8/2/2000

# DATA EVALUATION REPORT

ZIRAM

STUDY TYPE: MULTI GENERATION REPRODUCTION FEEDING - 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. 96-07A

Primary Reviewer:
Carol S. Forsyth, Ph.D. Signature: _____
Date: _____

Secondary Reviewers:
Kowetha A. Davidson, Ph.D., D.A.B.T. Signature: _____
Date: _____

Robert H. Ross, M.S. Group Leader Signature: _____
Date: _____

Quality Assurance:
Susan Chang, M.S. Signature: _____
Date: _____

Disclaimer

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

Managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

EPA Reviewer: P.S. Gaunt, DVM, PhD _____, Date _____
CEB2(7509C)
EPA Secondary Reviewer:Sanjivani Diwan, PhD _____ , Date _____
CEB2(7509C)

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction - Rat
OPPTS 870.3800 [§83-4]

DP BARCODE: D223815
P.C. CODE: 034805

SUBMISSION CODE: S501475
TOX. CHEM. NO.: N/A

TEST MATERIAL (PURITY): Ziram (97.8% a.i.)

SYNONYMS: Zinc dimethyldithiocarbamate

CITATION: Nemec, M.D. (1996) A dietary two-generation reproduction and developmental neurotoxicity study of ziram in rats, WIL Research Laboratories, Inc., 1407 George Road, Ashland, OH 44805-9281. Laboratory study number WIL-223003, January 30, 1996. MRID 43935801. Unpublished.

SPONSOR: The Ziram Task Force, NPC, Inc., 22636 Glenn Drive, Suite 304, Sterling, VA 22170

EXECUTIVE SUMMARY: Ziram (97.8% a.i.) was administered to male and female Sprague-Dawley CD rats in the diet at concentrations of 0, 72, 207, or 540 ppm for two generations (MRID 43935801). Premating doses for the $F_0$ males were 5.3, 14.8, and 37.5 mg/kg, respectively and for the $F_0$ females were 6.1, 16.8, and 42.8 mg/kg, respectively. Premating doses for the $F_1$ males were 5.6, 16.7, and 42.7 mg/kg, respectively, and for the $F_1$ females were 6.3, 18.4, and 47.5 mg/kg, respectively. Each generation contained 30 animals/sex/dose which were given test or control diet for at least 10 weeks then mated within the same dose group. $F_1$ animals were weaned on the same diet as their parents. Sibling matings were avoided and at least 23 litters were produced in each generation. All animals were exposed to test material either in the diet or during lactation until sacrifice. The time course for the study was as follows: weeks 1-10, $F_0$ premating; weeks 11-18, $F_0$ breeding, gestation, and lactation; weeks 19-30, $F_1$ premating; week 39, end of study.

All $F_0$ and $F_1$ parental animals survived to scheduled necropsy. Generalized, clinical signs in the adult animals, such as hair loss and sores, were observed in the control and treated animals equally and there was no correlation with dose.

No treatment-related effects were seen in the 72 or 207 ppm groups of either generation as compared with controls. High-dose $F_0$ males initially had lower body weights (90-93%) than controls at weeks 1, 2 ($p \leq 0.01$), and 3 ($p \leq 0.05$) due to a significantly ($p \leq 0.01$) lower body weight gain (71%) during week 0-1. Throughout the remainder of the study, there were no significant differences in absolute body weights of the treated $F_0$ male groups as compared

to controls. Food consumption by the high-dose $F_0$ males was significantly ($p \leq 0.01$) less than controls for the first 4 weeks of the study and at weeks 8-9, 9-10 ($p \leq 0.05$), and 10-11. Body weights of the high-dose $F_0$ females were significantly ($p \leq 0.01$) less than the controls for the entire premating period (92-94%). However, body weight gains were significantly less than controls only during week 0-1 (44%; $p \leq 0.01$), week 1-2 (76%; $p \leq 0.05$), and week 6-7 (67%; $p \leq 0.01$). High-dose $F_0$ females ate significantly ($p \leq 0.01$) less than the controls throughout the entire premating period.

High-dose $F_1$ males had significantly ($p \leq 0.01$) lower body weights (97-90%) as compared to controls throughout the entire premating period and continuing until study termination. Body weight gains in the high-dose males were significantly less than the controls during study weeks 18-19, 20-21 (83%; $p \leq 0.01$), and 21-22 (90%; $p \leq 0.05$) of the premating period. Food consumption was significantly less than the controls for the high-dose $F_1$ males ($p \leq 0.01$) throughout the entire premating period. Absolute body weights of the high-dose $F_1$ females were significantly lower than the controls for the entire premating period (89-92%; study weeks 19-23, $p \leq 0.05$; weeks 24-30, $p \leq 0.01$); significantly lower body weight gains (67-87%) occurred only during study weeks 18-19 ($p \leq 0.05$), 23-24, and 24-25 ($p \leq 0.01$). Food consumption by the high-dose $F_1$ females was also significantly less than the controls throughout premating ($p \leq 0.01$; weeks 21-22 and 28-29, $p \leq 0.05$).

There were no treatment-related gross- or histological abnormalities observed in either generation. Differences in absolute and relative organ weights of the high-dose male and female $F_0$ and $F_1$ groups as compared to controls are consistent with reduced body weights of these animals.

**Therefore, the systemic toxicity LOAEL is 540 ppm (37.5 mg/kg/day) based on reduced body weights, body weight gains, and decreased food consumption by $F_0$ and $F_1$ males and females. The systemic toxicity NOAEL is 207 ppm (14.8 mg/kg/day).**

High-dose $F_0$ animals had significantly ($p \leq 0.01$) lower body weights as compared to controls throughout gestation and until day 14 of lactation; body weight gains were significantly ($p \leq 0.05$) less than controls during the day 10-14 interval of gestation. Some recovery was apparent in the high-dose $F_0$ females with body weight gains significantly ($p \leq 0.01$) greater than the controls during lactation days 14-21; this resulted in overall body weight gains during lactation significantly greater than the controls. On gestation day 20 and lactation day 21 body weights of the high-dose $F_0$ animals were 90% and 98% of the control level. High-dose $F_0$ females also had significantly ($p \leq 0.01$) lower food consumption as compared to controls throughout gestation and during days 4-7 ($p \leq 0.05$) and 7-14 of lactation. The high-dose $F_1$ females had significantly lower body weights throughout gestation (days 0 and 7, $p \leq 0.05$; day 10, 14, and 20, $p \leq 0.01$) and lactation ($p \leq 0.01$) as compared to controls. Body weight gains were significantly lower in the high-dose ($p \leq 0.01$) group as compared to controls during days 14-20 of gestation. No significant differences occurred for body weight gains during lactation for any treated group as compared to controls. On gestation day 20 and lactation day 21 body weights of the high-dose $F_1$ animals were 89% and 93% of the control level. Food consumption was significantly ($p \leq 0.05$ or $p \leq 0.01$) lower than controls by the high-dose group throughout gestation and lactation.

No dose- or treatment-related effects were noted on the reproductive performance of adults from either generation. $F_1$ pups from high-dose group dams had consistently lower body weights than controls beginning at day 4 precull with significance (92%; $p \leq 0.01$) reached on day 14. High-dose $F_2$ pups also had lower body weights than the controls throughout lactation with significance reached on days 1, 4 precull (92-93%; $p \leq 0.05$), 14, and 21 (88-91%; $p \leq 0.01$).

