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

Office of Prevention, Pesticides
and
Toxic Substances

January 15, 2002

TXR#: 0050254

MEMORANDUM

SUBJECT: CARBARYL - Review of Multi-generation Reproduction Study in Rats (MRID 45448101)

PC Code: 056801
DP Barcode: D276230
Submission: S600197

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer
Reregistration Branch I, Health Effects Division (7509C)

Virginia A Dobozy 1/15/02

THRU: Whang Phang, Ph.D., Branch Senior Scientist
Reregistration Branch I, Health Effects Division (7509C)

TO: Betty Shackleford/Anthony Britten
Special Review and Reregistration Division (7508C)

Action Requested: Review two-generation reproduction study in rats (MRID 45448101)

Recommendation: The study has been reviewed and found acceptable/guideline. The Executive Summary follows and the Data Evaluation Record is attached.

CITATION: Tyl. R., C. Myers, M. Marr. (2001). Two-generation reproductive toxicity evaluation of carbaryl (RPA007744) administered in the feed to CD® (Sprague-Dawley) rats. Reproductive and Developmental Laboratory, Center for Life Sciences and Toxicology, Chemistry and Life Sciences. Research Triangle Institute, Life Sciences and Toxicology, Research Triangle Park, NC 27709. Laboratory report number 65C-07407-400, May 24, 2001. MRID 45448101. Unpublished

EXECUTIVE SUMMARY: In a two-generation reproduction study (MRID 45448101), carbaryl (99.1% a.i, Lot No. E1208008) was given in the diet to groups of 30 male and 30 female F_0 and F_1 rats (CD®[SD] IGS BR (Sprague-Dawley)) at concentrations of 0, 75, 300, or 1500 ppm. The dietary concentrations corresponded to doses of 4.67, 31.34, and 92.43 mg/kg/day for F_0 males; 0, 5.56, 36.32, and 110.78 mg/kg/day for F_0 females; 0, 5.79, 23.49, and 124.33 mg/kg/day for F_1 males; and 0, 6.41, 26.91, and 135.54 mg/kg/day for F_1 females averaged over the premating period. Each group received treated or control diet continuously for 70 days prior to mating and during mating, gestation, and lactation of one litter per generation. F_1 pups selected to parent the F_2 generation were weaned onto the same food as their parents. Parental males were sacrificed after delivery of their litters and parental females were sacrificed after weaning of their litters,

No treatment-related deaths, clinical signs, organ weight changes, gross lesions, or microscopic lesions were observed in adult rats of either generation. No treatment-related effects were observed on body weights, weight gain, feed consumption, or food efficiency in 75- or 300-ppm group F_0 or F_1 male or female rats at any time during the study including the gestation and lactation periods of the females. F_0 and F_1 male and female rats fed the 1500-ppm diet weighed significantly ($p<0.01$ or <0.05) less and gained less weight during the premating period. The F_0 males weighed 5-6% less than controls during premating, gained 14-23% less weight during three weekly intervals up to day 45, and gained 9% less weight over the entire premating period; they also gained 8% less weight than controls over the mating/postmating period. The F_1 males weighed 10-19% less than controls during the entire study, gained 16% and 11% less weight during the first two weekly intervals, and gained 8% less weight than controls averaged over the entire premating period. The F_0 females weighed 4-5% less than controls during the first 42 days of premating, gained 27% less weight during the first week, and 7% (N.S.) less averaged over the entire premating period. The F_1 females weighed 8-22% less than controls throughout premating and gained 9% less weight during the first week; weight gain for the remaining weekly intervals and for the entire premating period was similar to that of controls. Food consumption and food efficiency for F_0 and F_1 rats followed patterns similar to that of body weight and weight gain; the largest difference between the 1500-ppm groups and controls occurred during the early part of the premating period. When averaged over the entire premating period, F_0 and F_1 males consumed 6-7% less food than control and had food efficiency values similar to those of the controls. Feed consumption and food efficiency for the F_0 females were similar to those of the control group, whereas F_1 females consumed 9% ($p<0.01$) less feed and had a food efficiency value 10% ($p<0.01$) greater than that of controls. F_0 and F_1 females in the 1500 ppm group weighed less and gained less weight than controls during gestation, with the effect being greater in the F_1 females. During lactation weight gain was markedly reduced in F_1 females during the first 4 days, but was greater than that of controls averaged over the entire lactation period.

The lowest-observed-effect level (LOAEL) for parental systemic toxicity is 1500 ppm (92.43-124.33 mg/kg/day for males and 110.78-135.54 mg/kg/day for females) based on decreased body weight, weight gain, and feed consumption. The no-observed-adverse-effect (NOAEL) level is 300 ppm (23.49-31.34 mg/kg/day for males and 26.91-36.32 mg/kg/day for females).

