0, 4, 7 and 21 of lactation. The litters were culled on Day 4 of lactation, all litters with greater than 8 pups were reduced to that number with equal numbers/sex when possible.

F. Postmortem Examination

Males in the parental generations were sacrificed soon after the last litters were delivered. All females [both mated and unmated] were sacrificed as a group soon after weaning of the last litter. Excess $F_1$ pups and all $F_2$ pups were sacrificed on Day 21 of lactation.

Adults were exsanguinated following an overdose of inhaled carbon dioxide. Pups were sacrificed by an overdose of inhaled carbon dioxide.

(a) Gross Postmortem Examinations - Adults

Gross postmortem examinations were performed on all adult generation animals and included a count of uterine implantation scars, when present. Abnormal tissues, i.e., gross lesions, and

reproductive tissues were preserved for all animals in 10% neutral buffered formalin.

(b) Gross Postmortem Examinations - Pups

At weaning, the unselected $F_1$ pups and all pups from the $F_2$ generation were given a gross external and internal examination including internal sexing determinations and then discarded. Any abnormal tissue was saved in 10% neutral buffered formalin.

Pups (intact) found dead during lactation, stillborn pups, or those culled at Day 4, were weighed and given a gross external and internal examination including internal sexing. Unusual observations and the presence or absence of milk in the stomach of dead pups was also noted. These pups were then preserved in 10% neutral buffered formalin.

(c) Sperm Evaluation

The left testis, epididymis and vas deferens from each animal was removed and evaluated for sperm analyses on 10 randomly selected males per group from both the $F_0$ and $F_1$ generations as follows:

- spermatid count (homogeneity resistant) - left testes
- total cauda epididymal sperm count - left epididymis
- assessment of morphology from sperm collected from the left cauda epididymis
- assessment of motility from sperm collected from the vas deferens - left side
- assessment of fluid collected from the left cauda epididymis for debris and unexpected cell types (immature cells of spermatogenesis).

(d) Organs Weights

The following organs were weighed at necropsy of the $F_0$ and $F_1$ parental animals and organ/body weight ratios were calculated.

Males: testes (right and left testes individually weighed), epididymides (both weighed individually) as the entire structure and cauda alone [the cauda epididymides (left side) were weighed for the males used in the sperm assessment component. For all other males, the epididymides were weighed and retained intact], seminal vesicles (weighed together with and without their fluid content), prostate, pituitary.

Females: pituitary

(d) Histopathology

The following tissues were preserved in 10% neutral buffered formalin [testis and epididymis from males not selected for sperm evaluation were first preserved in Bouin's solution], stained with hematoxylin and eosin and microscopically examined for the all the $F_0$ and $F_1$ generation animals in the control and high dietary levels. Additionally, all gross lesions from all groups were microscopically evaluated.

The right testis of all animals selected for sperm evaluation (10/group/generation) were processed for plastic embedding, sectioned at 2 microns and stained with PAS for microscopic evaluation.

| Males | Females |
|---|---|
| testes<br>epididymides<br>seminal vesicles<br>prostate<br>coagulating gland<br>pituitary<br>gross lesions | vagina<br>uterus<br>ovaries<br>pituitary<br>gross lesions |

G. Statistical Analysis

Data for the treated groups were compared to the respective controls.

The following tests were used: Bartlett's, ANOVA, Dunnett's, Kruskal-Wallis, Summed Rank Test (Dunn), Regression Analysis [trend, lack of fit], Jonckheere's test for monotonic trend and/or Arc Sine transformation to evaluate the (1) mean body weight and mean body weight change, (2) mean food consumption data, (3) litter information - mean gestation length and mean number of pups, (4) mean pup weight data, (6) mean sperm assessment data and, (5) mean pup viability and weaning indices.

The following tests were used: Chi-square, Fisher Exact Test, Bonferroni Inequality, Armitage's Test to evaluate the (1) mating indices, (2) pregnancy rates, (3) male fertility indices, (4) litter survival index and (5) mortality rates.

H. Regulatory Compliance

Signed statements of compliance with Good Laboratory Practice

Standards, Data Confidentiality and Flagging criteria were included in the report in Vol. I. A Quality Assurance statement was included in Vol. II. The flagging statement indicates that this study exceeds the criteria (#9) for a reproduction study, that the "reproduction effects NOEL is less than 100 times the current ADI", as per 40CFR §158.34.

**IV. RESULTS and DISCUSSION:**

**A. Analytical Analyses**

(a) Homogeneity

Extractions from the 1.0 gm samples of the 100 ppm dose group using the GC/NPD method revealed that the average concentration at the top, middle and bottom of the container, respectively, were 125.0, 124.4 and 104.0% of the nominal concentration. Confirmatory analyses of the sample using AAS showed the 100 ppm dose groups to be 78.8, 92.3 and 89.6%, of the nominal concentration, respectively, in the top, middle and bottom parts of the container.

Extractions from the 1.0 gm samples of the 1000 ppm dose group were analyzed by AAS. [Extracts of the 1000 ppm dose group by GC/NPD was not performed]. The analysis showed the concentration of the test material in this dose group to be 101.2, 105.6 and 109.0% of nominal concentration (top, middle and bottom, respectively).

(b) Stability

The mean stability for all samples and for all time periods was: 108.7% - 110.0% [GC/NPD] and 86.9% - 100.2% [AAS]. These results indicated that the diets were stable over the required 21 day interval.

(c) Dietary Levels

Confirmation of mean concentration levels for all samples at all time periods was: 89.6% - 97.7% [GC/NPD] and 89.1 - 103.7 [AAS]. Dietary levels were considered to be within acceptable limits of the laboratory.

B. Mortality

(a) $F_0$ Generation

No unscheduled mortality was seen in the mid-or high-dose groups or the low levels males. One low dietary level female died on Day 21 of gestation. On Day 20 of gestation, this female showed a r. vaginal discharge; parturition was considered to have been

initiated. No pups, however, were delivered. At necropsy, 12 dead term pups and two early resorptions were found in utero. Since no other mortality occurred among the low-dose females and in the other higher levels, this effect was not considered to be treatment-related.

One accidental death occurred in 1 control male during the post-mating period. No other mortality occurred among the control animals.

(b) $F_1$ Generation

All $F_1$ parental animals in the control, low and mid-dose groups survived to scheduled sacrifice. One high dietary level female died early during the second week of the pre-mating period. Males were not affected. Macroscopic and histopathological examinations did not reveal the cause of death. The death of this high dietary level female, therefore, was not considered to be related to the administration of the test compound.

C. Observations - $F_0$ and $F_1$ Generations

The types and frequency of observations were similar between the control and treated groups for this strain of rat.