**Therefore, the LOAEL for offspring toxicity is 540 ppm (42.8 mg/kg/day) based on reduced pup body weights at birth in $F_2$ pups and during lactation in both $F_1$ and $F_2$ pups. The corresponding NOAEL for offspring toxicity is 207 ppm (16.8 mg/kg/day).**

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

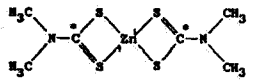
This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a multigeneration reproduction feeding study (83-4) in rats.

# I. MATERIALS AND METHODS

## A. MATERIALS

### 1. Test material: Ziram

Description: white powder
Lot/Batch No.: V528/8331AA
Purity: 97.8%, a.i.
Stability of compound: stable
CAS No.: 137-30-4
Structure:



### 2. Vehicle and/or positive control

Purina® Certified Rodent Chow® #5002, meal form, was used as vehicle and negative control. No positive control was used in this study.

### 3. Test animals

Species: rat
Strain: Sprague-Dawley Crl:CD®BR
Age and weight at start of study: approximately 6 weeks; males: 144-218 g, females: 117-174 g
Source: Charles River Breeding Laboratories, Inc., Portage, Michigan
Housing: Parental animals were individually housed in wire mesh cages until mating. Mated females were transferred to plastic maternity cages with nesting material (ground corn cob).
Diet: Purina® Certified Rodent Chow® #5002, meal form, was available *ad libitum*.
Water: Tap water was available *ad libitum* via an automatic watering system.
Environmental conditions:
Temperature: 68 - 79°F
Humidity: 20 - 92%
Air Changes: 10/hour
Photoperiod: 12 hour light/dark
Acclimation period: 12 days

4. Diet preparation and analysis

Fresh diets were prepared weekly and stored refrigerated. All diet formulations were adjusted for the per cent purity of the test article (97.8%). The appropriate amount of test article was added to 5 kg of diet and mixed in a Hobart blender for 5 minutes. This premix was added to a sufficient amount of diet to obtain 17 kg of the appropriate concentration of test diet and blended in a V-twin shell blender for 10 minutes. An intensifier bar was used during the first and last three minutes of this blending time. Samples from each diet were collected weekly and analyzed for concentration. Duplicate samples from the top, middle, and bottom of each dietary mixture were taken prior to initiation of dosing. One set of samples was analyzed for homogeneity while the other was stored refrigerated for 10 days and analyzed for stability.

Results

Homogeneity analysis: Results from the analyses of samples taken from the top, middle, and bottom of each diet formulation showed the preparations to be adequately mixed. Concentrations varied 97.2-107% of target for all formulations.

Stability analysis: Following 10 days at refrigerated storage, the low-, mid-, and high-dose diets were within 76%, 87%, and 96% of their initial measured concentrations. To compensate for the loss in the low- and mid-dose diets, these formulations were fortified by 20% and 15%, respectively. This insured that target concentrations were maintained over the course of the study.

Concentration analysis: Absence of test article was confirmed in the control diet. Concentrations of test article in the low-, mid-, and high-dose diets varied 87-101%, 88-103%, and 96-108% of nominal, respectively. If the measured concentration was >15% from target, fresh diets were prepared.

Results of the dietary analyses indicate that mixing was adequate and that administered concentrations were within 13% of target. Loss of test article in the diet due to storage was compensated for by increasing the initial concentrations of the low- and mid-dose diets.

B. STUDY DESIGN

1. In life dates

Start: May 24, 1994; end: March 31, 1995

2. Animal assignment

$F_0$ animals were randomly assigned to groups by the use of a computerized stratification block design based on body weight. $F_1$ pups were randomly selected, at least one male and one female from each litter, and weaned onto the same diet as their respective parents. Thirty $F_2$ pups/sex/group were also randomly selected for developmental neurotoxicity testing; these results are presented in a separate DER. Animal assignment is given in Table 1.

TABLE 1. Animal assignment

| Dose Group | Conc. in Diet[a] (ppm) | No. of Animals per Group | | | |
|---|---|---|---|---|---|
| | | $F_0$ Generation | | $F_1$ Generation | |
| | | Male | Female | Male | Female |
| 0 (Control) | 0 | 30 | 30 | 30 | 30 |
| 1 (Low) | 72 | 30 | 30 | 30 | 30 |
| 2 (Mid) | 207 | 30 | 30 | 30 | 30 |
| 3 (High) | 540 | 30 | 30 | 30 | 30 |

Data taken from pp. 28, MRID 43935801.
[a]Diets were administered from the beginning of the study until the animals were sacrificed.

3. Dose selection rationale

Doses were selected on the basis of a dose range-finding study conducted previously by the testing laboratory (WIL-223002). Only data on the analysis of dietary formulations were included with the main study.

C. METHODS

1. Mating procedure and schedule

$F_0$ animals were fed control or treated diets for 10 weeks prior to mating and continuing throughout mating, gestation, and lactation. $F_1$ animals were weaned onto the same diets as were fed their respective parents. After weaning, the $F_1$ animals were maintained on treatment for 10 weeks prior to mating. For mating, animals of the same dose group were paired one male to one female. Sibling matings were avoided. Females were examined each morning for evidence of mating which consisted of sperm in a vaginal lavage or a copulatory plug. Day 0 of gestation was designated as the day evidence of mating was seen. Each female was left with its first male for a maximum of 10 days. If no sign of mating was observed, the female was placed with a proven male of the same treatment group for an additional 5 days. When evidence of mating was not detected after the total 15-day period, the female was returned to individual housing.

2. Observation schedule

a. Parental animals - All animals were observed twice daily for morbidity, mortality, overt signs of toxicity, and dystocia. Body weights of the $F_0$ and $F_1$ males were recorded weekly throughout the study and prior to necropsy. Females were weighed weekly until evidence of copulation then on days 0, 7, 10, 14, and 20 of gestation and on days 1, 4, 7, 14, and 21 of lactation. Food consumption for the $F_0$ and $F_1$ adults was measured daily until mating. After mating food consumption for males was measured daily until necropsy and for females daily throughout gestation and lactation. Food consumption was calculated and reported as g/animal/day and g/kg/day at weekly intervals. Compound consumption was calculated from the mean food consumption data and the nominal concentration of test article in the diet for each sex/group.

b. Reproductive performance - The duration of gestation for each female and the following indices were calculated:

Female mating index = (No. females with evidence of mating/Total no. females paired)×100

Male mating index = (No. males with evidence of mating/Total no. males paired)×100

Female fertility index = (No. females pregnant/No. females paired)×100

Male fertility index = (No. males siring a litter/No. males paired)×100

c. Litter observations - All females were allowed to litter, the pups were examined for gross malformations, and the number of live and dead was pups recorded. Offspring were individually

identified by application of tattoo markings on the digits on lactation day 0. Live pups were counted and examined daily for survival and changes in appearance or behavior. Pups were individually sexed on lactation days 0, 4, and 21. All intact pups dying prior to weaning were necropsied. Each pup was weighed and received a detailed physical examination on lactation days 1, 4, 7, 14, and 21, and at weekly intervals thereafter until euthanasia. On lactation day 4, all litters were standardized to 4 males and 4 females, where possible, and the remaining pups euthanized and discarded. The following indices were calculated:

Live litter size = (No. live pups on lactation day 0/No. litters with viable pups)

Viability index (precull) = (No. live pups at lactation day 1 or 4/No. live pups on day 0)×100

Viability index (postcull) = (No. live pups at lactation day N/No. live pups on day 4 postcull)×100; where N = 7, 14, or 21 [same as lactation index].