No treatment-related effects were observed on the estrous cycle of either F_0 or F_1 females at any dose level or on percent motile sperm, sperm count, percent progressively motile sperm, epididymal sperm count, spermatid head count, daily sperm production, or efficiency of daily sperm production in F_0 or F_1 males at any dose level. There was a dose-related increase in the percentage of abnormal sperm in the treated males but no statistical significance at any dose level. No treatment-related gross or microscopic effects were observed in male or female rats of either generation. No treatment-related effects were observed on any parameter of reproductive performance including, mating and fertility indexes, gestation index, pregnancy index, precoital duration, gestation length, or number of females producing live litters.

The LOAEL for reproductive toxicity could not be established because no effects were observed at any dose level; therefore, the NOAEL is ≥1500 ppm (92.43-124.33 mg/kg/day for males and 110.78-135.54 mg/kg/day for females).

No treatment-related effects were observed on implantation sites/litter, number of live pups born/litter, number of dead pups born/litter, live birth index, sex ratio, clinical signs, or organ weight or necropsy findings in pups surviving to 21 days. Pup survival was decreased at 300 and 1500 ppm for both generations. Increased number of deaths in the F_2 generation males and females resulted in an 18-19% decrease in mean litter size on postnatal day 4 ($p<0.01$ or <0.05) and decreased viability and lactation indexes at 1500 ppm. A large number of pups that died had no milk in their stomachs. In addition, pup weight/litter and pup weight gain in the 1500-ppm group pups were reduced for both generations starting with postnatal day 4 (11-15% for F_1 and 13-23% for F_2 pups); body weight gain was reduced throughout lactation with the greatest effect occurring during the first 7 days for F_1 pups and the first 14 days for F_2 pups. Sexual maturation was delayed in 1500-ppm group F_1 offspring as evidenced by delayed balanopreputial separation in the males (+2.1 days) and vaginal patency in the females (+1.4 days). The differences remained statistically significant after adjustment for body weight decreases. Anogenital distance was significantly reduced in F_2 male pups in the 1500-ppm group, but not when the distance was adjusted for body weight.

The LOAEL for offspring toxicity was 300 ppm (23.49-31.34 mg/kg/day for males and 26.91-36.32 mg/kg/day for females) based on increased numbers of F_2 pups with no milk in the stomach and decreased pup survival. The NOAEL is 75 ppm (4.67-5.79 mg/kg/day for males and 5.56-6.41 mg/kg/day for females).

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

DP BARCODE: D276230 REREG CASE # 0080

CASE: 818954 DATA PACKAGE RECORD DATE: 01/23/02
SUBMISSION: S600197 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REREGISTRATION ACTION: 606 GENERIC DATA
CHEMICALS: 056801 Carbaryl (ANSI) %

ID#: 056801-
COMPANY:
PRODUCT MANAGER: 53 BETTY SHACKLEFORD ROOM: CS1
PM TEAM REVIEWER: ANTHONY BRITTEN 703-308-8179 ROOM: CM2 604W24
RECEIVED DATE: 07/03/01 DUE OUT DATE: 09/01/01

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 276230 EXPEDITE: N DATE SENT: 07/16/01 DATE RET.: 01/15/02
CHEMICAL: 056801 Carbaryl (ANSI)
DP TYPE: 999 Miscellaneous Data Package
CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT	ADMIN DUE DATE: 09/14/01
DIV : HED	07/17/01	01/15/02	NEGOT DATE: / /
BRAN: RRB1	07/17/01	01/15/02	PROJ DATE: / /
SECT: IO	07/26/01	01/15/02	
REVR : VDOBOZY	07/26/01	01/15/02	
CONTR:	/ /	/ /	

* * * DATA REVIEW INSTRUCTIONS * * *

Attn: V. Dobozy. MRID# 45448101. Guideline 83-4. Two generation reproduction study. Registrant submitted under 6(a)(2) -- screening results expected soon. Results of study lower NOAEL observed in previous multigeneration reproduction study by Aventis(MRID#00139647). SRRD sending new study to HED prior to 6a2 screen because study fills data gap in preliminary HED risk assessment now in Phase I. SRRD contact: Tony Britten, 308-8179.

* * * DATA PACKAGE EVALUATION * * *

No evaluation is written for this data package

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL

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DATA EVALUATION RECORD

CARBARYL

STUDY TYPE: MULTIGENERATION FEEDING - RAT [OPPTS 870.3800 (83-4)]
MRID 45448101

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
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 01-133

Primary Reviewer:
K.A. Davidson, Ph.D., D.A.B.T.

Signature: K A Davidson
Date: OCT 2 6 2001

Secondary Reviewers:
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Signature: Carol Forsyth
Date: OCT 2 6 2001

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: OCT 2 6 2001

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: OCT 2 6 2001

Disclaimer

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

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Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy **Date** 11/4/01
Reregistration Branch 1, Health Effects Division (7509C)
Branch Senior Scientist, Whang Phang, PhD: _____ **Date** 11/5/01
Reregistration Branch 1, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn. Stewart, PhD Joycelyn Stewart 11/9/2001 **Date**
Toxicology Branch, Health Effects Division (7509C)

TXR#: 0050254

DATA EVALUATION RECORD

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

PC CODE: 056801

DP BARCODE: D276230
SUBMISSION NO.: S600197

TEST MATERIAL (PURITY): Carbaryl technical (99.1% a.i.)

