# DATA EVALUATION RECORD

## BAS 510 F

## STUDY TYPE: MULTIGENERATION REPRODUCTION - RAT (870.3800 [83-4])
## MRID 45404906

Prepared for

7/23/2002

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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**DATA EVALUATION RECORD**
**TXR#: 0050193**

**STUDY TYPE:** Reproduction and Fertility Effects Study - Rat; OPPTS 870.3800 [§83-4]; OECD 416.

**PC CODE:** 128008

**DP BARCODE:** D278384
**SUBMISSION NO.:** S604279

**TEST MATERIAL (PURITY):** BAS 510 F (94.4% a.i.)

**SYNONYMS:** 2-chloro-N-(4'-chloro-biphenyl-2-yl)-nicotinamide

**CITATION:** Schilling, K., Gembardt, C., and van Ravenzwaay, B. (2001) BAS 510 F two generation reproduction toxicity study in Wistar rats continuous dietary administration. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, D-67056 Ludwigshafen, Germany. Laboratory report number 70R0179/97136, February 22, 2001. MRID 45404906. Unpublished.

**SPONSOR:** BASF Corporation, Agricultural Products Division, RTP, NC 27709

**EXECUTIVE SUMMARY:** In a two-generation reproduction study (MRID 45404906), BAS510F (94.4% a.i., batch #N 37) was administered to 25 Wistar (Chbb=THOM(SPF)) rats/sex/dose in the diet at concentrations of 0, 100, 1000, or 10,000 ppm. One litter was produced in each generation. Premating doses for the treated $F_0$ parental animals were 10.1, 101.2, and 1034.5 mg/kg/day, respectively, for males and 10.7, 106.8, and 1062.0 mg/kg/day, respectively, for females. Premating doses for the treated $F_1$ parental animals were 12.3, 123.9, and 1295.4 mg/kg/day, respectively, for males and 12.5, 124.7, and 1299.6 mg/kg/day, respectively, for females. $F_0$ and $F_1$ parental animals were administered test or control diet for at least 74 or 76 days, respectively, prior to mating, throughout mating, gestation, and lactation, and until sacrifice.

All parental animals of both generations survived to scheduled sacrifice. No treatment-related clinical signs of toxicity were observed in any animal during the study. Food consumption was not affected by treatment with the test article in either sex of either generation. Absolute body weights and body weight gains of the $F_0$ animals were similar between the treated and control groups throughout the study. For the treated $F_1$ females, body weights and body weight gains were similar to those of the control group throughout premating. Absolute body weights of the high-dose $F_1$ males were significantly (91-94% of controls; $p \leq 0.05$ or 0.01) less than the controls beginning at week 1 of premating and continuing until termination. Weight gain by the

high-dose $F_1$ males was significantly ($p \leq 0.01$) less than that of the control group during weeks 0-1 (89% of control), 3-4 (84% of control), and 13-14 (62% of control), with premating weight gain 93% of the controls and overall weight gain 91% of the control level.

At necropsy, no treatment-related gross lesions were found in any animal of either sex or generation. An increased incidence and severity of centrilobular hepatocyte hypertrophy in many mid- and all high-dose animals corresponded with increased liver weights only at the high dose. Additionally in the high-dose groups, hepatocyte degeneration was observed in three $F_0$ males, one $F_0$ female, and eight $F_1$ males. **The parental systemic LOAEL is 10,000 ppm for males (1034.5-1295.4 mg/kg/day) based on decreased body weights and body weight gains of the $F_1$ males and hepatocyte degeneration in $F_0$ and $F_1$ males; the systemic LOAEL was not identified for females. The parental systemic NOAELs are 1000 ppm for males (101.2-123.9 mg/kg/day) and 10,000 ppm for females (1062.0-1299.6 mg/kg/day).**

No treatment-related differences in estrous cycle length and periodicity were found in females of either generation. All control and almost all treated $F_0$ and $F_1$ animals showed regular estrous cycles and became sperm positive within a few days after pairing. No treatment-related differences were observed in any sperm measures. No treatment-related lesions occurred in the reproductive tracts from males or females from either generation. No differences in mating, fertility, or gestation indices were seen between the treated and control groups of either generation. The copulatory interval and gestation length of the treated groups were comparable to the control groups in both generations. **The reproductive toxicity NOAEL is ≥10,000 ppm (1034.5-1295.4 mg/kg/day for males and 1062.0-1299.6 mg/kg/day for females) and the reproductive toxicity LOAEL was not identified.**

For the $F_1$ litters, live birth, viability, and lactation indices, mean litter sizes, and sex ratios were similar between the treated and control groups. Pup survival in the high-dose $F_2$ litters was decreased during lactation days 0-4 as indicated by a significantly lower viability index (86% vs 93% for controls; $p \leq 0.01$). Post-implantation loss was significantly ($p \leq 0.05$) greater for the high-dose $F_1$ females, resulting in a mean live litter size of 12.5 pups for the high-dose group (n.s.) compared with 13.8 pups/litter for the control group. The live birth index, the lactation index, and pup sex ratio were similar between the treated and control groups. No treatment-related clinical signs of toxicity were observed in the $F_1$ or $F_2$ pups during lactation. For the $F_1$ pups, no differences in the rate of sexual maturation were noted between the treated and control groups.

Pups from the high-dose litters of both generations and mid-dose $F_2$ male pups had significantly reduced body weights and body weight gains during lactation as compared with their respective control group. High-dose male and female $F_1$ pups had significantly ($p \leq 0.05$) lower body weights on lactation day 21 compared with the controls due to consistently reduced body weight gains ($p \leq 0.05$ or 0.01; 89-93% of the control level) for all intervals after lactation day 4. Body weights of the high-dose $F_2$ male and female pups were 86-90% ($p \leq 0.01$) of the control levels on lactation days 14 and 21 due to consistently reduced body weight gains ($p \leq 0.05$ or 0.01; 83-88% of the control level) for all intervals after lactation day 4. In addition, body weights were significantly ($p \leq 0.05$) reduced for the mid- and high-dose $F_2$ males on lactation day 7 and the mid-dose $F_2$ males on day 21. Body weight gains by the mid-dose $F_2$ males were 88-93% ($p \leq 0.05$ or 0.01) of the control levels during lactation days 4-21. **The offspring toxicity LOAEL is**

**1000 ppm for males (101.2-123.9 mg/kg/day) based on decreased body weights and body weight gains by the $F_2$ male pups and 10,000 ppm for females (1062.0-1299.6 mg/kg/day) based on decreased body weights and weight gains. The offspring toxicity NOAEL is 100 ppm for males (10.1-12.3 mg/kg/day) and 1000 ppm for females (106.8-124.7 mg/kg/day).**

This study is **Acceptable/Guideline** and satisfies the guideline requirement for a two-generation reproduction study (OPPTS 870.3800; OECD 416) in rats.

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

## I. MATERIALS AND METHODS

### A. MATERIALS:

1. **Test material:** BAS 510 F

| | |
|---|---|
| **Description:** | white powder |
| **Lot/Batch #:** | N 37 |
| **Purity:** | 94.4% a.i. |
| **Compound Stability:** | proven by reanalysis |
| **CAS # of TGAI:** | 188425-85-6 |
| **Structure:** | Not available |

2. **Vehicle and/or positive control:** Ground Kliba maintenance diet rat/mouse/hamster, 343 meal, (Klingentalmühle AG, Kaiseraugst, Switzerland) was used as the vehicle and negative control. No positive control was used in this study.