3. Postmortem Studies

a. Sacrifice - All animals were euthanitized by carbon dioxide inhalation.

b. Necropsy -

1) Parental animals - The $F_0$ adults were euthanized following selection of the $F_1$ generation. The $F_1$ adults were euthanized following weaning of the $F_2$ pups. A complete necropsy and selective histopathological examination was performed on all animals.

2) Offspring - Offspring dying from lactation days 0 to 4 were necropsied using a fresh dissection technique. Offspring dying on or after day 4 were subjected to a detailed gross necropsy and tissues saved for histopathological examination as appropriate. All surviving non-selected $F_1$ pups were euthanized and necropsied on postnatal day 28. All $F_2$ weanlings not selected for developmental neurotoxicity testing were euthanized and necropsied on postnatal day 22.

3) Necropsy observations - $F_0$ and $F_1$ parental animals were subjected to a gross necropsy consisting of external and internal examinations. The following tissues (X) were preserved and weighed (XX). All organs and tissues were fixed in 10% neutral buffered formalin. A qualitative evaluation of spermatogenesis was conducted on males rats that failed to sire a litter. The * tissues, as well as the cervix and vas deferens, were examined histologically from the control and high-dose groups. Organ and tissue samples from the $F_2$ pups were collected and preserved only as deemed necessary by gross findings.

| X | DIGESTIVE SYSTEM | X | CARDIOVASC./HEMAT. | X | NEUROLOGIC |
|---|---|---|---|---|---|
| | Tongue | X | Aorta | XX | Brain |
| X | Salivary glands | X | Heart | X | Periph. nerve |
| X | Esophagus | X | Bone marrow | X | Spinal cord |
| X | Stomach | X | Lymph nodes | XX | (cervical) |
| X | Duodenum | X | Spleen | X | Pituitary* |
| X | Jejunum | X | Thymus | | Eyes (optic n.) |
| X | Ileum | | | | |
| X | Cecum | | UROGENITAL | X | |
| X | Colon | XX | Kidneys* | | GLANDULAR |
| X | Rectum | X | Urinary bladder | X | Adrenal gland |
| XX | Liver* | XX | Testes* | | Lacrimal gland |
| | Gall bladder | XX | Epididymides* | X | Mammary gland |
| X | Pancreas | X | Prostate* | | Parathyroids |
| | | X | Seminal vesicle* | | Thyroids |
| | RESPIRATORY | X | Coagulating gland* | X | |
| X | Trachea | XX | Ovaries* | X | |
| X | Lung | X | Uterus with | X | OTHER |
| | Nose | | vagina* | X | Bone |
| | Pharynx | | | | Skeletal muscle |
| | Larynx | | | | Skin |
| | | | | | All gross lesions and masses* |

*These tissues from the control and high dose groups were examined histologically.

D. STATISTICAL ANALYSES

Statistical tests were two-tailed except where noted. Tests were run on a Digital® MicroVAX® 3400 computer. Pup sex ratios, parental mating and fertility indices, numbers of stillborn and dead pups, and pup viability indices were analyzed by a Chi-square test with Yates' correction factor. Parental and pup body weights and parental body weight changes, parental food consumption, mean gestation length, absolute and relative organ weights, and live litter size were analyzed by a one-way analysis of variance (ANOVA) with Dunnett's test. Histopathological findings were analyzed by the Kolmogorov-Smirnov test (one-tailed). Data from nongravid animals was excluded from statistical analyses following the mating period. Significance level was set at 0.05.

## II. RESULTS

### A. SYSTEMIC TOXICITY

#### 1. Mortality and clinical signs

All $F_0$ and $F_1$ parental animals survived to scheduled necropsy. Clinical signs of toxicity in the adult animals consisted of hair loss, sores or scabbing around the ears and eyes, and

malaligned upper incisors. In the $F_0$ generation, control and treated animals were affected equally and there was no correlation with dose. Hair loss from the limbs was more common in the $F_1$ male and female high-dose groups than the controls, but this trend was not dose-related.

2. Body weight and food consumption

a. Premating - Body weight and food consumption data for the $F_0$ males and females are given in Tables 2 and 3, respectively. High-dose males initially had lower body weights than controls at weeks 1, 2 ($p \leq 0.01$), and 3 ($p \leq 0.05$). This was due to a significantly ($p \leq 0.01$) lower body weight gain (53%) for the high-dose males during week 0-1. Throughout the remainder of the study, there were no significant differences in absolute body weights of the treated male groups as compared to controls. At the end of the premating period, body weights of the high-dose males were 97% of the control value. After week 1, body weight gains of the treated male groups were occasionally greater ($p \leq 0.05$ or 0.01) than the controls. Body weights of

TABLE 2. $F_0$ Males: mean body weights and food consumption

| Week of study | Treatment group | | | |
|---|---|---|---|---|
| | 0 ppm | 72 ppm | 207 ppm | 540 ppm |
| **Body weight (g)** | | | | |
| 0 | 183 ± 13.5 | 183 ± 14.3 | 184 ± 13.8 | 183 ± 14.0 |
| 1 | 234 ± 18.3 | 234 ± 20.5 | 231 ± 17.6 | 210 ± 19.2** (90)[a] |
| 2 | 275 ± 23.2 | 279 ± 26.0 | 278 ± 21.6 | 252 ± 23.8** (92) |
| 4 | 341 ± 30.5 | 352 ± 34.0 | 353 ± 27.0 | 323 ± 30.0 (95) |
| 6 | 389 ± 35.7 | 403 ± 40.2 | 404 ± 31.9 | 372 ± 31.4 (96) |
| 8 | 429 ± 39.0 | 447 ± 44.5 | 448 ± 36.3 | 417 ± 34.1 (97) |
| 10 (end of premating) | 460 ± 40.6 | 476 ± 45.5 | 478 ± 35.1 | 445 ± 34.4 (97) |
| 20 (end of study) | 544 ± 40.8 | 553 ± 57.8 | 561 ± 52.1 | 519 ± 45.6 (95) |
| Weight gain weeks 0-10[b] | 277 | 293 | 294 | 262 (94) |
| Weight gain weeks 0-20[b] | 361 | 370 | 377 | 336 (93) |
| **Weekly food consumption prior to mating (g/rat/day)** | | | | |
| 0-1 | 24 ± 1.8 | 24 ± 2.3 | 23 ± 1.6 | 17 ± 2.7** (71) |
| 1-2 | 24 ± 2.2 | 24 ± 2.6 | 25 ± 2.0 | 22 ± 2.3** (92) |
| 3-4 | 25 ± 1.9 | 26 ± 2.5 | 26 ± 1.8 | 23 ± 2.3** (92) |
| 5-6 | 25 ± 2.3 | 26 ± 2.7 | 26 ± 2.0 | 24 ± 1.9 (96) |
| 7-8 | 25 ± 2.2 | 26 ± 2.8 | 26 ± 2.3 | 24 ± 1.9 (96) |
| 9-10 | 25 ± 2.3 | 26 ± 2.6 | 25 ± 2.0 | 24 ± 2.6* (96) |

Data taken from Tables 4 and 10, pp. 92-101 and 114-121, respectively, MRID 43935801.