D. Body Weight

(a) $F_0$ Generation

(i) *Males*

At pre-mating [week 0-14], mean body weight and mean body weight gains were somewhat lower than the controls at all levels. Mid-dietary level males showed a 6.0% decrease [$p<0.05$] in the mean weight at Week 14 [last week of the pre-mating period] and statistically significant [$p<0.05$] reduction [10.1%] in mean weight gain over the entire pre-mating period. Since no statistical significance or dose relationship was seen when the high dietary level was analyzed, no test compound effect was suggested (Table 1).

On study week 21, during the mating and post-mating period, mean weekly body weights were somewhat lower than the controls, i.e., low [-2.2%], mid [-5.6%, $p<0.05$] and high [-4.7%]. When the individual mean weekly body weights were evaluated, statistical significance was frequently seen at the mid ($p<0.05$) and high ($p<0.01$) levels. Mean body weight gain [week 0-21] showed statistically significant ($p<0.05$) decreases of ≈9% at the mid and high dietary levels suggesting a compound related effect (Table 1).

In summary, a mean body weight gain decrease during the 0-21 week interval at the mid and high levels suggested a compound related effect.

(ii) *Females*

Mean body weight values during the pre-mating and gestation periods were comparable to the respective controls. During the lactation period a concentration related increase was seen in the mean body weight gain of treated group values compared to the controls. Mean body weight gains during the lactation period were increased at the low [14 gm], mid [20 gm] and high [22 gm] dietary levels compared to the control [10 gm], although no statistical significance was seen. No correlation with the administration of the compound could be made for this time period due to variations in the litter size and suckling efficiency (Table 2).

In summary, changes in mean body weight or body weight gain seen in treated females during the entire first generation were comparable to the controls.

(b) $F_1$ Generation

(i) *Males*

Mean body weights and mean weight gain data over the entire pre-mating period [Weeks 24-38] for the low-dose $F_1$ males were comparable to the control (Table 1).

Mean body weights at initiation of the pre-mating period [week 24] were lower than controls in the mid (8.3%) and high [9.2%, $p<0.01$] levels. Throughout the pre-mating period, mean weekly body weight data continued to be lower than the control and these differences were statistically significant [$p<0.01$] for study weeks 25-29 for the mid dietary and throughout the entire study for the high dietary level. At week 38 [end of pre-mating period] there was only a 3.4% decrease in mean body weight at the mid dietary level. Since mean body weight gain over the entire 14 week period was comparable to the control, no adverse effects of treatment were suggested at this level (Table 1).

At week 38 [end of pre-mating period], the high dietary level showed an ≈7.8% ($p<0.01$) decrease in mean body weight compared to the control and the mean weight gain over the entire period was 7% lower than the control. Statistical significance was not seen. These facts suggested a treatment related effect (Table 1).

Body weights during the mating and post-mating period (week 39-44) showed a slight decrease at the low (6.3%) levels while the high dietary level showed a statistically significant ($p<0.01$) decrease (-9.2%).

In summary, at the low and mid level, weight changes over the entire 44 week period were comparable to the controls. A statistically significant ($p<0.05$) mean body weight gain decrease (-9.0%) was seen at the high dietary level suggesting a test compound related effect (Table 1).

(ii) *Females*

At the pre-mating period, a sporadic statistically significant ($p<0.01$) increase in the mean body weight was seen at the low dietary level compared to the control.

These increases were not attributable to treatment since the mean body weight and mean weight gain values at the other levels were comparable to the respective controls (Table 3). Mean body weights and body weight gains during gestation were comparable between control and treated groups.

During the lactation period, an increase was seen in the mean body weight at the low [5.7%, $p<0.05$] and mid [9.8%, $p<0.01$] dietary levels. The high level mean body weight during lactation was similar to the control. Mean body weight gains were increased at the low [20 gm] mid [32 gm, $p<0.01$] and high [20 gm] dietary levels compared to the control [11 gm]. No correlation with the administration of the test compound could be made for this time period due to variations in the litter size and suckling efficiency (Table 3).

E. Food Consumption

(a) $F_0$ Generation

(i) *Males*

At the pre-mating period, mean weekly food consumption for the low and mid-dose animals were considered comparable to the control.

In the high dietary level, mean weekly food consumption showed statistical significant increases ($p<0.01$) relative to the control at all periods [except for week 1], i.e., 10.5% at Week 7 and 12.5% at Week 14. Mean food consumption throughout the Week 1 thru Week 14 period increased 10.3% compared to the controls (Table 4).

At the mid and high levels during the post-mating period [week 18-21], individual mean weekly food consumptions showed statistically significant increases ($p<0.05$ to $p<0.01$) relative to the control at all periods [except for week 18 at the mid level] and culminated in mean food consumption increases in the mid [6.4%] and high [14.9%] levels during weeks 18 thru 21 [Table 4].

The food consumption increases seen at the pre-mating and post-mating period in the $F_0$ generations at the mid dietary level suggested a weak compound related effect; at the high dietary level a *definite* compound-related effect was seen.

(ii) *Females*

In the pre-mating period [weeks 1-14], mean food consumption in the low and mid dietary level females was equivalent to or slightly higher than the controls at all time periods. In the high dietary level, individual mean weekly food consumption showed statistically significant ($p<0.05$ to $p<0.01$) increases at 6/14 time periods. Mean food consumption ranged from 3.2% to 7.7% of Control throughout Weeks 1 thru 14 with a mean increase over the period of 6.8% compared to the control (Table 5).

Mean weekly food consumption for the low and mid dietary level females during the gestation period [Days 0-20] were considered comparable to the control. At the high level, food consumption showed statistically significant ($p<0.01$) increases at the various weekly time intervals, 0-7 [11.4%], 7-14 [7.6%] and 14-20 [13.7%] compared to the respective controls. Throughout the gestation period [Days 0-20] a 10.4% increase in food consumption was seen compared to the control (Table 5).

Mean food consumption was comparable to the control at the mid dietary level; at the high dietary level a definite compound-related effect was seen.

(b) $F_1$ Generation

(ii) *Males*

In the premating period [weeks 25-38], mean weekly food consumption for the low dietary level males were considered comparable to the control. During the same time period, mean weekly food consumption in the mid dietary level showed statistically significant [$p<0.01$] increases compared to the controls at 12/14 weekly intervals. The mid dietary level males showed a mean increase [6.0%] during weeks 25 thru 38 and increases were seen at the sampled time periods [25, 31 and 38 weeks] of from 4.2 - 7.7% compared to the controls. During the post-mating period [week 42-44], an increase in food consumption [4.3%] was also seen compared to the control (Table 4).