3. **Test animals:**

| | | |
|---|---|---|
| **Species:** | Rats | |
| **Strain:** | Wistar (Chbb = THOM (SPF)) | |
| **Age at study initiation:** | ($F_0$) 35±2 days; ($F_1$) 21 days | |
| **Wt. at study initiation:** | ($F_0$) Males: 100.1-145.7 g; Females: 93.3-132.1 g<br>($F_1$) Males: 48.1-91.6 g; Females: 48.9-87.9 g | |
| **Source:** | Boehringer Ingelheim, Pharma KG, Biberach/Riss, FRG | |
| **Housing:** | Rats were housed individually in type DK III stainless steel wire mesh cages. From GD 18 until lactation day 14, pregnant females and their litters were housed in Makrolon type M III cages with nesting material. | |
| **Diet:** | Ground Kliba maintenance diet rat/mouse/hamster, 343 meal, was available *ad libitum*. | |
| **Water:** | Drinking water was available *ad libitum*. | |
| **Environmental conditions:** | **Temperature:** | 20-24°C |
| | **Humidity:** | 30-70% |
| | **Air changes:** | Not stated |
| | **Photoperiod:** | 12 hrs dark/12 hrs light |
| **Acclimation period:** | 8 days | |

## B. PROCEDURES AND STUDY DESIGN:

1. **Mating procedure:** Male and female animals were mated overnight at a ratio of 1:1 for a maximum of 3 weeks. Matings occurred by placing the female in the cage of the male. A vaginal smear was prepared after each mating and examined for sperm. The day on which sperm was detected was designated as GD 0 and pairing of the animals was discontinued. Sibling matings were avoided.

2. **Study schedule:** $F_0$ and $F_1$ parental animals were administered test or control diet for at least 74 or 76 days, respectively, prior to mating, throughout mating, gestation, and lactation, and until sacrifice. One litter was produced in each generation.

3. **Animal assignment:** $F_0$ animals were assigned according to body weight to test groups in Table 1 using a randomization program. $F_1$ animals were chosen by lot during rearing; one male and one female were taken from each litter where possible.

**TABLE 1. Animal Assignment**

| Test Group | Concentration in Diet [a] (ppm) | Animals/group | | | |
|---|---|---|---|---|---|
| | | $F_0$ Males | $F_0$ Females | $F_1$ Males | $F_1$ Females |
| Control | 0 | 25 | 25 | 25 | 25 |
| Low (LDT) | 100 | 25 | 25 | 25 | 25 |
| Mid (MDT) | 1000 | 25 | 25 | 25 | 25 |
| High (HDT) | 10,000 | 25 | 25 | 25 | 25 |

Data taken from p. 32, MRID 45404906.
[a]Diets were administered from beginning of the study until sacrifice

4. **Dose selection rationale:** The dietary concentrations were selected such that the low-dose was the expected NOAEL, the mid-dose was an intermediate level, and the high-dose was guaranteed a test substance intake of about the limit dose, or 1000 mg/kg/day. No additional details or preliminary data were given.

5. **Dosage preparation and analysis:** Test diets were prepared at "intervals considering the different demand during the study periods and the proven stability." Dietary mixtures were stored at room temperature. For each concentration, the test article was weighed into a beaker and thoroughly mixed with a small amount of food using a spatula. Then a premix was prepared which was adjusted to the desired concentrations with appropriate amounts of food and mixed for about 10 minutes in a Ruberg (EM 100) laboratory mixer. Homogeneity was determined in three samples (location in mix not stated) from the low and high concentration diets prior to study initiation. Stability of the test article in the diet was assessed prior to study initiation in a sample diet stored at room temperature for up to 32 days.

**Results:**

**Homogeneity analysis:** Triplicate samples from the low and high concentration diets were 94.5-95.2% and 90.4-93.8%, respectively, of nominal.

**Stability analysis:** After 32 days of storage at room temperature, the mean concentration of the test article in the diet was 97.9% of the initial measured concentration.

**Concentration analysis:** Absence of test article was confirmed in the control diets. Throughout the study, concentrations of the test article in the low-, mid-, and high-dose diets were 92.6-113.3%, 91.5-95.1%, and 92.2-104.1%, respectively, of nominal.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

## C. OBSERVATIONS:

1. **Parental animals:** Adults were checked once daily for dead or moribund animals and for clinical signs of toxicity. Littering behavior of the dams was observed twice daily. Body weights were recorded weekly throughout the study. Food consumption was measured weekly during premating. Dams were weighed on GD 0, 7, 14, and 20 and on lactation days 1, 4, 7, 14, and 21. Food consumption for dams was measured on GD 0, 7, 14, and 20 and on lactation days 1, 4, 7, and 14. Test article intake was calculated from body weight and food consumption data and nominal dietary concentrations.

   Estrous cycle length and normality were evaluated daily for all $F_0$ and $F_1$ parental females for a minimum of 3 weeks prior to mating and throughout mating until evidence of mating was found. At necropsy a vaginal smear was examined to determine the stage of the estrous cycle for each female. For males, sperm parameters (motility, morphology, head count in cauda epididymis and testis) were evaluated at necropsy. Sperm morphology and head counts were determined for control and high-dose $F_0$ males and for all $F_1$ males.

2. **Litter observations:** Litter observations were made as shown in Table 2. All females were allowed to litter naturally with the day of birth designated as lactation day 0. On the day of birth, the sex of the pups was determined by observing the anogenital distance. Pups were weighed on lactation days 1, 4, 7, 14, and 21. On lactation day 4, litters were culled to 4 pups/sex, where possible. Pups were weaned on lactation day 21.

   For all $F_1$ pups selected as parental animals, females were examined for vaginal opening beginning on day 27 and males were examined for preputial separation beginning on day 40.

| TABLE 2. $F_1$ / $F_2$ Litter Observations | | | | | | | |
|---|---|---|---|---|---|---|---|
| Observation | Time of observation (lactation day) | | | | | | |
| | Day 0 | Day 1 | Day 4[a] | Day 4[b] | Day 7 | Day 14 | Day 21 |
| Number of live pups | X | | X | X | X | X | X |
| Pup weight | | X | X | | X | X | X |
| External alterations | daily | | | | | | |
| Clinical signs | daily | | | | | | |
| Dead/moribund pups | 2 × daily | | | | | | |
| Sex of each pup | X | X | X | | X | X | X |

Data obtained from text on pages 44-46.
[a]Before standardization (culling)
[b]After standardization (culling)

3. **Postmortem observations:**

1) **Parental animals:** Moribund animals were sacrificed and examined grossly. Parental animals were sacrificed after weaning of their pups and subjected to gross examination. All animals were sacrificed by decapitation under $CO_2$ anesthesia.

For animals sacrificed on schedule, the following tissues (X) were prepared for microscopic examination and weighed (XX). All tissues from the control and high-dose animals, the reproductive organs from any animal suspected of infertility, the liver from all animals, and the spleen from all $F_0$ males were examined microscopically. In addition, differential ovarian follicle counts were performed on all control and high-dose $F_1$ females and on all $F_0$ and $F_1$ females that were not pregnant. Uteri from all females were stained in 10% ammonium sulfide solution to determine the number of implantation sites.

| | | | |
|---|---|---|---|
| XX | *Ovaries* | XX | *Testes* |
| XX | Uterus | XX | Epididymides |
| X | Vagina | XX | Prostate gland |
| X | Cervix | XX | Seminal vesicles |
| X | Oviducts | X | Coagulating gland |
| | | | |
| | | X | Urinary bladder |
| XX | Spleen | XX | Thymus |
| XX | Liver | XX | Brain |
| XX | Kidney | XX | Pituitary gland |
| X | Lesions | XX | Adrenal gland |

2) **Offspring:** The $F_1$ offspring not selected as parental animals and all $F_2$ offspring were sacrificed at culling or weaning by $CO_2$ inhalation. These animals and all stillborn pups and pups that died during lactation were subjected to gross necropsy. Pups with noted findings were examined by a modified Dawson's method and the stained skeleton was evaluated. All

pups without notable findings or abnormalities were discarded after gross evaluation. At scheduled sacrifice of $F_1$ and $F_2$ weanlings the brain, spleen, and thymus from 1 pup/sex and litter were weighed.

## D. DATA ANALYSIS:

1. **Statistical analyses:** Data for food consumption, body weights, estrous cycle duration, number of mating days, duration of gestation, numbers of pups, and duration of sexual maturation were analyzed with Dunnett's test for equal means. Reproductive and offspring indices, data for live born, stillborn, and pup deaths, numbers of litters with affected pups at necropsy, and sexual maturation data (proportions) were analyzed by Fisher's exact test. Sperm parameters and numbers of follicles were assessed with the Wilcoxon test. Organ weight data were analyzed by the Kruskal-Wallis test followed by the Wilcoxon test.