[a]Numbers in parentheses are percent of control.

[b]Calculated by reviewer from week 0 and 10 group means.

Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

TABLE 3. $F_0$ Females: mean body weights and food consumption prior to mating

| Week of study | Treatment group | | | |
|---|---|---|---|---|
| | 0 ppm | 72 ppm | 207 ppm | 540 ppm |
| Body weight (g) | | | | |
| 0 | 141 ± 10.4 | 142 ± 11.6 | 141 ± 10.4 | 142 ± 10.4 |
| 1 | 160 ± 10.6 | 158 ± 11.8 | 157 ± 13.0 | 150 ± 9.5** (94)[a] |
| 2 | 176 ± 11.6 | 175 ± 13.9 | 172 ± 14.0 | 163 ± 10.0** (93) |
| 4 | 201 ± 13.8 | 199 ± 16.9 | 198 ± 14.9 | 187 ± 10.7** (93) |
| 6 | 222 ± 16.5 | 221 ± 20 | 219 ± 16.0 | 207 ± 11.9** (93) |
| 8 | 239 ± 17.9 | 238 ± 21.9 | 235 ± 16.6 | 221 ± 11.4** (92) |
| 10 | 251 ± 18.8 | 250 ± 22.4 | 244 ± 17.6 | 231 ± 13.0** (92) |
| Weight gain weeks 0-10[b] | 110 | 108 | 103 | 89 (81) |
| Food consumption (g/rat/day) | | | | |
| 0-1 | 17 ± 1.2 | 17 ± 1.3 | 16 ± 1.4** | 12 ± 1.6** (71) |
| 1-2 | 16 ± 1.1 | 16 ± 1.3 | 16 ± 1.8 | 15 ± 1.3** (94) |
| 3-4 | 17 ± 1.8 | 17 ± 1.3 | 17 ± 1.7 | 15 ± 1.2** (88) |
| 5-6 | 18 ± 1.8 | 17 ± 1.4 | 17 ± 1.9 | 16 ± 1.3** (89) |
| 7-8 | 17 ± 2.0 | 18 ± 1.7 | 17 ± 1.4 | 16 ± 1.4** (94) |
| 9-10 | 17 ± 1.5 | 18 ± 2.7 | 17 ± 1.5 | 16 ± 1.5** (94) |

Data taken from Tables 4 and 10, pp. 92-101 and 114-121, respectively, MRID 43935801.
[a]Numbers in parentheses are percent of control.
[b]Calculated by reviewer from week 0 and 10 group means.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

the high-dose $F_0$ females were significantly ($p \leq 0.01$) less than the controls for the entire premating period. However, body weight gains were significantly less than controls only during week 0-1 ($p \leq 0.01$), week 1-2 ($p \leq 0.05$), and week 6-7 ($p \leq 0.01$). At the end of the premating period, body weights of the high-dose females were 92% of the control value. Body weight gains of the mid-dose females were sporadically greater than or less than ($p \leq 0.05$) the controls.

Food consumption by the high-dose $F_0$ males was significantly ($p \leq 0.01$) less than controls for the first 4 weeks of the study and at weeks 8-9, 9-10 ($p \leq 0.05$), and 10-11. Week 1 food consumption by high-dose males was 71% of the control amount, but up to 92-96% thereafter. High-dose $F_0$ females ate significantly ($p \leq 0.01$) less than the controls throughout the entire premating period. Food consumption by the high-dose females for week 1 was 71% of the control amount and ranged from 88-94% thereafter. Mid-dose females had significantly ($p \leq 0.01$) lower food consumption as compared to controls during week 0-1.

Body weight and food consumption data for the $F_1$ males and females are given in Tables 4 and 5, respectively. High-dose males had significantly ($p \leq 0.01$) lower body weights as compared to controls throughout the entire premating period and continuing until study termination. At the end of the premating period, body weights of the high-dose males were 89% of the control value. Body weight gains in the high-dose males were significantly less than the controls only during study weeks 18-19, 20-21 ($p \leq 0.01$), and 21-22 ($p \leq 0.05$) of the $F_1$ premating period. Absolute body weights of the high-dose $F_1$ females were significantly lower than the controls for the entire premating period (weeks 19-23, $p \leq 0.05$; weeks 24-30, $p \leq 0.01$). However, significantly lower body weight gains occurred only during weeks 18-19 ($p \leq 0.05$), 23-24, and 24-25 ($p \leq 0.01$) for the high-dose females as compared to controls. At the end of the premating period, body weights of the high-dose females were 90% of the control value.

Food consumption was significantly less than that of the controls for the high-dose $F_1$ males ($p \leq 0.01$) throughout the entire premating period and for the mid-dose males ($p \leq 0.05$) for study weeks 18-19 and 19-20. Food consumption by the high-dose $F_1$ females was also

TABLE 4. $F_1$ Males: mean body weights and food consumption

| Week of study | Treatment group | | | |
|---|---|---|---|---|
| | 0 ppm | 72 ppm | 207 ppm | 540 ppm |
| Body weight (g) | | | | |
| 19 | 111 ± 21.8 | 113 ± 16.2 | 104 ± 21.5 | 97 ± 14.7* (87)[A] |
| 21 | 215 ± 30.0 | 219 ± 20.7 | 202 ± 31.3 | 188 ± 21.7** (87) |
| 23 | 312 ± 38.3 | 317 ± 24.0 | 299 ± 39.8 | 276 ± 29.9** (88) |
| 25 | 379 ± 41.2 | 384 ± 34.2 | 370 ± 40.2 | 339 ± 34.5** (89) |
| 27 | 427 ± 43.5 | 431 ± 38.6 | 420 ± 44.0 | 383 ± 38.2** (90) |
| 30 (end of premating) | 477 ± 47.4 | 478 ± 41.0 | 471 ± 46.0 | 426 ± 38.1** (89) |
| 39 (end of study) | 550 ± 49.7 | 549 ± 43.8 | 548 ± 53.7 | 494 ± 45.0** (90) |
| Weight gain weeks 19-39[b] | 439 | 436 | 444 | 397 (90) |
| Food consumption prior to mating (g/rat/day) | | | | |
| 19-20 | 19 ± 2.4 | 19 ± 1.6 | 18 ± 2.4* | 16 ± 1.8** (84) |
| 21-22 | 23 ± 2.8 | 24 ± 2.0 | 23 ± 2.7 | 21 ± 2.1** (91) |
| 23-24 | 25 ± 3.2 | 25 ± 2.2 | 25 ± 2.7 | 23 ± 2.3** (92) |
| 25-26 | 25 ± 2.6 | 25 ± 2.3 | 25 ± 2.6 | 23 ± 2.4** (92) |
| 27-28 | 26 ± 2.6 | 26 ± 2.3 | 25 ± 2.6 | 23 ± 2.0** (88) |
| 30-31 | 26 ± 2.6 | 25 ± 2.0 | 26 ± 2.6 | 22 ± 2.0** (85) |

Data taken from Tables 32 and 38, pp. 179-188 and 203-210, respectively, MRID 43935801.
[A]Numbers in parentheses are percent of control.
[b]Calculated by reviewer from week 19 and 39 group means.
Significantly different from controls, *$p \leq 0.05$; **$p \leq 0.01$.