SYNONYMS: 1-naphthyl methylcarbamate, RPA117744

CITATION: Tyl, R., C. Myers, M. Marr. (2001). Two-generation reproductive toxicity evaluation of carbaryl (RPA007744) administered in the feed to CD® (Sprague-Dawley) rats. Reproductive and Developmental Laboratory, Center for Life Sciences and Toxicology, Chemistry and Life Sciences, Research Triangle Institute, Life Sciences and Toxicology, Research Triangle Park, NC 27709. Laboratory report number 65C-07407-400, May 24, 2001. MRID 45448101. Unpublished

SPONSOR: Adventis Crop Science, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709

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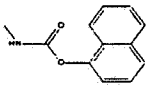
This study is **Acceptable/Guideline** and satisfies the guideline requirement for a two-generation reproductive study (OPPTS 870.3800; OECD 416) in the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test material:** Carbaryl
Description: White to light tan powder
Lot/Batch #: E12018008
Purity: 99.1% a.i.
CAS # of TGAI: 63-25-2
Structure:



2. **Vehicle and/or positive control:** none

3. **Test animals:**

Species:	Rat
Strain:	CD®[SD] IGS BR (Sprague-Dawley)
Age at study initiation:	(P) 7 wks; (F_1) 3 wks
Wt. at study initiation:	(F_0) Males: 225.7 - 276.5 g; Females: 162.7 - 203.9 g (F_1) Males: 105.1 ± 3.4 g to 130.2 ± 3.6 g; Females: 89.4 ± 2.2g to 115.6 ± 2.3 g
Source:	Charles River Breeding Laboratory, Raleigh, NC
Housing:	All animals were housed individually in solid bottom polycarbonate cages with stainless-steel wire lids except when one male and one female were co-housed during mating.
Diet:	Purina Certified Rodent Chow No. 5002 was available *ad libitum*
Water:	Tap water from the municipal supply was available *ad libitum*
Environmental conditions:	**Temperature** 18-26°C **Humidity:** 30 - 70% **Air changes:** Not reported **Photoperiod:** 12 hours dark/12 hours light
Acclimation period:	~ 1 week

B. PROCEDURES AND STUDY DESIGN

1. **Mating procedure:** One female was co-housed with one randomly selected male from the same dose group for 2 weeks or until evidence of mating (vaginal sperm or copulatory plug) was obtained. If evidence of successful mating was not observed, females were not mated to a second male. The day evidence of mating was obtained was designated as gestation day 0. Each generation was mated only one time and sibling matings were avoided. Females were housed individually after evidence of mating was obtained.

2. **Study schedule:** F_0 and F_1 females were fed control or treated diets continuously for 10 weeks before mating, throughout mating, gestation, and lactation of F_1 and F_2 litters, respectively. F_0 and F_1 males were fed control or treated diets continuously for a 10-week premating period and during the mating and postmating periods until sacrifice after weaning the F_1 and F_2 litters, respectively. F_0 rats were 17 weeks old and F_1 rats were 13-15 weeks old when mated. One F_1 male and one F_1 female pups were randomly selected from each litter (where possible) on day 21 postpartum to parent the F_2 generation. The pups were weaned onto the same diets as their parents and feeding of treated diets started immediately after weaning.

3. **Animal assignment:** F_0 rats were randomly assigned to the test groups listed in Table 1 based on body weight stratification such that the weights of all animals were within ±20% of the mean weight for each sex.

TABLE 1. Animal Assignment

Test Group	Conc. in Diet [a] (ppm)	Animals/group			
		F_0 Males	F_0 Females	F_1 Males	F_1 Females
Control	0	30	30	30	30
Low (LDT)	75	30	30	30	30
Mid (MDT)	300	30	30	30	30
High (HDT)	1500	30	30	30	30

Data taken from page 336. MRID 45448101.
[a]Diets were administered from the beginning of the study (F_0) or weaning (F_1) until sacrifice after weaning of offspring.

4. **Dose selection rationale:** The study report states that dose selection was based on a range finding study in which groups of ten male and ten female F_0 CD® rats were fed carbaryl at concentrations of 0, 100, 500, 2000, or 6000 ppm for 2 weeks before mating and during mating, gestation and lactation of F_1 litters. Ten weanlings per sex per group were fed the same diets as their parents for 2 weeks. F_0 parents had markedly reduced body weights and food consumption at 2000 and 6000 ppm, and F_1 offspring had reduced body weights and increased mortality at 6000 ppm and reduced body weights at 2000 ppm. Dietary concentrations selected for the current study were 75, 300, and 1500 ppm for the low-, mid-, and high-dose groups, respectively.