2. **Indices:**

**Reproductive indices:** The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Male mating index (%) = (No. males mated/No. males paired) × 100

Female mating index (%) = (No. females mated/No. females paired) × 100

Male fertility index (%) = (Males siring litters/No. males paired) × 100

Female fertility index (%) = (No. gravid females/No. females mated) × 100

Gestation index (%) = (No. live litters born/No. gravid females) × 100

**Offspring viability indices:** The following viability indices were calculated from lactation records of litters in the study:

Live birth index (%) = (No. pups born alive/No. pups born) × 100

Post-implantation loss (%) = [(No. implantations - No. pups delivered)/No. implantations] × 100

Viability index (%) = (No. pups alive on day 4 precull/No. pups born alive) × 100

Lactation index (%) = (No. pups alive on day 21/No. pups alive on day 4 post-cull) × 100

Sex ratio = (No. live male or female pups on day 0/21/No. live male and female pups on day 0/21) × 100

3. **Historical control data:** Historical control data for reproductive parameters were included. These data were from approximately 61 studies conducted between August 1989 and April 1998.

## II. RESULTS:

### A. PARENTAL ANIMALS:

#### 1. Mortality and clinical signs:

All parental animals of both generations survived to scheduled sacrifice. No treatment-related clinical signs of toxicity were observed in any animal during the study. Observations in 1-2 $F_1$ animals included chromodacryorrhea, cataract, and microphthalmia.

#### 2. Body weight and food consumption:

Selected body weight and body weight gain data for the $F_0$ and $F_1$ parental animals are given in Tables 3 and 4, respectively. Absolute body weights of the $F_0$ animals were similar between the treated and control groups throughout the study. Weekly body weight gains by the treated $F_0$ groups were occasionally greater than or less than those of the controls.

For the treated $F_1$ females, body weights and body weight gains were similar to those of the control group throughout premating. Absolute body weights of the high-dose $F_1$ males were significantly ($p \leq 0.05$ or 0.01) less than the controls beginning at week 1 of premating and continuing until termination. However, the body weights of the high-dose males were 91-94% of the control group levels throughout the study. Weight gain by the high-dose $F_1$ males was significantly ($p \leq 0.01$) less than that of the control group during weeks 0-1 (89% of control), 3-4 (84% of control), and 13-14 (62% of control), with premating weight gain 93% of the controls and overall weight gain 91% of the control level.

Food consumption was not affected by treatment with the test article in either sex of either generation. The high-dose $F_1$ females had significantly ($p \leq 0.05$ or 0.01) increased food consumption compared with that of the controls during several weeks of the premating interval.

| TABLE 3. Selected body weight (g) and body weight gain (g) data for the $F_0$ adults | | | | |
|---|---|---|---|---|
| Endpoint and day or interval | 0 ppm | 100 ppm | 1000 ppm | 10,000 ppm |
| **Males** | | | | |
| Week 0 | 126.2 ± 11.43 | 126.4 ± 11.87 | 126.3 ± 10.65 | 125.8 ± 10.94 |
| Week 2 | 228.9 ± 17.93 | 228.1 ± 17.34 | 224.7 ± 16.02 | 221.8 ± 15.51 |
| Week 4 | 306.5 ± 23.54 | 309.1 ± 20.00 | 302.0 ± 19.91 | 301.1 ± 20.67 |
| Week 8 | 399.0 ± 30.88 | 397.7 ± 28.52 | 388.6 ± 25.83 | 388.6 ± 30.21 |
| Week 10 (end of premating) | 425.5 ± 32.74 | 425.8 ± 30.03 | 415.1 ± 28.09 | 412.1 ± 40.15 |
| Week 18 (termination) | 485.6 ± 41.94 | 489.0 ± 35.50 | 478.9 ± 37.48 | 484.4 ± 46.81 |
| Weight gain premating[a] | 299.3 | 299.4 | 288.8 | 286.3 |
| Weight gain overall | 359.4 ± 37.50 | 362.6 ± 28.80 | 352.6 ± 36.10 | 358.6 ± 42.44 |
| **Females** | | | | |
| Week 0 | 112.8 ± 10.23 | 112.9 ± 10.30 | 112.2 ± 10.06 | 112.0 ± 9.75 |
| Week 2 | 163.3 ± 14.89 | 161.6 ± 13.59 | 160.8 ± 11.98 | 162.5 ± 12.95 |
| Week 4 | 199.3 ± 16.24 | 198.5 ± 16.11 | 196.6 ± 15.70 | 195.4 ± 14.40 |
| Week 8 | 243.1 ± 20.28 | 241.7 ± 19.25 | 242.3 ± 19.82 | 237.9 ± 17.85 |
| Week 10 (end of premating) | 255.1 ± 22.62 | 253.0 ± 19.09 | 252.7 ± 19.33 | 248.5 ± 22.0 |
| Weight gain premating | 142.3 ± 16.34 | 140.1 ± 15.50 | 140.6 ± 15.45 | 136.5 ± 17.44 |

Data taken from Table IA-09 - IA-014, pp. 119-124, MRID 45404906.
[a]Calculated by reviewer from group means.

| TABLE 4. Selected body weight (g) and body weight gain (g) data for the $F_1$ adults | | | | |
|---|---|---|---|---|
| **Endpoint and day or interval** | **0 ppm** | **100 ppm** | **1000 ppm** | **10,000 ppm** |
| **Males** | | | | |
| Week 0 | 72.2 ± 10.48 | 75.6 ± 8.80 | 71.8 ± 8.34 | 67.4 ± 8.71 |
| Week 2 | 176.5 ± 17.55 | 178.5 ± 15.63 | 175.8 ± 15.20 | 164.7* ± 12.27 (93)[a] |
| Week 4 | 278.0 ± 24.20 | 277.2 ± 20.06 | 273.1 ± 20.31 | 254.6** ± 16.29 (92) |
| Week 8 | 391.7 ± 37.27 | 386.9 ± 32.29 | 388.1 ± 33.75 | 368.1* ± 24.73 (94) |
| Week 10 (end of premating) | 428.6 ± 45.63 | 421.2 ± 35.15 | 422.6 ± 38.63 | 400.1* ± 28.58 (93) |
| Week 18 (termination) | 519.5 ± 64.11 | 502.8 ± 46.47 | 499.5 ± 51.96 | 473.0** ± 36.31 (91) |
| Weight gain premating[b] | 356.4 | 345.6 | 350.8 | 332.7 (93) |
| Weight gain overall | 447.4 ± 64.13 | 427.2 ± 42.19 | 427.7 ± 49.20 | 405.6* ± 34.98 (91) |
| **Females** | | | | |
| Week 0 | 67.1 ± 8.54 | 68.1 ± 8.73 | 69.2 ± 6.57 | 64.8 ± 7.93 |
| Week 2 | 137.6 ± 9.10 | 138.3 ± 11.97 | 139.6 ± 11.21 | 137.2 ± 8.28 |
| Week 4 | 181.1 ± 13.78 | 181.3 ± 17.51 | 181.9 ± 15.09 | 181.9 ± 11.58 |
| Week 8 | 240.1 ± 20.06 | 238.1 ± 21.95 | 235.2 ± 21.44 | 237.0 ± 14.19 |
| Week 10 (end of premating) | 256.3 ± 21.30 | 253.5 ± 22.31 | 251.6 ± 23.10 | 257.1 ± 16.95 |
| Weight gain premating | 189.2 ± 21.64 | 185.4 ± 18.56 | 182.4 ± 21.16 | 192.4 ± 15.48 |

Data taken from Table IA-051 - IA-056, pp. 161-166, MRID 45404906.
[a]Number in parentheses is percent of control; calculated by reviewer.
[b]Calculated by reviewer from group means.
Significantly different from control: **p≤ 0.01, *p ≤ 0.05.