TABLE 5. $F_1$ Females: mean body weights and food consumption prior to mating

| Week of study | Treatment group | | | |
|---|---|---|---|---|
| | 0 ppm | 72 ppm | 207 ppm | 540 ppm |
| Body weight (g) | | | | |
| 19 | 96 ± 16.7 | 98 ± 13.4 | 93 ± 13.4 | 87 ± 10.4* (91)[a] |
| 21 | 152 ± 20.8 | 151 ± 15.7 | 150 ± 13.3 | 140 ± 11.4* (92) |
| 23 | 192 ± 26.9 | 188 ± 21.5 | 191 ± 14.5 | 176 ± 16.5* (92) |
| 25 | 221 ± 29.1 | 213 ± 24.3 | 216 ± 17.3 | 197 ± 19.5** (89) |
| 27 | 242 ± 30.9 | 232 ± 26.2 | 235 ± 19.4 | 219 ± 20.9** (90) |
| 30 | 261 ± 32.3 | 254 ± 29.5 | 254 ± 24.1 | 235 ± 18.7** (90) |
| Weight gain weeks 19-30[b] | 165 | 156 | 161 | 148 (90) |
| Food consumption (g/rat/day) | | | | |
| 19-20 | 16 ± 2.1 | 16 ± 1.6 | 16 ± 1.5 | 15 ± 1.1** (94) |
| 21-22 | 17 ± 2.3 | 16 ± 2.4 | 17 ± 1.7 | 15 ± 1.2* (88) |
| 23-24 | 18 ± 2.5 | 17 ± 2.4 | 17 ± 1.8 | 16 ± 1.6** (89) |
| 25-26 | 18 ± 2.3 | 17 ± 2.5 | 17 ± 1.9 | 16 ± 1.4** (89) |
| 27-28 | 18 ± 2.1 | 18 ± 2.3 | 17 ± 2.0 | 16 ± 1.6** (89) |
| 30-31 | 17 ± 2.3 | 17 ± 2.1 | 17 ± 2.2 | 15 ± 1.4** (88) |

Data taken from Tables 32 and 38, pp. 179-188 and 203-210, respectively, MRID 43935801.
[a]Numbers in parentheses are percent of control.
[b]Calculated by reviewer from weekly group means.
Significantly different from controls, *$p \leq 0.05$; **$p \leq 0.01$.

significantly less than the controls throughout premating ($p \leq 0.01$; weeks 21-22 and 28-29, $p \leq 0.05$) except at week 24-25 which was not significant.

b. Gestation and lactation - Body weights, body weight gains, and food consumption during gestation and lactation for the $F_0$ and $F_1$ adult females are given in Table 6. High-dose $F_0$ animals had significantly ($p \leq 0.01$) lower body weights (~90%) as compared to controls throughout gestation and until day 14 of lactation. However, body weight gains were significantly ($p \leq 0.05$) less than controls only during day 10-14 of gestation. Recovery was apparent in the high-dose $F_0$ females with body weight gains significantly ($p \leq 0.01$) greater than the controls during lactation days 14-21; this resulted in overall body weight gains during lactation significantly ($p \leq 0.01$) greater than the controls. High-dose $F_0$ females also had significantly ($p \leq 0.01$) lower food consumption as compared to controls throughout gestation and during days 4-7 ($p \leq 0.05$) and 7-14 of lactation. $F_0$ females in the mid-dose group had significantly lower food consumption than controls on gestation days 0-7 ($p \leq 0.05$) and 14-20 ($p \leq 0.01$) and on lactation days 7-14 ($p \leq 0.05$).

The high-dose $F_1$ females had significantly lower body weights (89-92%) throughout gestation (days 0 and 7, $p \leq 0.05$; day 10, 14, and 20, $p \leq 0.01$) and lactation ($p \leq 0.01$) as compared to controls. Body weight gains were significantly lower in both the mid- ($p \leq 0.05$) and high-dose ($p \leq 0.01$) groups as compared to controls during days 14-20 of gestation. No significant differences occurred for body weight gains during lactation for any treated group as compared to controls. Food consumption was significantly ($p \leq 0.01$) lower than controls by the high-dose group throughout gestation and by the mid-dose group for the day 14-20 interval. During lactation, the high-dose group ate significantly less food than controls throughout (days 1-4 and 4-7, $p \leq 0.05$; days 7-14 and 14-21, $p \leq 0.01$) and the mid-dose group ate significantly less food during the days 7-14 and 14-21 intervals ($p \leq 0.01$).

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

| Observation | Treatment group | | | |
|---|---|---|---|---|
| | 0 ppm | 72 ppm | 207 ppm | 540 ppm |
| $F_0$ Generation | | | | |
| Mean body weight (g) | | | | |
| Day 0 of gestation | 257 ± 19.5 | 253 ± 24.9 | 247 ± 17.0 | 232 ± 13.0** (90)[a] |
| Day 20 of gestation | 365 ± 32.0 | 371 ± 32.4 | 355 ± 25.7 | 329 ± 23.8** (90) |
| Day 1 of lactation | 284 ± 18.8 | 283 ± 24.2 | 277 ± 18.8 | 259 ± 20.6** (91) |
| Day 21 of lactation | 323 ± 21.3 | 330 ± 23.2 | 325 ± 23.4 | 317 ± 16.1 (98) |
| Mean body weight gain (g) | | | | |
| Day 0-20 of gestation | 109 ± 25.1 | 118 ± 21.1 | 108 ± 18.9 | 98 ± 15.8 (90) |
| Day 1-21 of lactation | 39 ± 25.1 | 48 ± 14.2 | 48 ± 20.1 | 58 ± 13.4** (149) |
| Mean food consumption (g/rat/day) | | | | |
| Day 0-20 of gestation | 21 ± 2.1 | 21 ± 2.0 | 19 ± 1.4** | 17 ± 1.7** (81) |
| Day 1-21 of lactation | 50 ± 6.8 | 50 ± 4.7 | 48 ± 4.7 | 46 ± 3.5* (92) |
| $F_1$ Generation | | | | |
| Mean body weight (g) | | | | |
| Day 0 of gestation | 258 ± 25.0 | 264 ± 35.3 | 253 ± 18.1 | 237 ± 20.3* (92) |
| Day 20 of gestation | 375 ± 31.0 | 381 ± 42.9 | 363 ± 25.7 | 333 ± 28.8** (89) |
| Day 1 of lactation | 289 ± 25.1 | 298 ± 35.7 | 285 ± 22.6 | 263 ± 28.3** (91) |
| Day 21 of lactation | 324 ± 24.0 | 326 ± 31.2 | 319 ± 19.4 | 302 ± 28.2** (93) |
| Mean body weight gain (g) | | | | |
| Day 0-20 of gestation | 117 ± 17.5 | 117 ± 17.3 | 110 ± 14.7 | 96 ± 12.7** (82) |

TABLE 6. continued

| Observation | Treatment group | | | |
|---|---|---|---|---|
| | 0 ppm | 72 ppm | 207 ppm | 540 ppm |
| Day 1-21 of lactation | 35 ± 16.3 | 28 ± 18.3 | 34 ± 17.3 | 39 ± 25.0 (111) |
| Mean food consumption (g/rat/day) | | | | |
| Day 0-20 of gestation | 20 ± 1.8 | 20 ± 2.6 | 18 ± 1.7 | 16 ± 1.7** (80) |
| Day 1-21 of lactation | 48 ± 3.3 | 47 ± 4.3 | 44 ± 4.0** | 41 ± 4.7** (85) |

Data taken from Tables 6-9, 12, 14, 34-37, 40, and 42, pp. 110-113, 130, 132, 199-202, 223, and 225 respectively, MRID 43935801.
[a]Numbers in parentheses are percent of control.
**Significantly different from control, p≤ 0.01.