In the high-dose group, individual mean weekly food consumptions at all intervals showed statistically significant [p<0.01] increases compared to the controls and ranged from 14.4% to 16.7% on week 25, 31 and 38. During weeks 25 thru 38, the high dietary level males showed increases of 15.2% compared to the respective control (Table 4).

The food consumption increases seen at the pre-mating and post-mating period in the $F_1$ generation at the mid dietary level suggested a weak compound related effect; at the high dietary level, a definite compound-related effect was seen.

(ii) *Females*

In the premating period [weeks 25-38], mean weekly food consumption for the low dietary level females were considered comparable to the control. During the same time period, mean weekly food consumption at the mid dietary level showed statistically significant ($p<0.05$ to $p<0.01$) increases compared to the controls in 10/14 weekly intervals. The mid dietary level showed increases [7.6%] during weeks 25 thru 38 and increases were seen at the sampled time periods [25, 31 and 38 weeks] of from 6.3-9.5% compared to the controls. During the gestation period [Days 0-20], an increase in food consumption [2.7%] also was seen in the mid-dose compared to the control. Individual weekly values did not show statistical significance. (Table 6).

In the high dietary level, individual mean weekly food consumption showed statistical significant ($p<0.01$) increases relative to the control at all time periods. i.e., week 25 - 11.4%, week 31 - 15.6% and week 38 - 14.3%. Mean food consumption throughout the Weeks 25 thru 38 period increased 12.7% compared to the control (Table 6).

Mean weekly food consumption for the low and mid dietary level females at the gestation period [Days 0-20] were considered comparable to the control. At the high dietary level, a 12.3% increase was seen throughout the gestation period which reflected statistically significant ($p<0.05$ to $p<0.01$) increases at Days 0-7 [18.3%], 7-14 [10.8%] and 14-20 [6.8%] compared to the respective controls (Table 6).

The food consumption increases seen at the pre-mating and gestation periods in the $F_1$ generation at the mid dietary level suggested a weak compound related effect; at the high dietary level, a definite compound-related effect was seen.

Table 1. Selected Group Mean Body Weights and Weight Gains for $F_0$ and $F_1$ Males

| Body Weights (gm) | | | | | Body Weight Gains (gm) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dietary Levels | | | | | Dietary Levels | | | |
| Weeks | Cont. | Low | Mid | High | Weeks | Cont. | Low | Mid | High |
| $F_0$ Generation | | | | | | | | | |
| Pre-Mating Period | | | | | | | | | |
| 0 | 310 | 305 | 303 | 307 | | | | | |
| 14 | 558 | 540 [-3.2] | 524* [-6.0] | 538 [-3.5] | 0-14 | 247 | 234 [-5.3] | 222* [-10.1] | 231 [-6. |
| Mating and Post-Mating Periods | | | | | | | | | |
| 21 | 594 | 581 [-2.2] | 561* [-5.6] | 566 [-4.7] | 0-21 | 284 | 276 [-2.9] | 258* [-9.0] | 259 [-8. |
| $F_1$ Generation | | | | | | | | | |
| Pre-mating Period | | | | | | | | | |
| 24 | 212 | 216 [2.8] | 194 [-8.3] | 192** [-9.2] | | | | | |
| 38 | 557 | 542 [-2.7] | 538 [-3.4] | 514** [-7.8] | 24-38 | 345 | 325 [-5.8] | 344 [-0.3] | 321 [-7. |
| Mating and Post-Mating Period | | | | | | | | | |
| 44 | 601 | 582 [-3.1] | 581 [-3.3] | 546** [-9.2] | 24-44 | 389 | 365 [-6.3] | 387 [-0.6] | 354 [-9. |

*Adapted from original report, Vol I., p. 39, 40, 90-92, 96-98, 102, 104, 106-108, 112-114, 142-145.
*p<0.05; **p<0.01;
[] = % change from control.

Table 2. Selected Group Mean Body Weight and Body Weight Gain in F, Females

| Body Weights (gm) | | | | |
|---|---|---|---|---|
| | Dietary Levels | | | |
| Time | Cont. | Low | Mid | High |
| **Pre-Mating Period** | | | | |
| Weeks | | | | |
| 0 | 196 | 196 | 196 | 195 |
| 14 | 290 | 301 | 292 | 299 |
| **Gestation Period** | | | | |
| Days | | | | |
| 0 | 285 | 294 | 290 | 284 |
| 7 | 313 | 321 | 316 | 315 |
| 14 | 340 | 348 | 340 | 339 |
| 20 | 401 | 412 | 401 | 400 |
| **Lactation Period** | | | | |
| Days | | | | |
| 0 | 312 | 316 | 310 | 309 |
| 4 | 317 | 329 | 320 | 324 |
| 14 | 331 | 342 | 332 | 334 |
| 21 | 321 | 330 | 320 | 331 |

| Body Weight Gains (gm) | | | | |
|---|---|---|---|---|
| | Dietary Levels | | | |
| Weeks | Cont. | Low | Mid | High |
| 0-14 | 93 | 105 | 96 | 104 |
| 0-20 | 116 | 118 | 111 | 116 |
| 0-21 | 10 | 14 | 20 | 22 |

*Adapted from original report, Vol. I, p. 93-95, 103, 109-111, 150-151, 158-159.

Table 3. Selected Mean Body Weight and Mean Body Weight Gains in $F_1$ Females

| Body Weights (gm) | | | | | Body Weight Gains (gm) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dietary Levels | | | | | Dietary Levels | | | |
| Time | Cont. | Low | Mid | High | Week | Cont. | Low | Mid | High |
| **Pre-Mating Period** | | | | | | | | | |
| Weeks | | | | | | | | | |
| 24 | 149 | 161** | 147 | 147 | | | | | |
| 38 | 290 | 301 | 294 | 290 | 24-38 | 141 | 140 | 147 | 144 |
| **Gestation Period** | | | | | | | | | |
| Day | | | | | | | | | |
| 0 | 280 | 290 | 289 | 275 | | | | | |
| 7 | 306 | 317 | 317 | 303 | | | | | |
| 14 | 333 | 343 | 342 | 331 | | | | | |
| 20 | 396 | 407 | 398 | 391 | 0-21 | 115 | 117 | 109 | 116 |
| **Lactation Period** | | | | | | | | | |
| Days | | | | | | | | | |
| 0 | 306 | 315 | 314 | 306 | | | | | |
| 4 | 310 | 320 | 319 | 312 | | | | | |
| 14 | 320 | 333 | 332 | 319 | | | | | |
| 21 | 315 | 333* [5.7] | 346** [9.8] | 322 [2.2] | 0-21 | 11 | 20 | 32** | 20 |

[a]Adapted from original report, Vol. I, p. 99-101, 105, 115-117, 152-153, 160-161.
*$p<0.05$; **$p<0.01$.
[] = % change compared to the control.