During gestation of $F_0$ and $F_1$ rats, there were slight, but not of biological significance, lower body weight gains at 10000 ppm. For lactation, both generations had increased body weight gains for all treated groups. Food consumption by the $F_1$ dams during gestation was not affected by treatment. During lactation, food consumption by the high-dose $F_1$ dams was significantly ($p \leq 0.05$; 92% of control level) less than that of the controls for days 4-7 and 7-14.

| TABLE 5. Body weights (g) and body weight gains (g) during gestation and lactation | | | | |
|---|---|---|---|---|
| Day/Interval | 0 ppm | 100 ppm | 1000 ppm | 10000 ppm |
| $F_0$ | | | | |
| Day 0 gestation body wt | 261.0 ± 24.78 | 257.8 ± 19.15 | 255.3 ± 16.92 | 254.5 ± 21.00 |
| Day 20 gestation body wt | 395.6 ± 40.94 | 383.8 ± 25.96 | 384.6 ± 26.11 | 374.0 ± 39.45 |
| Days 0-20 gestation body wt gain | 134.6 ± 21.99 | 126.0 ± 13.81 | 129.3 ± 18.15 | 119.5 ± 24.35* |
| Day 1 lactation body wt | 302.1 ± 24.96 | 294.7 ± 23.49 | 283.9 ± 22.58* | 285.4 ± 25.28 |
| Day 21 lactation body wt | 324.8 ± 25.51 | 327.1 ± 20.66 | 318.8 ± 21.31 | 322.2 ± 25.92 |
| Days 1-21 lactation body wt gain | 22.7 ± 19.15 | 32.5 ± 14.16 | 34.9 ± 17.47* | 36.8 ± 10.62** |
| $F_1$ | | | | |
| Day 0 gestation body wt | 262.1 ± 21.52 | 259.5 ± 22.69 | 257.4 ± 20.18 | 260.1 ± 17.72 |
| Day 20 gestation body wt | 394.1 ± 30.66 | 384.6 ± 34.39 | 379.2 ± 29.51 | 385.1 ± 23.41 |
| Days 0-20 gestation body wt gain | 132.1 ± 16.60 | 125.1 ± 20.80 | 139.8 ± 17.00 | 125.0 ± 16.91 |
| Day 1 lactation body wt | 307.6 ± 25.73 | 299.8 ± 20.14 | 300.4 ± 24.77 | 295.2 ± 17.70 |
| Day 21 lactation body wt | 326.3 ± 23.56 | 325.2 ± 23.76 | 335.4 ± 24.13 | 333.4 ± 21.38 |
| Days 1-21 lactation body wt gain | 18.7 ± 14.93 | 25.4 ± 10.58 | 35.0 ± 11.20** | 38.2 ± 17.59** |

Tables 1A-015-1A-018 and 1A057-1A060, pp. 125-128 and 167-170, MRID 45404906.
Significantly different from control: * ≤ 0.05, ** ≤ 0.01.

3. **Test substance intake:** Based on food consumption, body weight, nominal dietary concentrations, doses expressed as mean daily mg test substance/kg body weight are presented in Table 6. Premating doses at the highest dietary concentration exceeded the limit dose of 1000 mg/kg/day for both sexes of both generations.

| TABLE 6. Mean test substance intake (mg/kg body weight/day) | | | |
|---|---|---|---|
| Sex/Study Interval | 100 ppm | 1000 ppm | 10,000 ppm |
| $F_0$ Males - premating | 10.1 | 101.2 | 1034.5 |
| $F_0$ Females - premating | 10.7 | 106.8 | 1062.0 |
| $F_0$ Females - gestation | 8.7 | 88.7 | 907.4 |
| $F_0$ Females - lactation (days 1-14) | 14.8 | 149.4 | 1456.7 |
| $F_1$ Males - premating | 12.3 | 123.9 | 1295.4 |
| $F_1$ Females - premating | 12.5 | 124.7 | 1299.6 |
| $F_1$ Females - gestation | 9.2 | 94.2 | 952.9 |
| $F_1$ Females - lactation (days 1-14) | 14.8 | 155.2 | 1456.1 |

Data taken from text tables pp. 63 and 79, MRID 45404906.

4. **Reproductive function:**

a. **Estrous cycle length and periodicity:** No treatment-related differences were found in females of either generation. All control and almost all treated $F_0$ and $F_1$ animals showed regular estrous cycles and became sperm positive within a few days after pairing. Three high-dose $F_1$ females showed slightly irregular cycles towards the end of the three-week determination period. Two of these animals failed to mate; however, the third mated and delivered pups. This low incidence is considered incidental to treatment.

b. **Sperm measures:** No treatment-related differences were observed for any parameter. Sperm counts and morphology were similar between the treated and control groups for both generations. The percent mobile sperm were similar between all groups of $F_0$ males. High-dose $F_1$ males had a significantly ($p \leq 0.05$) lower percentage of mobile sperm compared with the controls (83% vs 89% for the controls). However, the value for the high-dose group was within the range of historical control values (65-99%) and the slight decrease is considered incidental to treatment.

5. **Reproductive performance:** The reproductive performances of the $F_0$ and $F_1$ animals are summarized in Table 6. No differences in mating, fertility, or gestation indices were seen between the treated and control groups of either generation. The copulatory interval and gestation length of the treated groups were comparable to the control groups in both generations.

| TABLE 7. Reproductive performance of rats fed BAS 510 F for two generations | | | | |
|---|---|---|---|---|
| **Observation** | **0 ppm** | **100 ppm** | **1000 ppm** | **10,000 ppm** |
| **$F_0$ parental animals** | | | | |
| Male mating index (%) | 100 | 100 | 100 | 100 |
| Male fertility index (%) | 100 | 100 | 88 | 100 |
| Female mating index (%) | 100 | 100 | 100 | 100 |
| Female fertility index (%) | 100 | 100 | 88 | 100 |
| Gestation index (%) | 96 | 100 | 100 | 96 |
| Copulatory interval (days) | 2.8 ± 1.20 | 2.2 ± 0.96 | 2.4 ± 1.00 | 2.5 ± 1.12 |
| Gestation length (days) | 21.8 ± 0.41 | 21.8 ± 0.52 | 21.9 ± 0.47 | 21.8 ± 0.41 |
| **$F_1$ parental animals** | | | | |
| Male mating index (%) | 100 | 100 | 100 | 92 |
| Male fertility index (%) | 100 | 100 | 92 | 88 |
| Female mating index (%) | 100 | 100 | 100 | 92 |
| Female fertility index (%) | 100 | 100 | 92 | 96 |
| Gestation index (%) | 100 | 100 | 100 | 100 |
| Copulatory interval (days) | 2.3 ± 1.14 | 2.1 ± 1.05 | 2.7 ± 1.17 | 2.9 ± 2.07 |
| Gestation length (days) | 22.0 ± 0.00 | 22.0 ± 0.29 | 21.7* ± 0.45 | 21.8 ± 0.43 |

Data taken from Tables IA-024, IA-026, IA-066, and IA-068, pp. 134, 136, 176, and 178, respectively, MRID 45404906.
Significantly different from control: *$p \leq 0.05$.

## 6. Parental postmortem results:

a) **Organ weights:** Selected absolute and relative (to body weight) organ weight data for the $F_0$ and $F_1$ parental animals are given in Tables 7 and 8, respectively. For the $F_0$ males, absolute and relative spleen weights were significantly ($p \leq 0.05$ or 0.01) decreased in the mid- and high-dose animals compared with the controls. High-dose $F_0$ females had significantly ($p \leq 0.01$) increased absolute and relative liver weights and decreased absolute kidney weights. Absolute spleen weights for the high-dose $F_0$ females were also slightly less than that of the controls, but statistical significance was not attained.

For the high-dose $F_1$ males, terminal body weights, absolute and relative spleen weights, absolute kidney weights and absolute brain weights were significantly ($p \leq 0.05$ or 0.01) less than those of the controls. Absolute and relative spleen weights of the mid-dose $F_1$ males were also significantly ($p \leq 0.01$) decreased compared with the controls. High-dose $F_1$ females had significantly ($p \leq 0.01$) increased absolute and relative liver weights and decreased absolute and relative spleen and kidney weights. Absolute spleen weights for the mid-dose $F_1$ females were also significantly ($p \leq 0.05$) less than those of the controls.

Other statistical differences in organ weights between the treated and control groups were sporadic and not dose-related.