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5. **Dosage preparation and analysis:** Formulations were prepared monthly. A premix was prepared by mixing appropriate amounts of test substance (not corrected for purity) with a small amount of premix feed (ground Purina Certified Rodent Chow®) in a glass beaker. The contents of the beaker were poured into a Waring blender along with additional premix feed used to purge the beaker three times and mixed to break up lumps of test substance. The contents of the Waring blender were mixed in a stainless steel bucket along with additional premix feed used to purge the blender, layered in a Lowe (V-shell) blender with the remaining feed required to achieve the appropriate concentration, and mixed for 20 minutes. Homogeneity and stability were determined on 100- and 6000-ppm dietary preparations from the range-finding study prior to study initiation. Samples for evaluating homogeneity were taken from the left, center, and right in the blender. Samples for evaluating stability were stored in open containers at room temperature (cage-side simulation) for 0, 3-4 or 9-11 days or stored in sealed amber glass bottles at ~20°C (frozen) for 0, 9-11, 28-35, or 48-51 days. In addition, homogeneity and stability also were evaluated on samples taken from a 75-ppm dietary formulation. Homogeneity was evaluated as described above and stability was evaluated on samples stored for 0, 4-6 or 11-13 days under cage-side conditions or frozen in a sealed amber glass bottle for 0, 20-22 or 35-37 days. During the study, samples of all formulations from the first four preparation dates and for every second formulation date thereafter were verified for concentration of test substance. The animals received fresh diets every 7 days.

Results -

Homogeneity Analysis: The mean concentrations in samples taken from the right, left, and center of the blender ranged from 89.4 to 94.7% of nominal for the 75-ppm formulation, 90.8 to 105% of nominal for the 100-ppm formulation, and 98.6-99.9% of nominal for the 6000-ppm formulation.

Stability Analysis: Compared with the concentrations measured on day 0, the analytical concentration of treated diets stored under cage-side conditions ranged from 91.6% to 97.9% after 4 days and 91.2% to 106% after 10-11 days for the 75-, 100-, and 6000-ppm samples. The analytical concentrations in dietary formulations stored in the freezer ranged from 91.2% to 106% of day 0 concentration after 10-35 days (75, 100, and 6000 ppm). The 100-ppm diet stored frozen for 49 or 51 days was only 67.8-70.4% of the concentration measured on day 0, and the concentration in the 6000-ppm diet was 97.7-89.9% of the concentration measured on day 0.

Concentration Analysis: The mean concentrations of test substance in all dietary formulations were within ±10% 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.

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C. OBSERVATIONS

1. **Parental animals:** All animals were observed daily for general conditions and clinical signs and twice daily for mortality. Starting on gestation day (GD) 20, females were observed twice daily for delivery of litters. Body weights of F_0 and F_1 male and female rats were measured weekly during premating and mating. Pregnant females were weighed on GD 0, 7, 14, and 20 and females delivering litters were weighed on postnatal day (PND) 0, 4, 7, 14, and 21. Feed consumption for F_0 and F_1 male and female rats was measured weekly during the premating period, GD 0-7, 7-14, and 14-20 for pregnant F_0 and F_1 females, and PND 0-4, 4-7, 7-14, and 14-21 for lactating females. Feed consumption was not measured when the mating pairs were co-housed. Food efficiency was calculated as [(weight gain (g)/feed consumed (g)) × 100]. Body weights and clinical signs were recorded periodically during the postmating period until sacrifice of the males and the postlactational period until sacrifice of the females. Body weights and feed consumption were determined weekly until study termination for females that did not show evidence of mating.

   Vaginal smears were taken during the last 3 weeks of the premating period to evaluate the estrous cycles. Daily vaginal smears were also taken during the 2 week mating period or until mating was confirmed. Developmental milestones in F_1 rats were assessed by daily examination for the acquisition of vaginal patency in females starting on day 22 and acquisition of balanopreputial separation in males starting on day 35. The age and weight on the day of acquisition were recorded. At necropsy, the right cauda epididymides were used to assess sperm number, motility, and morphology; the right testes were used to assess the number of homogenization resistant spermatid heads and for calculation of the daily sperm production.

2. **Litter observations:** According to the report, the following litter observations (X) were made (Table 2). On the day of birth (PND 0), the number of viable and stillborn pups was determined, all pups were examined for physical abnormalities, and the live pups were sexed and individually weighed. Individual pup weights were also recorded on day 4, 7,14, and 21. On day 4, litters were culled to five of each sex, where possible, by random selection of pups to be discarded. Because of delays in acquisition of developmental milestones in F_1 rats, the anogenital distance was measured on the day of birth of all F_2 pups. Dead pups were examined grossly for external and internal abnormalities and the cause of death was determined for pups found dead, when possible.

TABLE 2. F_1 / F_2 Litter Observations

Observation	Time of observation (lactation day)				
	Day 0	Day 4	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X
Pup weight	X	X	X	X	X
Physical abnormalities	Daily				
Anogenital distance[a]	X				
Sex of each pup (M/F)	X	X	X	X	X

Data were taken from pages 26 and 27. MRID 45448101.
[a]Determined for F_2 pups only.