Table 4. Mean Food Consumption - $F_0$ and $F_1$ Male[a]

| Weeks | Mean Food Consumption (gm/kg/day) | | | |
|---|---|---|---|---|
| | Dietary Levels | | | |
| | Cont. | Low | Mid | High |
| | $F_0$ Generation | | | |
| | Pre-mating Period | | | |
| 1 | 83 | 85 | 82 [-1.2] | 82 [-1.2] |
| 7 | 97 | 98 | 98 [1.0] | 69** [10.5] |
| 14 | 48 | 50 | 51* [3.3] | 54** [12.5] |
| 1-14[b] | 58 | 59 | 59 [1.7] | 64 [10.3] |
| | Post-mating Period | | | |
| 18-21[b] | 47 | 47 | 50 [6.4] | 54 [14.9] |
| | $F_1$ Generation | | | |
| | Pre-mating Period | | | |
| 25 | 104 | 103 | 112** [7.7] | 119** [14.4] |
| 31 | 62 | 62 | 65** [4.8] | 71** [14.5] |
| 38 | 48 | 48 | 50 [4.2] | 56** [16.7] |
| 25-38[b] | 66 | 65 | 70 [6.0] | 76 [15.2] |
| | Post-mating Period | | | |
| 42-46[b] | 46 | 46 | 48 [4.3] | 53 [15.2] |

[a]Adapted from the original report, Vol I., p. 118-120, 124-126, 146, 147.
[b]Calculated by reviewer; statistical analyses not performed.
*p>0.05; **p>0.01.
[] = % difference compared to the control.

Table 5. Mean Food Consumption - $F_1$ Female[a]

| | Mean Food Consumption (gm/kg/day) | | | |
|---|---|---|---|---|
| | Dietary Levels | | | |
| Weeks | Cont. | Low | Mid | High |
| **Pre-mating Period** | | | | |
| 1 | 93 | 93 | 98 [5.2] | 93 [0.2] |
| 7 | 75 | 75 | 74 [1.4] | 76 [4.1] |
| 14 | 65 | 66 | 67 [3.1] | 70[**] [7.7] |
| 1-14[b] | 73 | 73 | 75 [2.7] | 78 [6.8] |
| **Gestation Period** | | | | |
| Days 0-7 | | 78 | 80 [1.3] | 88[**] [11.4] |
| 7-14 | 75 | 76 | 78 [-1.3] | 83[**] [7.6] |
| 14-20 | 73 | 71 | 75 [2.7] | 83[**] [13.7] |
| 0-20[b] | 77 | 75 | 78 [1.3] | 85 [10.4] |

[a]Adapted from the original report, Vol I, p. 121-123, 154.
[b]Calculated by reviewer; statistical analysis not performed.
[**]$p > 0.01$.
[] = % difference from control.

Table 6. Mean Food Consumption - $F_1$ Female[a]

| | Mean Food Consumption (gm/kg/day) | | | |
|---|---|---|---|---|
| | Dietary Levels | | | |
| Weeks | Cont. | Low | Mid | High |
| **Pre-mating Period** | | | | |
| 25 | 105 | 102 | 115[*] [9.5] | 117[**] [11.4] |
| 31 | 77[*] | 79 | 82 [6.5] | 89[**] [15.6] |
| 38 | 63 | 64 | 67[*] [6.3] | 72[**] [14.3] |
| 25-38[b] | 79 | 80 [1.3] | 85 [7.6] | 89 [12.7] |
| **Gestation Period** | | | | |
| Days 0-7 | 71 | 72 | 75 [5.6] | 84[**] [18.3] |
| 7-14 | 74 | 73 | 76 [2.7] | 82[**] [10.8] |
| 14-20 | 73 | 70 | 75 [2.7] | 78[*] [6.8] |
| 0-20[b] | 73 | 72 | 75 [2.7] | 82 [12.3] |

[a]Adapted from the original report, Vol. I., p. 127-129, 155.
[b]Calculated by reviewer; statistical analysis not performed.
[*]$p > 0.05$; [**]$p > 0.01$.
[] = % difference from control.

F. Test Substance Intake

Test substance intake [mg/kg/day] was derived from the food consumption data and based on the nominal dietary concentrations. The calculated mean test substance intake, based on the $F_0$ generation pre-mating data was ♂ 5.8, ♀ 7.5 mg/kg/day at the 100 ppm level, ♂ 17.8, ♀ 22.5 mg/kg/day at the 300 ppm dietary level and ♂ 63.5, ♀ 77.6 mg/kg/day at the 1000 ppm level. Test compound intake was 22 to 29% higher in the females compared to the males during the premating period (Table 7).

The test substance intake in the post mating period and gestation periods, respectively, were similar in the $F_0$ and $F_1$ generations when compared by sex (Table 7).

Table 7. Mean Test Substance Intake at Selected Time Periods[a]

| | Mean Test Substance Intake (mg/kg/day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $F_0$ Generation | | | | $F_1$ Generation | | | |
| Dietary Levels | Male | | Female | | Male | | Females | |
| | Pre.[b] | Post.[c] | Pre. | Gest.[d] | Pre. | Post. | Pre. | Gest. |
| Low | 5.8 | 4.7 | 7.5 | 7.5 | 6.5 | 4.6 | 7.9 | 7.2 |
| Mid | 17.8 | 14.9 | 22.5 | 23.4 | 21.1 | 14.3 | 25.4 | 22.7 |
| High | 63.5 | 54.1 | 77.6 | 85.2 | 75.8 | 52.8 | 88.6 | 81.2 |

[a]Adapted from original report, Vol. I, p. 38, 42, 46.
[b]Pre. = pre-mating period; [c]Post. = post-mating period; [d]Gest. = gestation period.

G. Mating Indices, Male Fertility Indices and Pregnancy Rates

(a) $F_0$ Generation

Slight decreases were seen in the male and female mating indices (Table 8). The mating indices for sexes were within the laboratory's historical controls (Appendix 1, Recent Historical Control Data).

Pregnancy rates for the low (82.8%) and mid-dose (82.8%) dietary level animals were comparable to the control (86.2%) but slightly outside the laboratory's historical control range [$F_1$ litters: 83-100%]. The pregnancy rate of the high dietary level animals was 74.1%, compared to 86.2% in the control, and outside the laboratory's historical control range. Although statistical significance was not seen, the decrease was considered to be a compound related response (Table 8).