3. Test substance intake

Based on weekly food consumption and nominal Ziram concentrations in the diet, the doses expressed as mg of test substance/kg body weight/day during the premating period for males and females and during gestation and lactation for females are presented in Table 7.

TABLE 7: Test substance intake in rats fed ziram for two generations (mg/kg/day)

| Sex - Study Interval | Concentration in Diet | | |
|---|---|---|---|
| | 72 ppm | 207 ppm | 540 ppm |
| $F_0$ Generation | | | |
| Males -Premating[a] | 5.3 ± 1.3 | 14.8 ± 3.9 | 37.5 ± 7.7 |
| Females - Premating[a] | 6.1 ± 0.9 | 16.8 ± 2.7 | 42.8 ± 4.8 |
| Females - Gestation | 5 ± 0.5 | 13 ± 0.9 | 33 ± 2.5 |
| Females - Lactation | 12 ± 1.2 | 33 ± 2.6 | 85 ± 3.3 |
| $F_1$ Generation | | | |
| Males -Premating[a] | 5.6 ± 2.1 | 16.7 ± 6.0 | 42.7 ± 14.5 |
| Females - Premating[a] | 6.3 ± 1.5 | 18.4 ± 4.9 | 47.5 ± 12.5 |
| Females - Gestation | 5 ± 0.5 | 13 ± 0.9 | 32 ± 2.2 |
| Females - Lactation | 11 ± 0.8 | 30 ± 2.4 | 79 ± 5.3 |

Data taken from Tables 16-18 and 44-46, pp. 134-143 and 227-238 MRID 43935801.
[a]Overall group means calculated by reviewer from weekly group means.

4. Necropsy results

a. Organ weights - Selected absolute and relative organ weights for the $F_0$ males and females are listed in Table 8. High-dose males had significantly ($p \leq 0.01$) increased liver weights relative to body weights. Females in the high-dose group had significantly ($p \leq 0.05$) lower absolute brain weights and increased relative brain weights as compared to controls.

Selected absolute and relative organ weights at necropsy for the $F_1$ males and females are given in Table 9. High-dose males had significantly ($p \leq 0.01$) lower absolute brain and kidney weights as compared to controls. Males in this group also had significantly increased relative brain ($p \leq 0.05$), epididymides, and testes ($p \leq 0.01$) weights as compared to controls. For females, kidney weights were significantly less than controls in the mid- ($p \leq 0.05$) and high-dose ($p \leq 0.01$) groups. Relative brain weights were significantly greater in the high-dose females ($p \leq 0.01$) and relative ovarian weights were significantly greater in the mid-dose females ($p \leq 0.05$) as compared to controls.

TABLE 8: Selected absolute and relative organ weights from $F_0$ rats

| Organ | Concentration in the diet | | | |
|---|---|---|---|---|
| | 0 ppm | 72 ppm | 207 ppm | 540 ppm |
| **Males** | | | | |
| Brain | | | | |
| absolute | 2.18 ± 0.139 | 2.17 ± 0.090 | 2.13 ± 0.089 | 2.14 ± 0.160 |
| relative | 0.402 ± 0.037 | 0.396 ± 0.040 | 0.383 ± 0.034 | 0.414 ± 0.042 |
| Liver | | | | |
| absolute | 19.69 ± 2.090 | 21.03 ± 3.345 | 21.39 ± 4.241 | 20.60 ± 2.694 |
| relative | 3.618 ± 0.259 | 3.792 ± 0.336 | 3.780 ± 0.438 | 3.952 ± 0.342** |
| **Females** | | | | |
| Brain | | | | |
| absolute | 1.99 ± 0.126 | 1.98 ± 0.104 | 1.96 ± 0.073 | 1.92 ± 0.051* |
| relative | 0.674 ± 0.057 | 0.671 ± 0.053 | 0.691 ± 0.048 | 0.709 ± 0.051* |
| Liver | | | | |
| absolute | 11.72 ± 1.267 | 11.53 ± 1.402 | 11.46 ± 1.128 | 11.21 ± 1.070 |
| relative | 3.957 ± 0.317 | 3.894 ± 0.374 | 4.024 ± 0.290 | 4.139 ± 0.300 |

Data taken from Tables 26 and 27, pp. 155-157 and 158-161, respectively, MRID 43935801.
Significantly different from control, *$p \leq 0.05$; **$p \leq 0.01$.

TABLE 9: Selected absolute and relative organ weights from $F_1$ rats

| Organ | Concentration in the diet | | | |
|---|---|---|---|---|
| | 0 ppm | 72 ppm | 207 ppm | 540 ppm |
| **Males** | | | | |
| Brain | | | | |
| absolute | 2.15 ± 0.082 | 2.16 ± 0.089 | 2.13 ± 0.101 | 2.06 ± 0.111** |
| relative | 0.387 ± 0.031 | 0.388 ± 0.030 | 0.387 ± 0.036 | 0.411 ± 0.030* |
| Kidneys | | | | |
| absolute | 3.94 ± 0.518 | 3.81 ± 0.410 | 3.70 ± 0.426 | 3.52 ± 0.410** |
| relative | 0.705 ± 0.059 | 0.683 ± 0.060 | 0.665 ± 0.046* | 0.699 ± 0.065 |
| Epididymes | | | | |
| absolute | 1.41 ± 0.121 | 1.43 ± 0.103 | 1.43 ± 0.203 | 1.41 ± 0.119 |
| relative | 0.253 ± 0.025 | 0.257 ± 0.025 | 0.258 ± 0.038 | 0.281 ± 0.033** |
| Testes | | | | |
| absolute | 3.73 ± 0.271 | 3.71 ± 0.293 | 3.72 ± 0.444 | 3.69 ± 0.271 |
| relative | 0.674 ± 0.075 | 0.668 ± 0.064 | 0.673 ± 0.094 | 0.737 ± 0.076** |
| **Females** | | | | |
| Brain | | | | |
| absolute | 1.97 ± 0.091 | 2.01 ± 0.087 | 1.96 ± 0.094 | 1.91 ± 0.094 |
| relative | 0.641 ± 0.071 | 0.671 ± 0.060 | 0.663 ± 0.059 | 0.699 ± 0.052** |
| Kidneys | | | | |
| absolute | 2.36 ± 0.287 | 2.26 ± 0.238 | 2.19 ± 0.199* | 2.06 ± 0.176** |
| relative | 0.761 ± 0.061 | 0.747 ± 0.044 | 0.742 ± 0.074 | 0.749 ± 0.043 |
| Ovaries | | | | |
| absolute | 0.141 ± 0.020 | 0.146 ± 0.019 | 0.149 ± 0.016 | 0.132 ± 0.022 |
| relative | 0.046 ± 0.008 | 0.048 ± 0.005 | 0.050 ± 0.007* | 0.048 ± 0.008 |

Data taken from Tables 49 and 50, pp. 245-247 and 248-251, respectively, MRID 43935801.
Significantly different from control, *$p \leq 0.05$; **$p \leq 0.01$.

b. Pathology

1) Gross pathology - In the $F_0$ males, there was a significant increase in the number of animals in the mid- and high-dose groups with reddened mesenteric lymph nodes: 0/30, 0/30, 6/30 ($p \leq 0.05$), and 9/30 ($p \leq 0.01$) affected in the control, 72, 207, and 540 ppm groups, respectively.