3. Postmortem observations:

a. **Parental animals:** Parental males were sacrificed after delivery of their respective litters and parental females were sacrificed after weaning their respective litters. All F_0 and F_1 parental animals were sacrificed by carbon dioxide asphyxiation and subjected to a detailed gross examination. The stage of the estrous cycle was determined in all F_0 and F_1 parental females on the day of sacrifice and necropsy. Male rats that died before study termination were subjected to a detailed gross examination. Gross examination of all rats consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera. The following tissues were weighed and examined histologically: ovaries, vagina, uterus with oviducts and cervix, liver, kidneys, testis (left), epididymides (left and right without cauda), seminal vesicles with coagulating gland and fluids, and prostate from ten randomly selected high-dose and control F_0 and F_1 rats. Organs exhibiting treatment-related lesions also were examined in the remaining high-dose and control animals and in the low- and mid-dose groups. Detailed microscopic examinations were conducted on the testes (and epididymides) and ovaries. The left testes were examined for evidence of abnormal spermatids or spermatogenesis and the ovaries were examined for changes in numbers of small, growing, and antral follicles and corpora lutea that could be attributed to treatment with the test substance.

Gross lesions and reproductive organs from rats that exhibited reduced reproductive performance (failed to mate, conceive, sire, or deliver healthy offspring) or fertility were also examined microscopically. The fixed uteri from females that failed to produce a litter were stained with potassium ferricyanide to confirm pregnancy status. The following organs were weighed and retained in fixative from all surviving F_0 and F_1 parental animals at scheduled sacrifices: ovaries, uterus with oviducts and cervix, pituitary, kidney, adrenal gland, epididymides, prostate, seminal vesicles, testes, liver, brain and spleen.

2) **Offspring:** Three F_1 and three F_2 weanlings/sex/litter in each dose group were randomly selected for detailed gross external and internal examinations. Gross lesions and brain, spleen, and thymus, which were weighed, were retained in fixative for possible microscopic

examination if deemed necessary. Pups that died or appeared moribund also were examined grossly. All F_1 weanlings not selected to parent the next generation or for necropsy and all F_2 weanlings not selected for necropsy were examined for external abnormalities, sacrificed by carbon dioxide asphyxiation, and discarded.

D. DATA ANALYSIS

1. **Statistical analyses:** Treatment groups were compared with concurrent controls using the methods described below.

   Except as indicated, parametric data were analyzed by Analysis of Variance (ANOVA) followed by Dunnett's test for pairwise comparisons. ANOVA was weighed according to litter size for litter data. Dunnett's test was one-tailed for all data sets except parental and pup body weights, feed consumption, sex ratios, and anogenital distance, which was two-tailed.

   Levene's test was utilized to test for homogeneity of variance; if homogeneity was rejected ($p<0.05$), then a robust regression method was used to test all treatment effects followed by pairwise comparison using Wald chi-square test Acquisition of developmental landmarks was analyzed by Analysis of Covariance (ANCOVA) using body weight at acquisition as the covariate. Frequency data such as the reproductive indices were analyzed by Chi-Square Test for independencies of differences among treatment groups by Cochran-Armitage Trend test followed by Fisher's Exact Probability Test when the Chi-Square Test was significant ($p<0.05$). A statistical test for outliers was performed for parental body weights, feed consumption, and organ weights and weanling organ weights. Outliers were not included unless there was biological reason for inclusion. Statistical significance was indicated by $p<0.05$.

2. **Indices:**

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

   Female mating index = (No. sperm positive females/No. females paired) × 100

   Male mating index = (No. males impregnating females/No. males paired) × 100

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

   Male fertility index = (No. males siring litters/No. males impregnating females) × 100

   Gestational index = (No. females producing live litters/No. females pregnant) × 100

   Pregnancy index = (No. pregnant females/No. males that mated) × 100

Offspring viability indices:

Live birth index (%) = (No. live pups at birth/Total No. pups born) × 100

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

Lactation index (%) = (No. pups alive, day 21/No. pups alive, day 4 (postcull)) × 100

3. **Historical control data:** Data were provided for body weights, body weight changes, feed consumption, reproduction parameters, and pup/litter data.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and clinical signs:** One F_0 male rat in the 300-ppm group was found dead on day 60 of the study and one escaped from the cage on day 12 and was removed from the study. The remaining F_0 male rats and all F_0 female rats survived until scheduled sacrifice. One F_1 male in the 1500-ppm group died on day 1 of the premating period and one F_1 male in the 300-ppm group died on day 92 of the study. The remaining F_1 males and all F_1 females survived until scheduled sacrifice.

   No treatment-related clinical signs were observed in F_0 or F_1 male or female rats receiving any dose of the test substance during the premating period. Clinical signs were observed with equal frequency and type in treated and control groups. No clinical sign occurred in more than three animals per group.