Male fertility indices for the low and mid dietary levels were comparable to the respective controls. At the high dose, the male

fertility index was 79.2% compared to 95.7% in the concurrent control. Although no statistical significance was seen, the decrease was outside the laboratory's historical control range [87.0-100%] suggesting that the effect was compound related (Table 8).

(b) $F_1$ Generation

The mating indices for the treated groups were comparable to the respective control. The 70% mating index for the mid-dose group was decreased compared to the control index of 83.3% and the laboratory's historical control range [72-92%]. No treatment related effect was suggested since no statistical significance was seen and because of the lack of a similar response at the high dietary level (Table 8).

Pregnancy rates and male fertility indices for the low and mid-dose groups were comparable to the concurrent control values and within the range of the laboratory's historical controls.

In the high level, the pregnancy rate [75.9%] and male fertility index [77.8%] were lower than the respective control [89.3, 100%] but only the fertility rate was statistically significant ($p<0.05$). The pregnancy rates and male fertility indices for the $F_1$ high dietary level were within the historical control range ($F_2$ litters) for this laboratory. Since the responses were similar to those seen in the high dietary level $F_0$ parental animals, however, these changes were considered indicative of a treatment related effect (Table 8).

H. Gestation Length and Parturition Data

$F_0$ and $F_1$ Parental Generations - $F_1$ and $F_2$ Litters

Mean gestation lengths and gestation indices for the treated groups in each litter interval were comparable to the control (Table 9, 10).

One females died during delivery. One $F_0$ low-dose female presented a red vaginal discharge on Day 20 of gestation. This female was found dead; no pups were delivered prior to death. At necropsy, 12 dead pups and two resorption were found in utero.

The mean numbers of liver, dead and total pups at birth for the treated groups were comparable to control for each litter intervals (Table 9, 10).

I. Litter Size Data - Lactation Periods

$F_0$ and $F_1$ Parental Generations - $F_1$ and $F_2$ Litters:

Mean litter size on Day 4 (pre- and post-cull) and throughout the remaining lactation period for the treated groups was comparable to the control for each litter interval (Table 9, 10).

Table 8. Mating, Pregnancy and Fertility Indices

| Dietary Levels | Mating | | | | Pregnancy | | Male Fertility | |
|---|---|---|---|---|---|---|---|---|
| | ♀ Mated[a]/Total | | ♂ Mated[b]/Total | | ♂ No. Pregnant[c]/ No. Mated | | No. Impregnating/ No. Mated | |
| | No. | % | No. | % | No. | % | No. | |
| | $F_0$ Generation | | | | | | | |
| Cont. | 29/30 | 96.7 | 28/30 | 93.3 | 25/29 | 86.2 | 24/28 | 85 |
| Low | 29/30 | 96.7 | 27/30 | 90.0 | 24/29 | 82.8 | 23/27 | 85 |
| Mid | 29/30 | 96.7 | 24/30 | 80.0 | 24/29[e] | 82.8 | 22/24 | 91 |
| High | 27/30 | 90.0 | 24/30 | 80.0 | 20/27 | 74.1 | 19/24 | 79 |
| | $F_1$ Generation | | | | | | | |
| Cont. | 28/30 | 93.3 | 25/30 | 83.3 | 25/28 | 89.3 | 25/25 | 100 |
| Low | 28/30 | 93.3 | 26/30 | 86.7 | 25/28[f] | 89.3 | 23/26 | 88 |
| Mid | 29/30 | 96.7 | 21/30 | 70.0 | 23/29 | 79.3 | 19/21 | 90 |
| High | 29/29 | 100.0 | 27/30 | 90.0 | 22/29 | 75.9 | 21/27* | 77 |

[a]Number of animals showing evidence of mating [plug and/or sperm and/or pregnancy].
[b]Number of males in which mating was confirmed in at least one female.
[c]Number of females showing evidence of pregnancy (parturition and/or uterine implantation scars at gross postmortem examination).
[d]Number of males mated with at least one female for which pregnancy was evident.
[e]Pregnancy rates include one female which showed two uterine implantation scars at the gro postmortem examination.
[f]Pregnancy rates include one female which showed one uterine implantation scar at the gros postmortem examination.
*$p<0.05$.

J. Pup Data

(a) Pup Weights [$F_1$ and $F_2$ Pups]

Mean pup weights for lactation days 0, 4, 7, 14 and 21 for the low and mid-dose groups were comparable to the control. In the high dietary level, mean pup weights at days 14 [-5.1%] and 21 [-5.9%] were slightly lower than the corresponding controls. Statistical significance was not seen and these data were within the range of the laboratory's historical control data suggesting that these results were not due to the test compound. Mean pup weight data for the high-dose group at Days 0, 4 and 7 were comparable to the controls (Table 9, 10).

(b) Pup Survival

(i) $F_1$ Litters

Mean pup survival indices [Pup Viability and Pup Lactation Indices] over the Day 0-4 and 4-21 lactation intervals, respectively, in the treated groups were comparable to the controls. In the high level, the mean pup viability index (89.9%) for Day 0-4 was lower than the control index (96.4%). This difference was not statistically significant. This decrease was caused by one high dietary level female which delivered a litter containing 14 live pups but none survived to Day 4. Excluding the data for this one litter, the mean Day 0-4 pup survival index for the high dietary level (94.9%) was similar to the mean control value of 96.4%. Since the decrease in the survival index was largely attributable to increased mortality within a single litter, and statistical significance was not seen, these facts suggested that this decrease was not due to the administration of the test compound (Table 9).

The mean Day 4-21 pup lactation index for the high dietary level (92.4% was lower than the control group of 100% and slightly outside the laboratory's historical control range [93.7-100%] suggesting a treatment related effect (Table 9). Although the study author judged this effect to be a treatment-related response, the evidence is insufficient to arrive at this conclusion especially since neither the slight reduction in lactation index nor mean litter size (Days 7, 14, and 21) were statistically significant.

(ii) $F_2$ Litters

Mean pup viability indices (Day 0 - 4) for treated groups were comparable to Control.

The low dietary level mean pup lactation index (97.8%) over the 4-21 lactation period was comparable to the mean control value (97.4%) (Table 10).

At the mid-dose level, mean pup survival over the Day 4-21 lactation interval (pup lactation index) was 88.1% [lowest value seen in the treated group] vs. the control mean value of 97.4%. The difference was not statistically significant. This decrease in the pup lactation index was largely attributable to increased pup mortality in a single litter [one mid-dose female had a litter of 8 pups at Day 4 post-cull but none survived to weaning]. When this litter was excluded, the mean pup lactation index was 92.7% [just outside the range of the laboratory's historical control of range of 92.9-100%]. Since the mean pup lactation index did not differ statistically from the control data and in the absence of any effect on pup survival in the $F_1$

litters, the decrease in the $F_2$ litter survival rate was, therefore, not considered indicative of a treatment-related effect (Table 10).