**Table 8. Selected organ weights from $F_0$ parental animals**

| Organ | 0 ppm | 100 ppm | 1000 ppm | 10,000 ppm |
|---|---|---|---|---|
| **$F_0$ Males** | | | | |
| Terminal body wt. (g) | 459.43 ± 38.88 | 462.80 ± 35.49 | 452.14 ± 35.90 | 454.98 ± 43.00 |
| Liver<br>absolute (g)<br>relative (% body wt.) | 16.64 ± 2.70<br>3.61 ± 0.45 | 16.24 ± 2.68<br>3.50 ± 0.40 | 16.59 ± 3.05<br>3.66 ± 0.50 | 17.13 ± 2.43<br>3.76 ± 0.28 |
| Spleen<br>absolute (g)<br>relative (% body wt.) | 0.87 ± 0.14<br>0.19 ± 0.03 | 0.86 ± 0.09<br>0.19 ± 0.02 | 0.77* ± 0.10 (89)[a]<br>0.17** ± 0.02 (89) | 0.71** ± 0.11 (82)<br>0.16** ± 0.02 (84) |
| **$F_0$ Females** | | | | |
| Terminal body wt. (g) | 273.18 ± 20.41 | 267.23 ± 20.19 | 266.60 ± 17.61 | 263.73 ± 22.54 |
| Liver<br>absolute (g)<br>relative (% body wt.) | 9.86 ± 1.49<br>3.60 ± 0.43 | 9.75 ± 1.15<br>3.65 ± 0.34 | 10.08 ± 1.06<br>3.79 ± 0.37 | 11.46** ± 1.26 (116)<br>4.35** ± 0.41 (121) |
| Spleen<br>absolute (g)<br>relative (% body wt.) | 0.61 ± 0.10<br>0.22 ± 0.03 | 0.59 ± 0.08<br>0.22 ± 0.03 | 0.56 ± 0.06<br>0.21 ± 0.03 | 0.55 ± 0.06<br>0.21 ± 0.04 |
| Kidney<br>absolute (g)<br>relative (% body wt.) | 2.08 ± 0.17<br>0.76 ± 0.05 | 1.98* ± 0.17 (95)<br>0.74 ± 0.06 | 2.01 ± 0.20<br>0.76 ± 0.07 | 1.93** ± 0.17 (93)<br>0.73 ± 0.04 |

Data taken from Tables IB-1 - IB-4, pp. 190-193, MRID 45404906.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

| TABLE 9. Selected organ weights from $F_1$ parental animals | | | | |
|---|---|---|---|---|
| Organ | 0 ppm | 100 ppm | 1000 ppm | 10,000 ppm |
| **$F_1$ Males** | | | | |
| Terminal body wt. (g) | 503.26 ± 70.03 | 483.51 ± 44.15 | 483.04 ± 54.01 | 455.74** ± 38.72 (91)* |
| Liver | | | | |
| absolute (g) | 17.77 ± 3.93 | 18.34 ± 4.07 | 17.73 ± 3.10 | 18.09 ± 1.87 |
| relative (% body wt.) | 3.53 ± 0.55 | 3.79 ± 0.74 | 3.68 ± 0.58 | 3.97** ± 0.25 (112) |
| Brain | | | | |
| absolute (g) | 2.12 ± 0.09 | 2.13 ± 0.10 | 2.11 ± 0.07 | 2.06* ± 0.07 (97) |
| relative (% body wt.) | 0.43 ± 0.05 | 0.44 ± 0.03 | 0.44 ± 0.05 | 0.46 ± 0.03 |
| Kidney | | | | |
| absolute (g) | 3.06 ± 0.39 | 3.01 ± 0.22 | 2.96 ± 0.35 | 2.74** ± 0.24 (90) |
| relative (% body wt.) | 0.61 ± 0.04 | 0.62 ± 0.04 | 0.61 ± 0.04 | 0.60 ± 0.04 |
| Spleen | | | | |
| absolute (g) | 0.90 ± 0.12 | 0.87 ± 0.17 | 0.76** ± 0.09 (84) | 0.71** ± 0.14 (79) |
| relative (% body wt.) | 0.18 ± 0.02 | 0.18 ± 0.03 | 0.16** ± 0.02 (89) | 0.16** ± 0.03 (89) |
| **$F_1$ Females** | | | | |
| Terminal body wt. (g) | 283.26 ± 21.85 | 278.61 ± 19.85 | 275.23 ± 23.11 | 282.16 ± 18.97 |
| Liver | | | | |
| absolute (g) | 10.41 ± 1.09 | 10.48 ± 1.23 | 10.51 ± 1.11 | 12.61** ± 1.62 (121) |
| relative (% body wt.) | 3.68 ± 0.37 | 3.77 ± 0.47 | 3.83 ± 0.36 | 4.47** ± 0.51 (121) |
| Kidney | | | | |
| absolute (g) | 1.99 ± 0.16 | 1.93 ± 0.18 | 1.94 ± 0.20 | 1.86** ± 0.29 (93) |
| relative (% body wt.) | 0.71 ± 0.05 | 0.69 ± 0.06 | 0.71 ± 0.06 | 0.66** ± 0.09 (93) |
| Spleen | | | | |
| absolute (g) | 0.63 ± 0.07 | 0.61 ± 0.08 | 0.58* ± 0.07 (92) | 0.57** ± 0.13 (90) |
| relative (% body wt.) | 0.22 ± 0.03 | 0.22 ± 0.03 | 0.21 ± 0.03 | 0.20** ± 0.04 (91) |

Data taken from Tables IB-9 - IB-12, pp. 198-201, MRID 45404906.
*Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

b) **Pathology:**

1) **Macroscopic examination:** No treatment-related gross lesions were found in any animal of either sex or generation.

2) **Microscopic examination:** A treatment-related histopathological finding was increased incidence and severity of centrilobular hepatocyte hypertrophy which was observed in 24-40% of mid- and 100% of high-dose animals (Table 9). In addition, for the high-dose groups, hepatocyte degeneration was observed in three $F_0$ males, one $F_0$ female, eight $F_1$ males, and no $F_1$ females. Other microscopic lesions were found at low incidences and were not dose-related in incidence or severity. No treatment-related lesions occurred in the reproductive tracts of males or females from either generation.

Differential ovarian follicle counts were similar between the high-dose and control $F_1$ females.

**TABLE 10. Incidence and severity of liver lesions of male and female rats fed BAS 510 F for two generations**

| Sex/generation | 0 ppm | 100 ppm | 1000 ppm | 10,000 ppm |
|---|---|---|---|---|
| **Centrilobular hypertrophy** | | | | |
| $F_0$ Males | 0/25 | 0/25 | 9/25 (1.11)[a] | 25/25 (1.92) |
| $F_0$ Females | 0/25 | 0/25 | 6/25 (1.00) | 25/25 (1.72) |
| $F_1$ Males | 1/25 (1.00) | 0/25 | 10/25 (1.00) | 25/25 (1.84) |
| $F_1$ Females | 0/25 | 0/25 | 8/25 (1.75) | 25/25 (1.68) |
| **Centrilobular hepatocyte degeneration** | | | | |
| $F_0$ Males | 0/25 | 1/25 (3.00) | 0/25 | 3/25 (2.00) |
| $F_0$ Females | 0/25 | 0/25 | 0/25 | 1/25 (2.00) |
| $F_1$ Males | 0/25 | 0/25 | 0/25 | 8/25 (2.50) |
| $F_1$ Females | 0/25 | 0/25 | 0/25 | 0/25 |

Data taken from Tables IB-7 and IB-15, pp. 196 and 204, respectively, MRID 45404906.
[a]Number in parentheses is mean severity score; 1 = minimal, 2 = slight, 3 = moderate, 4 = marked/severe, 5 = massive/extreme.

## B. OFFSPRING

1. **Viability and clinical signs:** Mean litter size and viability (survival) results from the $F_1$ and $F_2$ pups during lactation are summarized in Table 10. For the $F_1$ litters, live birth, viability, and lactation indices, mean litter sizes, and sex ratios were similar between the treated and control groups. The mean number of implantation sites for the low- and high-dose $F_0$ females was significantly ($p \leq 0.05$) lower than that of the control females resulting in significantly ($p \leq 0.05$ or 0.01) fewer total pups/litter for these treated groups.