2. **Body weight and food consumption:** Selected mean absolute body weights and weight gain are summarized in Table 4a and 4b for the F_0 and F_1 generations, respectively. F_0 male rats fed the 1500-ppm diet weighed significantly ($p<0.01$) less than controls throughout the premating period and the last 2 weeks (days 98 and 105) of the postmating period. Differences between the treated and control group were 5-6%. Weekly weight gain during the premating period was 23% ($p<0.01$) less than that of controls from days 0-7 of treatment, 14% ($p<0.01$) less from days 14-21, 21% ($p<0.05$) less from days 35-42, and similar to controls for the remaining weeks in F_0 males fed the 1500-ppm diet. Weight gain for the entire premating period was 9% ($p<0.01$) less than that of controls. During the mating/postmating periods overall weight gain by 1500-ppm group males was 8% (no statistical analysis) less than that of controls. F_0 females fed the 1500-ppm diet weighed 4-5% ($p<0.01$ or 0.05) less than controls from day 7 to 42 of the premating period and gained 27% ($p<0.001$) less weight during the first 7 days of the study. Weight gain for the remaining weekly intervals was similar to that of the controls, and weight gain for the entire premating period was only 7% (N.S.) less than that of controls.

   F_1 males fed the 1500-ppm diet weighed significantly ($p<0.001$) less than controls throughout the premating, mating, and postmating periods: 19% less than controls on day 0, 18% less on day 7, 12% less on day 35, and 10% less on day 70, the last day of premating. During the mating/postmating periods, F_1 males in the 1500-ppm group weighed 9-10% less than controls. F_1 males in the 1500-ppm group also gained 16% ($p<0.01$) less weight from days 0-7, 11% less ($p<0.001$) from days 7-14, 20% and 15% (N.S.) less from days 21-28 and 28-35,

respectively, and 8% ($p<0.01$) less over the entire 70-day premating period. From day 14-21, F_1 males in the 300- and 1500-ppm group gained significantly more weight than controls. During the mating/postmating periods, weight gain by 1500-ppm F_1 males was not significantly different from that of controls. F_1 females fed the 1500-ppm diet weighed significantly ($p<0.01$or 0.001) less than controls throughout the 70-day premating period. The weights were 22% less on day 0, 19% less on day 7, 12% less on day 35, and only 8% less on day 70. Weight gain was 9% ($p<0.05$) less than that of controls from day 0-7, but was not significantly decreased at other weekly intervals during the premating period and was similar to that of controls for the entire premating period.

Mean weekly feed consumption was reduced by 5-8% in 1500-ppm group F_0 males during the premating, mating, and postmating periods; statistical significance was achieved at most weekly intervals (Table 4a). Overall feed consumption in the 1500-ppm group F_0 males averaged over the entire premating period was 7% ($p<0.01$) less than that of the controls. Food efficiency values for male rats fed the 1500-ppm diet were 18% less than that of controls for days 0-7, 16% less for days 35-42, and similar to that of controls when averaged over the entire premating period. F_0 females fed the 1500-ppm diet consumed 8% ($p<0.05$) less feed than controls from day 14-21, 9% ($p<0.01$) less from day 28-35, and amounts similar to that of controls when averaged over the entire premating period (Table 4a). Food efficiency for the first week of the study was significantly reduced by 26% ($p<0.001$) compared with that of the controls, but was similar to that of controls for the entire premating period.

In the F_1 generation, 1500-ppm group males consumed 11-13% ($p<0.01$) less feed than controls for days 0-7, 7-14, and 21-28, 7% ($p<0.05$) less for days 35-42, and 6% ($p<0.05$) less over the entire premating period (Table 4b). Food efficiency values for F_1 males in the 1500-ppm group were significantly less than that of controls for days 0-7 (-5% , $p<0.05$), significantly greater for days 14-21 (129% , $p<0.01$), and similar to that of controls when averaged over the entire premating period. F_1 females fed the 1500-ppm diet consumed 9-10% ($p<0.01$ or <0.05) less feed than controls for each weekly interval up to day 49 of the premating period and 9% ($p<0.01$) less averaged over the entire premating period (Table 4b). Weekly food efficiency values for 1500-ppm group F_1 females were 10-29% greater that those of controls for almost all weeks during the premating period; statistical significance was attained only at two weekly intervals. Food efficiency averaged over the entire premating period was 10% ($p<0.01$) greater than that of controls. No treatment-related effects were observed on body weights, weight gain, feed consumption, or food efficiency in 75- and 300-ppm group F_0 or F_1 male or female rats; sporadic statistically significant changes were observed at these dietary levels.