This effect did not reach statistical significance in the $F_1$ males, but 0/30, 0/30, 1/30, and 3/30 were affected, respectively. $F_1$ males also had a dose-related increase in the incidence of mottled lungs with 0/30, 1/30, 2/30, and 5/30 ($p \leq 0.05$) affected in the control, 72, 207, and 540 ppm groups, respectively. No dose- or treatment-related abnormalities were observed in either the $F_0$ or $F_1$ females.

2) Microscopic pathology - No treatment-related histological abnormalities were observed for either sex or generation.

B. REPRODUCTIVE TOXICITY

1. Reproductive performance

The reproductive performances of the $F_0$ and $F_1$ animals are summarized in Tables 10 and 11, respectively. No dose- or treatment-related effects were noted in either generation. However, $F_1$ males in the control group had a low fertility index due to only 23 of 30 animals siring litters.

TABLE 10. Reproductive performance in $F_0$ generation rats fed ziram for two generations

| Observation | Dietary concentration | | | |
|---|---|---|---|---|
| | 0 ppm | 72 ppm | 207 ppm | 540 ppm |
| Mean nights to positive mating | 3.6 ± 3.22 | 2.9 ± 2.29 | 2.7 ± 1.56 | 2.7 ± 1.33 |
| Males | | | | |
| Number paired | 30 | 30 | 30 | 30 |
| Number siring | 26 | 26 | 26 | 28 |
| Females | | | | |
| Number paired | 30 | 30 | 30 | 30 |
| Number pregnant | 27 | 27 | 28 | 28 |
| Number with sperm not detected, littered | 1 | 0 | 1 | 0 |
| Number delivering | 27 | 27 | 28 | 28 |
| Indices (%) | | | | |
| Male fertility index | 86.7 | 86.7 | 90.0 | 93.3 |
| Female fertility index | 90.0 | 90.0 | 93.3 | 93.3 |
| Male mating index | 90.0 | 93.3 | 96.7 | 93.3 |
| Female mating index | 96.7 | 96.7 | 96.7 | 93.3 |
| Mean gestation length (days) | 21.6 ± 0.50 | 21.6 ± 0.49 | 21.7 ± 0.48 | 21.8 ± 0.39 |

Data taken from Tables 2 and 19, pp. 85-87 and 144, respectively, MRID 43935801.

TABLE 11. Reproductive performance in $F_1$ generation rats fed ziram for two generations

| Observation | Dietary concentration | | | |
|---|---|---|---|---|
| | 0 ppm | 72 ppm | 207 ppm | 540 ppm |
| Mean nights to positive mating | 3.4 ± 2.96 | 3.1 ± 2.67 | 2.6 ± 1.17 | 2.8 ± 2.01 |
| Males | | | | |
| Number paired | 30 | 30 | 30 | 30 |
| Number siring | 23 | 24 | 26 | 28 |
| Females | | | | |
| Number paired | 30 | 30 | 30 | 30 |
| Number pregnant | 23 | 25 | 26 | 28 |
| Number with sperm not detected, littered | 1 | 2 | 0 | 1 |
| Number delivering | 23 | 25 | 26 | 28 |
| Indices (%) | | | | |
| Male fertility index | 76.7 | 80.0 | 86.7 | 93.3 |
| Female fertility index | 76.7 | 83.3 | 86.7 | 93.3 |
| Male mating index | 90.0 | 90.0 | 93.3 | 93.3 |
| Female mating index | 93.3 | 93.3 | 93.3 | 96.7 |
| Mean gestation length (days) | 21.8 ± 0.43 | 21.7 ± 0.45 | 21.8 ± 0.43 | 21.7 ± 0.48 |

Data taken from Tables 30 and 47, pp. 171-173 and 239, respectively, MRID 43935801.

2. Viability and clinical signs

Viability data for the $F_0$ generation offspring ($F_1$ pups) are given in Table 12 and for the $F_1$ generation offspring ($F_2$ pups) in Table 13. The percentage of $F_1$ male pups was significantly lower in the high-dose group than that of the control. Viability of pups in the low-dose $F_0$ group was significantly less than the control on lactation day 4 before culling. Pup deaths in this group did not correlate with any clinical or gross necropsy observation.

No differences were observed between treated and control groups for the $F_1$ generation offspring. The reduced viability index in all groups at lactation day 21 reflects the removal of 10 pups/sex/group on lactation day 11 for neuropathological evaluation and/or brain weight measurement (see corresponding DER).

TABLE 12: Viability of $F_0$ generation offspring during lactation

| Observation/ study time | 0 ppm | 72 ppm | 207 ppm | 540 ppm |
|---|---|---|---|---|
| Number of litters | 27 | 27 | 28 | 28 |
| Total number of pups | 369 | 364 | 360 | 337 |
| Number of pups born alive | 367 | 362 | 359 | 336 |
| Number of pups still born | 2 | 2 | 1 | 1 |
| Sex ratio (% male)[a] | 54.2 | 51.4 | 48.2 | 45.2* |
| Mean number live pups/litter (day 0) | 13.6 | 13.4 | 12.8 | 12.0* |
| Day 1[a] | 13.4 | 13.1 | 12.6 | 12.0 |
| Day 4 (precull)[a] | 13.3 | 12.6 | 12.5 | 11.9 |
| Day 4 (postcull)[a] | 7.8 | 7.9 | 7.9 | 7.9 |
| Day 14[a] | 7.8 | 7.7 | 7.9 | 7.9 |
| Day 21[a] | 7.8 | 7.7 | 7.8 | 7.9 |
| Number of litters weaned | 27 | 27 | 28 | 28 |
| Survival indices (%) | | | | |
| Live birth index[a] | 99.5 | 99.5 | 99.7 | 99.7 |
| Viability index (precull; d 0-4) | 97.8 | 94.2* | 97.8 | 98.8 |
| Viability index (postcull; d 4-21) | 100 | 98.6 | 98.6 | 100 |

Data taken from Table 20, pp. 145 and 146, MRID 43935801.
*Significantly different from control, $p \leq 0.05$.
[a]Calculated by reviewer from group summaries.

| TABLE 13: Viability of $F_1$ generation offspring during lactation | | | | |
|---|---|---|---|---|
| Observation/study time | 0 ppm | 72 ppm | 207 ppm | 540 ppm |
| Number of litters | 23 | 25 | 26 | 28 |
| Total number of pups | 285 | 310 | 322 | 336 |
| Number of pups born alive | 281 | 306 | 320 | 333 |
| Number of pups still born | 4 | 4 | 2 | 3 |
| Sex ratio (% male)[a] | 47.0 | 44.1 | 53.4 | 45.3 |
| Mean number live pups/litter (day 0) | 12.2 | 12.2 | 12.3 | 11.9 |
| Day 1[a] | 12.1 | 12.2 | 12.2 | 11.8 |
| Day 4 (precull)[a] | 12.1 | 12.1 | 12.2 | 11.7 |
| Day 4 (postcull)[a] | 7.9 | 8.0 | 8.0 | 8.0 |
| Day 14[a] | 7.0 | 7.2 | 7.2 | 7.2 |
| Day 21[a] | 7.0 | 7.2 | 7.2 | 7.2 |
| Number of litters weaned | 23 | 25 | 26 | 28 |
| Survival indices (%) | | | | |
| Live birth index[a] | 98.6 | 98.7 | 99.4 | 99.1 |
| Viability index (precull; d 0-4) | 98.9 | 98.7 | 98.8 | 98.5 |
| Viability index (postcull; d 4-21) | 89.0 | 89.9 | 90.4 | 90.6 |

Data taken from Table 52, pp. 259 and 260, MRID 43935801.
[a]Calculated by reviewer from group summaries.