   Pup survival in the high-dose $F_2$ litters was decreased during lactation days 0-4 as indicated by a significantly ($p \leq 0.01$) lower viability index compared with the controls. Post-implantation loss was significantly ($p \leq 0.05$) greater for the high-dose $F_1$ females compared with the controls, but the mean number of pups delivered by the high-dose group was only slightly (n.s.) less than that of the control group. The live birth index, the lactation index, and pup sex ratio were similar between the treated and control groups.

   No treatment-related clinical signs of toxicity were observed in the pups during lactation.

TABLE 11. Viability of $F_1$ and $F_2$ litters during lactation

| Observation/study time | 0 ppm | 100 ppm | 1000 ppm | 10,000 ppm |
|---|---|---|---|---|
| **$F_1$ litters** | | | | |
| Number of viable litters | 24 | 25 | 22 | 24 |
| Mean number of pups delivered | 15.8 ± 2.13 | 13.2* ± 2.23 | 14.2 ± 3.57 | 13.0** ± 4.18 |
| Mean live litter size on day 0 | 15.2 ± 2.70 | 13.1 ± 2.25 | 13.6 ± 3.65 | 12.9 ± 4.30 |
| Live birth index (%) | 96 | 99 | 96 | 99 |
| Viability index (%) | 95 | 93 | 92 | 94 |
| Lactation index (%) | 99 | 100 | 99 | 99 |
| Sex ratio day 0 (% male) | 48.9 | 51.1 | 50.2 | 47.2 |
| Implantation sites | 17.4 ± 1.69 | 15.6* ± 2.38 | 16.3 ± 2.59 | 15.2* ± 3.37 |
| Post-implantation loss (mean %) | 12.8 ± 20.29 | 14.7 ± 11.83 | 13.7 ± 15.08 | 18.6 ± 24.93 |
| **$F_2$ litters** | | | | |
| Number of viable litters | 25 | 25 | 23 | 22 |
| Mean number of pups delivered | 14.2 ± 2.49 | 13.4 ± 4.03 | 15.5 ± 2.29 | 13.0 ± 3.09 |
| Mean live litter size on day 0 | 13.8 ± 2.59 | 13.3 ± 3.97 | 15.1 ± 2.32 | 12.5 ± 3.10 |
| Live birth index (%) | 97 | 99 | 97 | 97 |
| Viability index (%) | 93 | 91 | 97 | 86** |
| Lactation index (%) | 100 | 100 | 100 | 99 |
| Sex ratio day 0 (% male) | 47.7 | 53.3 | 50.9 | 47.5 |
| Implantation sites | 15.4 ± 2.84 | 15.1 ± 4.25 | 17.1 ± 1.68 | 15.2 ± 2.64 |
| Post-implantation loss (mean %) | 7.0 ± 6.89 | 10.8 ± 9.28 | 9.1 ± 10.81 | 15.4* ± 14.98 |

Data taken from Tables IA-26 - IA-29 and IA-68 - IA-71, pp. 136-139 and 178-181, respectively, MRID 45404906.
Significantly different from control: *$p \leq 0.05$.

2. **Body weight:** Body weight and body weight gain data for the $F_1$ and $F_2$ pups are given in Tables 11 and 12, respectively. Pups from the high-dose litters of both generations and mid-dose $F_2$ male pups had significantly reduced body weights and body weight gains during lactation compared with their respective control group. High-dose male and female $F_1$ pups had significantly ($p \leq 0.05$) lower body weights on lactation day 21 compared with the controls due to consistently reduced body weight gains ($p \leq 0.05$ or 0.01; 89-93% of the control level) for all intervals after lactation day 4. Body weights of the high-dose $F_2$ male and female pups were 86-90% ($p \leq 0.01$) of the control levels on lactation days 14 and 21 due to consistently reduced body weight gains ($p \leq 0.05$ or 0.01; 83-88% of the control level) for all intervals after lactation day 4. In addition, body weights were significantly ($p \leq 0.05$) reduced for the mid- and high-dose $F_2$ males on lactation day 7 and the mid-dose $F_2$ males on

day 21. Body weight gains by the mid-dose $F_2$ males were 88-93% ($p \leq 0.05$ or 0.01) of the control levels during lactation days 4-21.

TABLE 12. Mean body weights (g) and body weight gains (g) of the $F_1$ pups during lactation

| Day of lactation | 0 ppm | 100 ppm | 1000 ppm | 10,000 ppm |
|---|---|---|---|---|
| **Litters** | | | | |
| Body weight | | | | |
| 1 | 6.0 ± 0.44 | 6.3 ± 0.65 | 6.2 ± 0.69 | 6.3 ± 0.62 |
| 4 (postcull) | 8.6 ± 1.13 | 8.9 ± 1.13 | 9.0 ± 1.40 | 8.7 ± 1.21 |
| 7 | 13.9 ± 1.75 | 14.3 ± 1.76 | 14.4 ± 2.02 | 13.5 ± 1.57 |
| 14 | 30.3 ± 2.39 | 30.3 ± 2.57 | 30.6 ± 2.78 | 28.7 ± 2.11 (95) |
| 21 | 49.5 ± 4.14 | 49.4 ± 5.46 | 51.0 ± 4.85 | 45.9* ± 3.76 (93)* |
| Body weight gain | | | | |
| 1-4 | 2.4 ± 0.74 | 2.5 ± 0.61 | 2.6 ± 0.83 | 2.4 ± 0.75 |
| 4-7 | 5.4 ± 0.71 | 5.4 ± 0.76 | 5.4 ± 0.86 | 4.8* ± 0.55 (89) |
| 7-14 | 16.3 ± 0.96 | 16.0 ± 1.14 | 16.3 ± 1.57 | 15.2** ± 1.33 (93) |
| 14-21 | 19.3 ± 2.11 | 19.1 ± 3.05 | 20.4 ± 5.79 | 17.1 ± 1.90 (89) |
| 4-21 | 41.1 ± 3.30 | 40.6 ± 4.61 | 42.2 ± 4.70 | 37.2** ± 3.16 (91) |
| **Males** | | | | |
| Body weight | | | | |
| 1 | 6.2 ± 0.44 | 6.4 ± 0.62 | 6.3 ± 0.50 | 6.4 ± 0.67 |
| 4 (postcull) | 8.7 ± 1.16 | 9.2 ± 1.16 | 8.9 ± 1.03 | 8.8 ± 1.21 |
| 7 | 14.2 ± 1.88 | 14.7 ± 1.88 | 14.5 ± 1.73 | 13.7 ± 1.69 |
| 14 | 30.5 ± 2.60 | 30.8 ± 2.84 | 31.0 ± 2.79 | 29.0 ± 2.57 |
| 21 | 50.3 ± 4.66 | 50.6 ± 6.14 | 51.8 ± 4.77 | 46.7* ± 4.48 (93) |
| Body weight gain | | | | |
| 1-4 | 2.5 ± 0.76 | 2.6 ± 0.63 | 2.5 ± 0.70 | 2.3 ± 0.74 |
| 4-7 | 5.4 ± 0.79 | 5.5 ± 0.84 | 5.5 ± 0.88 | 4.9 ± 0.73 |
| 7-14 | 16.4 ± 1.06 | 16.1 ± 1.29 | 16.5 ± 1.74 | 15.4* ± 1.47 (94) |
| 14-21 | 19.8 ± 2.45 | 19.8 ± 3.44 | 20.8 ± 5.89 | 17.6 ± 2.25 (89) |
| 4-21 | 41.7 ± 3.80 | 41.6 ± 5.29 | 43.0 ± 4.72 | 37.9* ± 3.81 (91) |
| **Females** | | | | |
| Body weight | | | | |
| 1 | 5.9 ± 0.46 | 6.0 ± 0.65 | 6.0 ± 0.77 | 6.1 ± 0.66 |
| 4 (postcull) | 8.4 ± 1.17 | 8.6 ± 1.12 | 8.8 ± 1.51 | 8.6 ± 1.31 |
| 7 | 13.7 ± 1.80 | 13.9 ± 1.75 | 14.1 ± 2.21 | 13.4 ± 1.67 |
| 14 | 30.0 ± 2.52 | 29.7 ± 2.54 | 30.5 ± 2.21 | 28.4 ± 1.86 |
| 21 | 48.7 ± 4.13 | 48.0 ± 5.11 | 49.3 ± 3.43 | 45.2* ± 3.39 (93) |
| Body weight gain | | | | |
| 1-4 | 2.3 ± 0.77 | 2.5 ± 0.61 | 2.6 ± 0.83 | 2.5 ± 0.81 |
| 4-7 | 5.3 ± 0.73 | 5.2 ± 0.77 | 5.3 ± 0.92 | 4.8* ± 0.51 (91) |
| 7-14 | 16.3 ± 1.09 | 15.8 ± 1.10 | 16.2 ± 1.29 | 15.1** ± 1.34 (93) |
| 14-21 | 18.7 ± 2.00 | 18.3 ± 2.80 | 18.7 ± 1.61 | 16.7** ± 1.77 (89) |
| 4-21 | 40.4 ± 3.26 | 39.4 ± 4.26 | 40.5 ± 2.69 | 36.6** ± 2.78 (91) |