TABLE 4a. Body weight, body weight gain, feed consumption, and food efficiency F_0 rats fed carbaryl

Observations/study week	Dietary concentration (ppm)			
	0	75	300	1500
F_0 Generation Males - Premating				
Mean body weight (g)				
Day 0	253.6 ± 1.8	252.1 ± 2.0	251.3 ± 2.0	250.8 ± 2.0
Day 7	312.5 ± 2.6	310.7 ± 2.4	305.9 ± 2.1	296.0 ± 2.9** (95)[a]
Day 35	450.5 ± 6.3	442.2 ± 5.3	441.1 ± 4.7	427.7 ± 4.8** (95)
Day 70 (end of premating)	542.8 ± 8.5	540.2 ± 6.5	534.2 ± 6.2	513.1 ± 6.5** (94)
Day 105 (end of postmating)	594.9 ± 10.0	587.5 ± 7.6	582.0 ± 7.3	561.2 ± 8.4** (94)
Mean weight gain (g)				
Day 0-7	58.9 ± 1.4	58.6 ± 1.2	54.6 ± 1.3 (93)	45.2 ± 1.5** (77)
Day 14-21	44.4 ± 1.3	43.4 ± 1.3	40.4 ± 1.1* (91)	38.4 ± 0.9** (86)
Day 35-42	30.8 ± 1.6	32.1 ± 2.5	25.5 ± 0.8	24.2 ± 1.1* (79)
Day 0-70	289.2 ± 7.7	288.2 ± 5.9	283.6 ± 5.3	262.3 ± 5.8** (91)
Day 70-105[b]	52.1	47.3	47.8	48.1 (92)
Mean food consumption (g/rat/day)				
Day 0-7	25.2 ± 0.3	25.0 ± 0.4	25.0 ± 0.3	23.6 ± 0.4** (94)
Day 0-70	27.2 ± 0.4	26.7 ± 0.3	26.6 ± 0.4	25.3 ± 0.3** (93)
Day 98-105 (postmating)	27.7 ± 0.6	26.8 ± 0.5	26.8 ± 0.5	25.8 ± 0.4* (93)
Food efficiency[c]				
Day 0-7	33.4 ± 0.6	33.5 ± 0.7	31.3 ± 0.7	27.4 ± 0.7*** (82)
Day 35-42	16.1 ± 0.9	17.9 ± 1.7	13.7 ± 0.4*	13.6 ± 0.6* (84)
Day 0-70	15.4 ± 0.3	15.4 ± 0.2	15.3 ± 0.2	14.8 ± 0.2
Day 98-105	5.5 ± 0.4	4.0 ± 0.4	5.3 ± 0.5	5.0 ± 0.4
F_0 Generation Females - Premating				
Mean body weight (g)				
Day 0	184.4 ± 1.6	182.9 ± 1.6	182.6 ± 1.7	182.0 ± 1.9
Day 7	209.0 ± 2.0	207.4 ± 2.0	206.0 ± 2.1	199.9 ± 2.4** (96)
Day 35	271.6 ± 3.1	269.0 ± 3.7	264.5 ± 2.9	258.3 ± 3.7* (95)
Day 70	303.6 ± 4.5	301.3 ± 3.8	298.8 ± 4.4	292.4 ± 4.8 (96)
Mean weight gain (g)				
Day 0-7	24.5 ± 1.3	24.5 ± 1.2	23.4 ± 1.1	17.9 ± 1.0***(73)
Day 0-70	119.1 ± 4.1	118.5 ± 3.3	116.2 ± 3.3	110.4 ± 3.6 (93)
Mean feed consumption (g/rat/day)				
Day 0-7	18.9 ± 0.5	19.6 ± 0.6	18.1 ± 0.3	18.9 ± 0.6
Day 14-21	20.1 ± 0.6	19.6 ± 0.4	18.8 ± 0.3	18.4 ± 0.5* (92)
Day 28-35	20.0 ± 0.6	19.8 ± 0.4	18.6 ± 0.3* (93)	18.1 ± 0.3** (91)
Day 0-70	19.1 ± 0.4	19.1 ± 0.3	18.5 ± 0.3	18.3 ± 0.3
Food efficiency[c]				
Day 0-7	18.5 ± 0.9	18.3 ± 0.9	18.4 ± 0.8	13.7 ± 0.8*** (74)
Day 0-70	8.9 ± 0.2	8.9 ± 0.2	9.0 ± 0.2	8.6 ± 0.2

Data taken from Tables 3-4 (pp. 93-102). 6-7 (pp. 110-119). and 22–23 (pp. 155-157). MRID 45448101.

[a]Numbers in parentheses are percent of control. calculated by the reviewer.

[b]Body weight gain calculated by the reviewer using mean absolute body weights.

[c]Food efficiency = (g body weight gain/g food consumed) × 100

*p<0.05. **p<0.01. ***p<0.001. statistically significant compared with controls.