3. Body weight

Selected body weights of the $F_1$ and $F_2$ pups during lactation are given in Table 14. $F_1$ pups from high-dose group dams had consistently lower body weights than controls beginning at day 4 precull with significance ($p \leq 0.01$) reached on day 14. High-dose $F_2$ pups also had lower body weights than the controls starting at birth and continuing throughout lactation with significance reached on days 1, 4 precull ($p \leq 0.05$), 14, and 21 ($p \leq 0.01$).

| TABLE 14: Selected group mean body weights of offspring during lactation (g) | | | | |
|---|---|---|---|---|
| Day of lactation | 0 ppm | 72 ppm | 207 ppm | 540 ppm |
| $F_1$ generation | | | | |
| Day 1 | 6.4 ± 0.75 | 6.5 ± 0.50 | 6.4 ± 0.52 | 6.4 ± 0.56 |
| Day 4 (precull) | 9.1 ± 1.41 | 9.3 ± 0.94 | 8.8 ± 0.93 | 8.8 ± 1.16 |
| Day 4 (postcull) | 9.1 ± 1.37 | 9.3 ± 0.95 | 8.8 ± 0.96 | 8.8 ± 1.15 |
| Day 14 | 31.4 ± 3.73 | 32.2 ± 2.82 | 29.7 ± 3.04 | 28.8 ± 2.82** |
| Day 21 | 43.1 ± 5.03 | 45.8 ± 3.80 | 41.5 ± 4.83 | 40.4 ± 3.53 |
| $F_2$ generation | | | | |
| Day 1 | 6.7 ± 0.61 | 6.6 ± 0.52 | 6.6 ± 0.74 | 6.2 ± 0.48* |
| Day 4 (precull) | 9.6 ± 1.28 | 9.8 ± 0.90 | 9.5 ± 1.20 | 8.8 ± 0.91* |
| Day 4 (postcull) | 9.5 ± 1.32 | 9.8 ± 0.93 | 9.5 ± 1.23 | 8.9 ± 0.94 |
| Day 14 | 32.9 ± 2.87 | 33.1 ± 2.60 | 31.6 ± 3.28 | 30.0 ± 3.03** |
| Day 21 | 46.8 ± 3.94 | 47.4 ± 3.60 | 45.1 ± 4.44 | 41.0 ± 4.68** |

Data taken from Tables 23 and 55, pp. 149 and 263, respectively, MRID 43935801.
Significantly different from control, *$p \leq 0.05$; **$p \leq 0.01$.

4. Testicular function and sperm assessment

Qualitative assessment of spermatogenesis of the $F_0$ and $F_1$ males not siring a litter did not show any dose- or treatment-related effect as compared with controls.

## III. DISCUSSION

Male and female Sprague-Dawley rats were fed up to 540 ppm ziram in the diet for two generations. At least 23 litters were produced in each generation.

### A. SYSTEMIC TOXICITY

No overt clinical signs of toxicity were observed in the adult animals of either sex or generation. However, high-dose males and females had significantly lower body weights as compared to controls. In the $F_0$ generation, reduced body weights directly correlated with lower food consumption especially during the initial few weeks of the study. This reduction in food consumption resulted in significantly decreased body weight gains for the first two weeks. Following week 1, body weight gains of the treated male groups were occasionally greater than the controls with recovery of absolute body weights to within 5% of

the control level. Body weights of the $F_0$ females were also less than the control values during the premating period, but final body weights were within 8% of the control level. Although the initial body weights of the high-dose $F_1$ males and females were significantly less than the controls, results for the premating period were similar to the $F_0$ generation. Reduced food consumption by the high-dose $F_1$ animals caused a reduction in body weight gains resulting in lower absolute body weights with the effect more pronounced during the first few weeks. Mid-dose $F_0$ females and $F_1$ males also had significantly reduced food consumption during the first week of feeding. Therefore, the reduced food consumption is most likely due to lack of palatability of ziram to rats.

Changes in absolute and relative organ weights of the high-dose groups did not correlate with gross or histological findings and are probably due to the lower body weights of these animals instead of a direct effect by the test article. At necropsy a dose-related increase in the incidence of reddened mesenteric lymph nodes was observed in males of both generations. However, histological examination was not performed on these organs. An expanded microscopic analysis of the affected lymph nodes might aid in determining whether the effect was directly compound related or due to some other secondary response. This effect was not observed in a subchronic feeding study in rats (MRID 42450301) at a maximum dose tested of 1000 ppm.

**Therefore, the systemic toxicity LOAEL is 540 ppm based on reduced body weights, body weight gains, and decreased food consumption by $F_0$ and $F_1$ males and females. The systemic toxicity NOAEL is 207 ppm.**

Although decreased food consumption also reached statistical significance in the mid-dose group, the effect only occurred during the first week of feeding. A corresponding effect on body weight was not observed at this dose. This supports the lack of palatability of ziram to rats and is not considered as a basis for establishing the LOEL.

B. REPRODUCTIVE TOXICITY

A similar profile of decreased food consumption, reduced body weight gains, and lower absolute body weights was observed in pregnant and nursing rats of both generation. There were no dose- or treatment-related effects on the reproductive performance of either generation. The low male and female fertility indices for the control $F_1$ parental animals is still within the historical control incidence for the testing laboratory. Excess $F_1$ pup deaths in the low-dose group are unexplained but do fall within the historical control range. Since pup deaths were not dose related or repeated in the second generation, this effect is probably not treatment related. The low sex ratio among the high-dose $F_1$ pups was significantly different from the control value, but, the percent male pups for both the control and high-dose groups are within the historical control range (See Appendix 1).

Pup body weights in the high-dose groups were consistently lower than the controls throughout lactation in both generations. Statistical significance was only reached at day 14 for the $F_1$ pups but the $F_2$ pup body weights were significantly less than the control at each interval except day 4 postcull.

**Therefore, the LOAEL for offspring toxicity is 540 ppm (42.8 mg/kg/day) based on reduced pup body weights at birth in $F_2$ pups and during lactation in both $F_1$ and $F_2$ pups. The corresponding NOAEL for offspring toxicity is 207 ppm (16.8 mg/kg/day).**

C. STUDY DEFICIENCIES

Data for $F_1$ animals were reported as week of study and not normalized for age. However all litters of a treatment group were born within 15 days of each other so at the time of breeding of the $F_1$ animals sexual maturity would have been reached.

An expanded histopathology of the mesenteric lymph nodes might cause the LOAEL/NOAEL values to change for systemic toxicity. However, the most pronounced effects are still on food consumption and body weight changes.

D. CORE CLASSIFICATION

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

| | |
|---|---|
| SignOff Date: | 8/2/00 |
| DP Barcode: | D172447 |
| HED DOC Number: | 014277 |
| Toxicology Branch: | RAB2 |