Data taken from Tables IA-030 - IA-033, pp. 140-144, MRID 45404906.
*Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

TABLE 13. Mean body weights (g) and body weight gains (g) of the $F_1$ pups during lactation

| Day of lactation | 0 ppm | 100 ppm | 1000 ppm | 10,000 ppm |
|---|---|---|---|---|
| **Litters** | | | | |
| Body weight | | | | |
| 1 | 6.3 ± 0.52 | 6.4 ± 0.62 | 6.1 ± 0.50 | 6.2 ± 0.58 |
| 4 (postcull) | 9.3 ± 1.16 | 9.2 ± 1.50 | 8.6 ± 1.25 | 8.9 ± 1.30 |
| 7 | 15.0 ± 1.74 | 14.8 ± 1.94 | 13.7 ± 2.15 | 13.7 ± 2.10 (91) |
| 14 | 32.1 ± 2.53 | 31.3 ± 2.88 | 30.7 ± 3.05 | 28.7** ± 3.34 (89)* |
| 21 | 53.1 ± 3.76 | 51.7 ± 4.91 | 50.1 ± 4.60 | 46.2** ± 4.84 (87) |
| Body weight gain | | | | |
| 1-4 | 2.9 ± 0.87 | 2.8 ± 1.00 | 2.4 ± 0.85 | 2.7 ± 0.83 |
| 4-7 | 5.7 ± 0.74 | 5.4 ± 0.95 | 5.1 ± 1.04 | 4.8** ± 0.93 (84) |
| 7-14 | 17.1 ± 1.31 | 16.5 ± 1.50 | 16.9 ± 1.33 | 15.1** ± 1.54 (88) |
| 14-21 | 21.0 ± 1.54 | 20.3 ± 2.3 | 19.4* ± 1.85 (92) | 17.5** ± 1.88 (83) |
| 4-21 | 43.8 ± 3.01 | 42.3 ± 4.21 | 41.6 ± 3.66 | 37.4** ± 3.71 (85) |
| **Males** | | | | |
| Body weight | | | | |
| 1 | 6.5 ± 0.51 | 6.4 ± 0.56 | 6.2 ± 0.50 | 6.3 ± 0.65 |
| 4 (postcull) | 9.6 ± 1.18 | 9.3 ± 1.16 | 8.7 ± 1.27 | 9.0 ± 1.45 (94) |
| 7 | 15.4 ± 1.79 | 14.7 ± 1.92 | 13.9* ± 2.20 (90) | 13.9* ± 2.25 (90) |
| 14 | 32.7 ± 2.57 | 31.6 ± 3.02 | 30.9 ± 3.14 | 29.1** ± 3.63 (89) |
| 21 | 54.6 ± 4.08 | 52.4 ± 5.19 | 50.8* ± 4.90 (93) | 47.0** ± 5.23 (86) |
| Body weight gain | | | | |
| 1-4 | 2.9 ± 0.94 | 2.9 ± 0.72 | 2.4 ± 0.87 | 2.7 ± 0.96 |
| 4-7 | 5.9 ± 0.75 | 5.4 ± 0.95 | 5.2* ± 1.07 (88) | 4.8** ± 0.93 (81) |
| 7-14 | 17.3 ± 1.35 | 16.8 ± 1.48 | 17.0 ± 1.38 | 15.2** ± 1.74 (88) |
| 14-21 | 21.9 ± 1.82 | 20.9 ± 2.56 | 19.8** ± 2.09 (90) | 17.9** ± 1.98 (82) |
| 4-21 | 45.2 ± 3.28 | 43.1 ± 4.52 | 42.2* ± 4.00 (93) | 37.9** ± 3.98 (84) |
| **Females** | | | | |
| Body weight | | | | |
| 1 | 6.2 ± 0.55 | 6.2 ± 0.58 | 5.9 ± 0.52 | 6.0 ± 0.50 |
| 4 (postcull) | 9.1 ± 1.17 | 9.0 ± 1.36 | 8.5 ± 1.29 | 8.7 ± 1.14 |
| 7 | 14.7 ± 1.75 | 14.7 ± 1.70 | 13.6 ± 2.18 | 13.5 ± 1.98 (92) |
| 14 | 31.5 ± 2.58 | 31.1 ± 2.82 | 30.4 ± 3.15 | 28.4** ± 3.15 (90) |
| 21 | 51.6 ± 3.64 | 50.8 ± 4.81 | 49.3 ± 4.66 | 45.5** ± 4.64 (88) |
| Body weight gain | | | | |
| 1-4 | 2.9 ± 0.85 | 2.8 ± 0.94 | 2.4 ± 0.85 | 2.7 ± 0.74 |
| 4-7 | 5.6 ± 0.76 | 5.5 ± 0.90 | 5.1 ± 1.06 | 4.7** ± 0.98 (84) |
| 7-14 | 16.9 ± 1.31 | 16.4 ± 1.60 | 16.8 ± 1.37 | 14.9** ± 1.43 (88) |
| 14-21 | 20.0 ± 1.53 | 19.7 ± 2.22 | 19.0 ± 1.83 | 17.1** ± 1.90 (86) |
| 4-21 | 42.5 ± 2.99 | 41.6 ± 4.22 | 41.0 ± 3.69 | 36.8** ± 3.67 (87) |

Data taken from Tables 1A-072 - 1A-075, pp. 182-185, MRID 45404906.
*Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

3. **Sexual maturation** ($F_1$): No differences in the rate of sexual maturation were noted between the treated and control groups. For females, the mean days to vaginal opening ranged from 30.6 to 31.6 days for all groups. For males, the average number of days to preputial separation was 44.0-44.5 for all groups.

4. **Offspring postmortem results:**

a. **Organ weights:** Selected absolute and relative (to body weight) organ weight values for the $F_1$ and $F_2$ pups at weaning are presented in Table 13. Final body weights of the high-dose pups of both generations were significantly ($p \leq 0.05$ or 0.01) less than those of the controls. For the high-dose $F_1$ pups, relative brain weights were significantly ($p \leq 0.01$) increased as compared with those of the controls. For the $F_2$ pups, significant ($p \leq 0.05$ or 0.01) differences in organ weights included decreased absolute thymus weights in the low- and high-dose groups and decreased absolute spleen weights in the mid- and high-dose groups. In addition, relative brain weights were significantly ($p \leq 0.01$) increased in the mid- and high-dose groups, relative thymus weights were decreased ($p \leq 0.01$) in the low-dose group, and relative spleen weights were decreased ($p \leq 0.01$) in the high-dose group.