At the high-dose level, mean pup survival over the Day 4-21 lactation interval (pup lactation index) was 91.3% vs. the control mean value of 97.4%. The difference was not statistically significant. This decrease in the pup lactation index was largely attributable to an increased pup mortality in a single litter [one high dietary level female had a litter of 8 pups at Day post 4 post-cull but none survived to weaning]. The study author stated that treatment related effect was suggested since the data were consistent with the results of the $F_1$ litter data (Table 10); however, lack of statistical significance for survival or mean litter size data provides insufficient evidence for this conclusion.

(c) Pup Sex Distribution [$F_1$ and $F_2$ Litters]

Sex distribution in the $F_1$ and $F_2$ litters was comparable to the respective controls at birth and at Day 4 post-cull (Table 9, 10).

(d) Dead Pup Observations [$F_1$ and $F_2$ Litters]

No adverse effect of the test compound were observed in either litter when compared to the respective controls (Table 9, 10).

Two malformed pups were noted at delivery. In one control animal of the $F_1$ litter, conjoined twins [single head with duplication of the torso] was found. A pup from the low dietary level showed severe multiple malformations involving facial cleft, cleft palate, protruding tongue, open eyes, spina bifida and exencephaly. No other malformations were seen in dead pups recovered from control or treated $F_1$ or $F_2$ females. The malformed pup recovered at the low dietary level from the $F_1$ litter was considered to be a sporadic occurrence and not related to the administration of the test compound since similar findings were not seen among pups at the higher dietary levels.

Table 9. Gestation, Parturition and Mean Litter Data - $F_0$ Generation - $F_1$ Litter[a]

| Observation | | Dietary Levels | | | |
|---|---|---|---|---|---|
| | | Control | Low | Mid | High |
| Mean Gestation Length | [Days] | 22.4 | 22.5 | 22.6 | 23.1 |
| Mean Pups Born | | 12.3 | 13.1 | 13.3 | 13.8 |
| Pups Alive at Birth | [Day 0] | 12.1 | 12.9 | 13.0 | 12.6 |
| No. of Litters with Live Pups/ No. of Litters Delivered | [Day 0] | 24/25 | 23/23 | 22/23 | 19/20 |
| Pups Dead at Birth | | 0.2 | 0.3 | 0.3 | 0.3 |
| Total Litter Death [No. of Litters] | [Days 0 - 4] | 0 | 0 | 0 | 1 |
| | [Days 4 -21] | 0 | 0 | 0 | 0 |
| Males/Females | [Day 0] | 6.6/5.6 | 6.2/6.5 | 6.0/7.0 | 6.3/6.3 |
| Pre-Cull No. of Pups Alive | [Day 4] | 12.1 | 12.1 | 12.6 | 12.6 |
| Post-Cull No. of Pups Alive | [Day 4] | 7.9 | 8.0 | 7.9 | 7.8 |
| Males/Females [Post-Cull] | [Day 4] | 4.2/3.7 | 4.4/3.6 | 3.9/4.0 | 3.8/4.0 |
| Pups Alive | [Day 7] | 7.9 | 7.9 | 7.9 | 7.8 |
| | [Day 14] | 7.9 | 7.9 | 7.7 | 7.4 |
| | [Day 21] | 7.9 | 7.9 | 7.7 | 7.2 |
| Pup Viability Index[b] | [%] | 96.4 | 94.9 | 96.6 | 89.9 |
| Pup Lactation Index[c] | [%] | 100 | 98.8 | 97.8 | 92.4 |
| Mean Pup Weight (gm) | [Day 0] | 6.3 | 6.3 | 6.4 | 6.2 |
| Mean Pup Weight (gm) | [Day 21] | 49.1 | 51.1 | 48.8 | 46.2 |

[a]Adapted from the original report, Vol. I, p. 163-166, 171, 174.
[b]Pup Viability Index = total number of live pups at Day 4 (pre-cull)/total number live pups at Day 0.
[c]Pup Lactation Index = total number of live pups at Day 21/total number of live pups at Day 4 (post-cull).

Table 10. Gestation, Parturition and Mean Litter Data - $F_1$ Generation - $F_2$ Litter[a]

| Observation | | Dietary Levels | | | |
|---|---|---|---|---|---|
| | | Control | Low | Mid | High |
| Mean Gestation Length | [Days] | 22.3 | 22.2 | 22.6 | 22.5 |
| Mean Pups Born | | 12.5 | 13.7 | 11.7 | 12.7 |
| Pups Alive at Birth | [Day 0] | 12.3 | 13.2 | 11.4 | 12.3 |
| No. of Litters with Live Pups/ No. of Litters Delivered | [Day 0] | 25/25 | 23/24 | 22/23 | 22/22 |
| Pups Dead at Birth | | 0.2 | 0.5 | 0.3 | 0.4 |
| Total Litter Death [No. of Litters] | [Days 0 - 4] | 1 | 0 | 2 | 1 |
| | [Days 4 -21] | 0 | 0 | 1 | 1 |
| Males/Females | [Day 0] | 6.6/5.7 | 6.4/6.7 | 5.5/6.0 | 6.2/6.1 |
| Pre-Cull No. of Pups Alive | [Day 4] | 12.2 | 13.4 | 12.1 | 12.1 |
| Post-Cull No. of Pups Alive | [Day 4] | 8.0 | 8.0 | 7.6 | 7.7 |
| Males/Females [Post-Cull] | [Day 4] | 4.3/3.8 | 4.0/4.0 | 3.7/3.9 | 4.0/3.7 |
| Pups Alive | [Day 7] | 8.0 | 8.0 | 7.4 | 7.6 |
| | [Day 14] | 7.8 | 7.8 | 6.9 | 7.4 |
| | [Day 21] | 7.8 | 7.8 | 6.9 | 7.3 |
| Pup Viability Index[b] | [%] | 92.4 | 97.2 | 89.0 | 92.2 |
| Pup Lactation Index[c] | [%] | 97.4 | 97.8 | 88.1 | 91.3 |
| Mean Pup Weight (gm) | [Day 0] | 6.1 | 5.9 | 5.9 | 6.3 |
| Mean Pup Weight (gm) | [Day 21] | 47.0 | 47.5 | 47.2 | 46.8 |

[a]Adapted from the original report, Vol I, p. 167-170, 172, 175.
[b]Pup Viability Index = total number of live pups at Day 4 (pre-cull)/total number of live pups at Day 0.
[c]Pup Lactation Index = total number of live pups at Day 21/total number of live pups at Day 4 (post-cull).

K. Postmortem Data

(a) Terminal Body Weights

In the $F_0$ parental generation, mean male terminal body weight showed a dose related decrease (<6%) which culminated in statistical significance ($p<0.05$) at the mid and high levels. In the $F_1$ generation a slight decrease in male body weight was seen

in both the low and mid dietary levels while at the high dietary level a statistically significant ($p<0.01$) decrease (11.9%) occurred (Table 11).

(b) Organ Weight Data

The prostate weight of the $F_0$ high-dietary level parental animals showed a dose-related decrease of <10% compared to the control. The high dietary level prostate gland to body weight ratio showed a 5% decrease compared to the control. Statistical significance was not seen. The testes [left and right] weights of the $F_0$ parental animals showed a dose-related decrease of <10% compared to the control which culminated in statistical significance ($p<0.01$) at the high dietary level in both left and right testes No change was seen in the testes (left and right) to body weight ratio at any dietary level when compared to the control values (Table 11).

The mean prostate weight of the $F_1$ parental animals showed a dose-related decrease of 9.8% - 19.4% which culminated in statistical significance ($p<0.05$) at the high dietary level compared to the control. The mean prostate to body weight ratio of the high dietary level showed a dose related 8.0% - 13.1% decrease compared to the control. Statistical significance was not seen (Table 11).

The testis weights of the $F_1$ parental animals showed a dose-related decrease in the left, but not the right testis. The right testis showed a slight decrease [5.4%, $p<0.05$] at the high dietary level compared to the control. No change was seen in the testes (left and right) to body weight ratio at any dietary level when compared to the control value (Table 11).

| Dietary Levels | Terminal Body Weight (gm) | Prostate | | Rt.Testis (gm) | Lt. Testis (gm) |
|---|---|---|---|---|---|
| | | Weight (gm) | Prostate/ Body Weight (x 1000) | (Testis/Body Weight Ratio x 1000) | (Testis/Body Weight Ratio x 1000) |
| | $F_0$ Generation | | | | |
| Control | 596 | 1.026 | 1.74 | 1.820 (3.07) | 1.826 (3.08) |
| Low | 582 | 1.008 | 1.74 | 1.786 (3.09) | 1.791 (3.09) |
| Mid | 562* | 0.973 | 1.74 | 1.739 (3.12) | 1.744 (3.12) |
| High | 563* | 0.930 | 1.66 | 1.673** (3.00) | 1.672** (2.99) |
| | $F_1$ Generation | | | | |
| Control | 596 | 1.035 | 1.76 | 1.718 (2.93) | 1.723 (2.94) |
| Low | 583 | 0.934 | 1.62 | 1.748 (3.02) | 1.722 (2.98) |
| Mid | 585 | 0.926 | 1.60 | 1.671 (2.88) | 1.670 (2.88) |
| High | 546** | 0.834* | 1.53 | 1.626* (3.00) | 1.638 (3.04) |

*Adapted from original report, Vol. I, p. 181-188; *p<0.05, **p<0.01.

(c) Gross Postmortem

(i) $F_0$ and $F_1$ Generations

Treated males presented findings comparable to the respective controls. Incidental findings in the reproductive tract of the 30 examined females of the $F_0$ generation showed that implantation scars of the uterus were present in the following number of animals: 0 ppm (25), 100 ppm (23), 300 ppm (24) and 1000 ppm (20). Similar findings occurred in the uterus of 30 examined females in the $F_1$ animals and showed that implantation scars of the uterus were present in the following number of animals: 0 ppm (25), 100 ppm (25), 300 ppm (23) and 1000 ppm (22).

Incidental findings in the kidneys of the 30 examined males of the $F_1$ generation showed kidney dilation in the following number of animals: 0 ppm (1), 100 ppm (4), 300 ppm (2) and 1000 ppm (4). The females showed the same lesion in the following number of animals: 0 ppm (2), 100 ppm (1), 300 ppm (4) and 1000 ppm (6). This change was not seen in the $F_0$ generation animals.

(d) Histological Evaluations

(i) $F_0$ and $F_1$ Generations

In the $F_0$ generation, the uterus presented an increase in squamous/squamoid metaplasia of the glands of the endometrium while the ovary presented an increase in mineral deposits [unilateral]. Both of these effects occurred in the high dietary level in 3/29 animals compared to 0/30 animals in the control. F1 animals, however, showed effects comparable to the controls suggesting sporadic occurrences not related to test compound administration. Other findings were considered to be not remarkable. The organ weight changes of the prostate gland and testes were not correlated with the histopathological findings.

(e) Sperm Assessment Parameters

(i) Cauda Epididymal Sperm Count and Testicular Spermatid Count

No treatment related effects were seen in either the $F_0$ or $F_1$ generations when compared to the respective controls (Table 12).

(i) Motility

The mean motility rate for the $F_1$ mid dietary level animals was reduced compared to the controls and was caused by a very low sperm motility rate in one animal [sperm motility rate 3.5% vs. 72.6% in the control]. Excluding data from this animal, the sperm motility rate for the $F_1$ mid-dose males was 68.1% and was similar to the sperm motility in the control group [72.6%]. This animal showed no mating activity during the study and at necropsy presented a small left testis and epididymis (Table 12).

In summary, mean motility rates for sperm collected from the vas deferens of treated males were comparable to the respective controls in both the $F_0$ and $F_1$ generations.

Table 12. Sperm Assessment Parameters*

| Dietary Levels | Testicular Spermatid Count [x $10^6$] | Cauda Epididymal Sperm Count [x $10^6$] | Mean Sperm Mortality [%] |
|---|---|---|---|
| | $F_0$ Generation | | |
| Control | 183 | 787 | 71.3 |
| Low | 176 | 722 | 79.3 |
| Mid | 177 | 731 | 75.7 |
| High | 150 | 795 | 73.3 |
| | $F_1$ Generation | | |
| Control | 177 | 718 | 72.6 |
| Low | 161 | 698 | 73.0 |
| Mid | 164 | 644 | 61.6 |
| High | 177 | 735 | 72.7 |

*Adapted from original report, Vol. I, p. 370-377.

(iii) Morphology

Sperm samples collected from the cauda epididymis of the $F_0$ and $F_1$ parental males for sperm morphology and immature sperm/unusual cell types presented no effects compared to the controls that could be attributed to the administration of the test compound.

The most common sperm abnormality seen in both the treated and control animals, at a low incidence, in both generations was taillessness followed by defects in the appearance of the hook of the sperm head. The highest incidence of abnormal sperm appeared in a $F_0$ high dietary level male and a $F_1$ mid-dose male. Both of these animals had testicular lesions at gross postmortem evaluation and reduced testicular and epididymal sperm counts; neither produced a litter.