**TABLE 14. Selected organ weights from $F_1$ and $F_2$ pups (male and female combined)**

| Organ | 0 ppm | 100 ppm | 1000 ppm | 10,000 ppm |
|---|---|---|---|---|
| **$F_1$ Pups** | | | | |
| Day 21 body wt. (g) | 49.5 ± 4.14 | 49.4 ± 5.46 | 51.0 ± 4.85 | 45.9* ± 3.76 (93)[a] |
| Brain<br>absolute (g)<br>relative (% body wt.) | <br>1.43 ± 0.06<br>2.87 ± 0.22 | <br>1.44 ± 0.04<br>2.91 ± 0.26 | <br>1.45 ± 0.06<br>2.83 ± 0.37 | <br>1.42 ± 0.05<br>3.07** ± 0.22 (107) |
| Thymus<br>absolute (g)<br>relative (% body wt.) | <br>0.18 ± 0.03<br>0.35 ± 0.04 | <br>0.18 ± 0.03<br>0.35 ± 0.03 | <br>0.19 ± 0.03<br>0.36 ± 0.08 | <br>0.16 ± 0.02<br>0.35 ± 0.04 |
| Spleen<br>absolute (g)<br>relative (% body wt.) | <br>0.23 ± 0.05<br>0.45 ± 0.06 | <br>0.21 ± 0.05<br>0.42 ± 0.06 | <br>0.22 ± 0.04<br>0.43 ± 0.08 | <br>0.20 ± 0.03<br>0.43 ± 0.06 |
| **$F_2$ Pups** | | | | |
| Day 21 body wt. (g) | 53.1 ± 3.76 | 51.7 ± 4.91 | 50.1 ± 4.60 | 46.2** ± 4.84 (87) |
| Brain<br>absolute (g)<br>relative (% body wt.) | <br>1.46 ± 0.04<br>2.77 ± 0.18 | <br>1.44 ± 0.05<br>2.81 ± 0.26 | <br>1.45 ± 0.04<br>2.96* ± 0.26 (107) | <br>1.44 ± 0.06<br>3.12** ± 0.31 (113) |
| Thymus<br>absolute (g)<br>relative (% body wt.) | <br>0.20 ± 0.02<br>0.37 ± 0.05 | <br>0.17** ± 0.03 (85)<br>0.33** ± 0.04 (89) | <br>0.19 ± 0.03<br>0.38 ± 0.05 | <br>0.17** ± 0.03 (85)<br>0.36 ± 0.04 |
| Spleen<br>absolute (g)<br>relative (% body wt.) | <br>0.25 ± 0.06<br>0.47 ± 0.08 | <br>0.24 ± 0.05<br>0.46 ± 0.07 | <br>0.22* ± 0.04 (88)<br>0.44 ± 0.06 | <br>0.19** ± 0.04 (76)<br>0.41** ± 0.06 (87) |

Data taken from Tables IA-034 - IA-035 and IA-076 - IA-077, pp. 141, 144-145 and 183, 186-187, respectively, MRID 45404906.

[a]Number in parentheses is percent of control; calculated by reviewer.

Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

b. Pathology:

1. Macroscopic examination: No treatment-related gross lesions were found in stillborn, found dead, culled, or surplus pups of either generation.

2. Microscopic examination: Tissues from pups were not examined microscopically.

## III. DISCUSSION and CONCLUSIONS

### A. INVESTIGATORS' CONCLUSIONS:

The study authors concluded that systemic toxicity occurred at the mid- and high-dose in parental animals of both generations. Clinical effects in the high-dose groups included decreases in food consumption, body weights, and body weight gains during various study intervals. Increased liver weights were found in high-dose males and females of both generations and microscopic lesions were seen in the livers of mid- and high-dose males and females.

Under the conditions of the study, the test article had no adverse effects on reproductive performance or fertility of the $F_0$ or $F_1$ parental animals. At the high dose, pup mortality was increased in the $F_2$ litters and body weights of surviving pups were decreased in the $F_1$ and $F_2$ litters. In addition, body weights of the $F_2$ male pups were decreased in the mid-dose group.

The NOAEL for reproductive performance and fertility was set at 10,000 ppm (about 1173 mg/kg/day), the NOAEL for systemic effects was 100 ppm (about 11 mg/kg/day), and the NOAEL for developmental toxicity was 1000 ppm (about 114 mg/kg/day) for male and female $F_1$ and female $F_2$ progeny and was 100 ppm (about 11 mg/kg/day) for male $F_2$ progeny.

B. REVIEWER COMMENTS: Only mild systemic toxicity was observed in the parental animals on this study. No deaths, clinical signs of toxicity, or effects on food consumption were observed in either sex of either generation. The only effects on body weights occurred in the high-dose $F_1$ males and consisted of reduced absolute body weights due to decreased weight gain during the first few weeks of premating.

Occasional decreases in body weights, body weight gains, or food consumption by the high-dose $F_0$ and $F_1$ dams during gestation or lactation were considered to be treatment-related by the study author. However, the decreases were not dose-related, were not sustained for more than one week, and did not occur consistently over both generations. Therefore, the reviewer disagrees with the study author and thinks that differences in these parameters during gestation and lactation were sporadic and not treatment-related.

At necropsy, spleen weights from the mid- and high-dose males and females of both generations were reduced as compared with those of the controls. However, no gross or microscopic lesions were found that corresponded with the decreased organ weights. Therefore, the reviewer concludes that the lower spleen weights were probably incidental to treatment and do not represent an adverse effect in the absence of histopathological

correlates. In another study, reduced spleen weights in the absence of other lesions were also observed in males administered 15,000 ppm of the test article for 3 months; females were not affected (MRID 45404822).

Histologically, centrilobular hepatocyte hypertrophy was observed in the mid- and high-dose groups. This lesion is considered an adaptive response to a xenobiotic and is not adverse. On the other hand, the low incidence of hepatocyte degeneration observed in high-dose $F_0$ and $F_1$ males is considered to be an adverse effect.

Neither reproductive function nor performance were affected by treatment in either generation. However, pup growth and survival were decreased. Pup survival in the high-dose $F_2$ litters was decreased during lactation days 0-4 as indicated by a significantly lower viability index compared with the controls. An increased post-implantation loss was observed in the high-dose $F_1$ dams, but the relationship to treatment is unclear. Pups from the high-dose litters of both generations and mid-dose $F_2$ male pups had significantly reduced body weights and body weight gains during lactation compared with their respective control group. Body weight gains by these groups were consistently reduced for all intervals after lactation day 4 suggesting both a lactational and a systemic effect. The systemic effect persisted in the high-dose $F_1$ males after weaning, although the $F_1$ females appeared to recover. In addition, the reduced pup growth was more pronounced in the $F_2$ pups which may indicate a cumulative effect.

**The parental systemic LOAEL is 10,000 ppm for males (1034.5-1295.4 mg/kg/day) based on decreased body weights and body weight gains of the $F_1$ males and hepatocyte degeneration in $F_0$ and $F_1$ males; the systemic LOAEL was not identified for females. The parental systemic NOAELs are 1000 ppm for males (101.2-123.9 mg/kg/day) and 10,000 ppm for females (1062.0-1299.6 mg/kg/day).**

**The reproductive toxicity NOAEL is ≥10,000 ppm (1034.5-1295.4 mg/kg/day for males and 1062.0-1299.6 mg/kg/day for females) and the reproductive toxicity LOAEL was not identified.**

**The offspring toxicity LOAEL is 1000 ppm for males (101.2-123.9 mg/kg/day) based on decreased body weights and body weight gains by the $F_2$ male pups and 10,000 ppm for females (1062.0-1299.6 mg/kg/day) based on decreased body weights and weight gains. The offspring toxicity NOAEL is 100 ppm for males (10.1-12.3 mg/kg/day) and 1000 ppm for females (106.8-124.7 mg/kg/day).**

C. <u>STUDY DEFICIENCIES</u>: No deficiencies were identified in the conduct of this study.

73

# DATA FOR ENTRY INTO ISIS

Reproductive Study - rats (870.3800)

| PC code | MRID # | Study type | Species | Duration | Route | Dosing method | Dose range mg/kg/day | Doses tested mg/kg/day | NOAEL mg/kg/day | LOAEL mg/kg/day | Target organ(s) | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 128008 | 45404906 | reproductive | rats | 2 generat | oral | diet | 10.1-1299.6 | 0, 10.1-12.5, 101.2-124.7, 1034.5-1299.6 | 101.2-124.7 | 1034.5-1299.6 | males: body wt decr; liver | Parental/ systemic |
| 128008 | 45404906 | reproductive | rats | 2 generat | oral | diet | 10.1-1299.6 | 0, 10.1-12.5, 101.2-124.7, 1034.5-1299.6 | 10.1-12.5 | 101.2-124.7 | decr body wt of $F_2$ male pups | Offspring |
| 128008 | 45404906 | reproductive | rats | 2 generat | oral | diet | 10.1-1299.6 | 0, 10.1-12.5, 101.2-124.7, 1034.5-1299.6 | ≥1034.5-1299.6 | not identified | none | Reproductive |