In summary, no adverse effect of treatment was presented for the sperm morphology for the $F_0$ and $F_1$ males.

IV. DISCUSSION:

Dose levels used in this study were based on the results of a preliminary study [as noted by the study author]. No data, however, were supplied in this report. The resulting NOEL and LOEL for both parental and reproductive toxicity were acceptable for this definitive study.

The mean concentrations of the test material in chow samples expressed as a percentage of the nominal concentration as analyzed by GC/NPD during the course of the study were as follows: 97.7±31.8% for the Group II (100 ppm) dose group, 95.5% ±29.0% for the Group III (300 ppm) dose group and 89.6±27.6% for the Group IV (1000 ppm) dose group. Because of this large standard deviation, even with duplicate samples, AAS was chosen to confirm the GC/NPD findings.

The mean concentrations of the test material in chow samples expressed as a percentage of the nominal concentration analyzed by AAS during the course of the study were as follows: 99.1±16.0% for the Group II (100 ppm) dose group, 100.0±16.6% for the Group III (300 ppm) dose group and 103.7±11.1% for the Group IV (1000 ppm) dose group.

Both analytical methods used gave a valid indication of the true mean, but the AAS method presented a smaller standard deviation of ≈15% compared to the standard deviation of ≈30% in the GC/NPD method. The reason for the observed variability in the standard deviation between the two methods is unknown at the present time.

In conclusion, although the expected variability existed, all concentration levels were considered to be within the acceptable range of the laboratory with regard to homogeneity/stability and sampled dietary levels. Confirmation of homogeneity in the dietary mixes used in the study was not absolutely confirmed, however, because homogeneity was performed only on mock samples prepared before the start of the study. This fact, however, would not materially affect the integrity of the study.

No adverse effect of treatment occurred at 100 ppm in the evaluated $F_0$ and $F_1$ parental generation parameters except for a slight [<6%] and toxicological insignificant decrease in the efficiency of food utilization. The neonatal parameters for the $F_1$ and $F_2$ generation were not remarkable.

$F_0$ parental males at the 300 ppm dietary level, showed a suggestion of an adverse effect as evidenced by a reduction [≈9%] in mean body weight gain over the entire treatment interval correlated with a suggestion of an increase [≈6%] in food consumption during the post-mating period and resulted in a decrease in the efficiency of food utilization of 11.5% when the body weight gain and food consumption parameters were sampled during the premating period. A suggestion of an increase in food consumption also occurred in the $F_1$ parental generation in both sexes but correlation with mean body weight/body weight gain did not occur. Even though a treatment related decrease in the efficiency of food utilization of 6.1% was noted when the body weight gain and food consumption parameters were analyzed during the premating period, this effect was not considered to be of any

toxicological importance for no body weight change occurred. No adverse effect of treatment occurred at 300 ppm in the evaluation of $F_0$ or $F_1$ parental reproduction or of the $F_1$ and $F_2$ neonatal parameters.

At the 1000 ppm dietary levels, treatment effects over the entire treatment interval included reduction in mean body weight [≈8%] for the $F_1$ parental males; reduced mean body weight gain [≈9%] for the $F_0$ and $F_1$ parental males which correlated with an increased food consumption in these males [≈15%]. $F_0$ and $F_1$ females were not affected.

These facts suggested a treatment related decrease in the efficiency of food utilization in the $F_0$ generation males of 15.3% and $F_1$ males of 19.3% when the pre-mating period body weight gain and food consumption parameters were analyzed.

The $F_0$ generation females [all treatment levels] did not show a treatment-related decrease compared to the control in the efficiency of food utilization. The $F_1$ generation females showed a dose related decrease which reached -9.0% compared to the control at 1000 ppm. This decrease, however, was not accompanied by a change in body weight gain but was caused by an increase in food consumption over the pre-mating period and was, therefore, not considered to be of any toxicological significance.

The study author stated that organ weight data did not demonstrate an adverse effect of treatment. However, the decreased weights of the prostate gland, testes and prostate to body weight ratio in the high dietary level in both the $F_0$ and $F_1$ parental generations were suggestive of a compound related effect, for there was (1) a dose-relationship [except for the $F_0$ prostate /body weight and $F_1$ right testis], (2) statistical significance in absolute organ weights of the testes [$F_0$/$F_1$ high level], and prostate [$F_1$ high level], (3) involvement of both the $F_0$ and $F_1$ parental generations. These decreases, moreover, were not exclusively attributable to the decrease in the terminal body weights of the $F_0$ and $F_1$ parental generations since relative prostate weights were also decreased at the high-dose level (although not significantly) and absolute testis weight is often independent of less than severe body weight decrements in rats.

Evidence of reproductive toxicity was noted at 1000 ppm for both parental generations ($F_0$ and $F_1$) where decreased pregnancy rates and male fertility rates were seen. Organ weight changes also occurred in the prostate gland [and prostrate to body weight ratio] and testes which were not correlated with either gross or histopathology. No other indication of altered reproductive function was observed for the parental females for either gestation.

Reproductive effects were not seen in other subchronic or chronic studies using this compound except for the 52 week Chronic Toxicity Study in the Dog [MRID 405461/412664-01] which revealed a decreased incidence of estrus combined with an absence of corpora lutea in all females at the high dose level [35 mg/kg/day] compared to the respective controls. The study authors, however, attributed these changes to the severe debilitation caused by the test compound.

V. CONCLUSION:

No adverse effect of treatment occurred at 100 ppm in the evaluated $F_0/F_1$ parental generation parameters.

$F_0$ parental males at the 300 ppm dietary level, showed a suggestion of an adverse effect, as evidenced by a reduction [≈9%] in mean body weight gain over the entire treatment interval correlated with a suggestion of an increase [≈6%] in food consumption during the post-mating period. Analyses of the premating period parameters showed a decrease in the efficiency of food utilization of ≈12%.

At the 1000 ppm dietary levels, treatment effects over the entire treatment interval included reduction in mean body weight [≈8%] for the $F_1$ parental males; reduced mean body weight gain [≈9%] for the $F_0$ and $F_1$ parental males which correlated with an increased food consumption in these males [≈15%]. Analyses of the premating period parameters showed a treatment related decrease in the efficiency of food utilization in the $F_0$ generation males of ≈15% and $F_1$ males of ≈19%.

Evidence of a reproductive toxicity effect was noted at 1000 ppm for both parental generations ($F_0$ and $F_1$) where decreased pregnancy rates and male fertility rates, and decreased weights of the prostate gland and testes and prostate to body weight ratio occurred.

The neonatal parameters for the $F_1$ and $F_2$ generations were not remarkable compared to the respective control at any dietary level.

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