TABLE 4b. Body weight, body weight gain, feed consumption, and food efficiency F_1 rats fed carbaryl

Observations/study week	Dietary concentration (ppm)			
	0	75	300	1500
F_1 Generation Males - Premating				
Mean body weight (g)				
Day 0	130.2 ± 3.6	130.1 ± 2.6	123.4 ± 3.8	105.1 ± 3.4*** (81)
Day 7	194.7 ± 4.7	193.3 ± 3.4	182.0 ± 4.4	159.3 ± 4.6*** (82)
Day 35	388.6 ± 6.5	389.9 ± 5.5	372.9 ± 5.6	340.2 ± 5.9*** (88)
Day 70 (end of premating)	531.5 ± 6.6	535.3 ± 8.8	512.5 ± 8.9	475.8 ± 8.3*** (90)
Day 105 (end of postmating)	598.9 ± 7.1	609.5 ± 10.7	589.4 ± 9.4	541.5 ± 9.9*** (90)
Mean weight gain (g)				
Day 0-7	64.6 ± 1.4	63.1 ± 1.2	58.6 ± 1.2** (91)	54.2 ± 1.5** (84)
Day 7-14	57.8 ± 1.0	59.5 ± 1.0	56.9 ± 1.1	51.5 ± 1.3***(89)
Day 21-28	41.2 ± 4.4	34.5 ± 3.6	28.1 ± 4.4 (68)	32.8 ± 2.8 (80)
Day 28-35	55.4 ± 3.6	53.4 ± 3.2	51.8 ± 2.7	47.1 ± 1.8 (85)
Day 0-70	401.4 ± 5.5	405.1 ± 7.1	389.1 ± 8.7	370.8 ± 7.2** (92)
Day 70-105[b]	67.0	74.2	76.9	65.7
Mean food consumption (g/rat/day)				
Day 0-7	20.4 ± 0.4	20.5 ± 0.3	19.5 ± 0.5	18.1 ± 0.5** (89)
Day 7-14	24.2 ± 0.5	24.4 ± 0.4	23.5 ± 0.4	22.0 ± 0.5 ** (91)
Day 21-28	30.4 ± 0.8	28.1 ± 0.8	27.4 ± 0.7*	26.4 ± 0.9** (87)
Day 35-42	31.2 ± 0.5	31.6 ± 0.6	30.1 ± 0.6	29.1 ± 0.5* (93)
Day 0-70	28.1 ± 0.4	28.4 ± 0.4	27.5 ± 0.4	26.4 ± 0.5* (94)
Day 98-105 (postmating)	29.1 ± 0.4	29.9 ± 0.6	29.2 ± 0.6	27.6 ± 0.5
Food efficiency[c]				
Day 0-7	45.3 ± 0.5	44.1 ± 0.5	43.2 ± 0.7* (95)	42.9 ± 0.5* (95)
Day 14-21	23.4 ± 1.8	27.9 ± 1.1*	29.8 ± 1.1**	30.3 ± 0.7***(129)
Day 0-70	20.4 ± 0.4	20.4 ± 0.2	20.2 ± 0.4	20.1 ± 0.3
Day 98-105	6.6 ± 0.6	7.4 ± 0.5	7.7 ± 1.3	6.6 ± 0.4
F_1 Generation Females - Premating				
Mean body weight (g)				
Day 0	114.6 ± 2.0	115.6 ± 2.3	107.8 ± 3.2	89.4 ± 2.2*** (78)
Day 7	154.7 ± 2.4	156.0 ± 2.6	145.5 ± 3.5	125.7 ± 2.4*** (81)
Day 35	251.2 ± 3.7	255.0 ± 4.1	241.2 ± 4.2	220.0 ± 3.7*** (88)
Day 70	309.3 ± 5.3	314.3 ± 5.6	302.8 ± 6.2	283.6 ± 5.1** (92)
Mean weight gain (g)				
Day 0-7	40.1 ± 1.3	40.4 ± 1.0	37.8 ± 1.1	36.3 ± 0.9* (91)
Day 0-70	194.6 ± 5.0	198.6 ± 4.6	195.0 ± 5.7	194.2 ± 4.6
Mean feed consumption (g/rat/day)				
Day 0-7	16.7 ± 0.3	16.8 ± 0.3	16.2 ± 0.3	15.1 ± 0.2*** (90)
Day 0-70	20.5 ± 0.4	20.4 ± 0.4	20.3 ± 0.4	18.7 ± 0.3** (91)
Food efficiency[c]				
Day 0-7	34.3 ± 0.9	34.4 ± 0.7	33.5 ± 1.0	34.5 ± 0.8
Day 0-70	13.6 ± 0.2	14.0 ± 0.2	13.8 ± 0.3	14.9 ± 0.2*** (110)

Data taken from Tables 34-35 (pp. 183-192), 37-38 (pp. 200-208), and 53–54 (pp. 247-250). MRID 45448101.

[a]Numbers in parentheses are percent of control, calculated by the reviewer.

[b]Body weight gain calculated by the reviewer using mean absolute body weights.

[c]Food efficiency = (g body weight gain/g food consumed) × 100

*p<0.05, **p<0.01, ***p<0.001,statistically significant compared with controls.

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Selected group mean body weight, body weight gain, feed consumption, and food efficiency values for pregnant or nursing dams are summarized in Table 5. During gestation, F_0 females fed the 1500-ppm diet weighed 6% ($p<0.01$) less than controls on GD 20; gained 19 and 12% ($p<0.01$ or <0.05) less weight from GD 0-7 and 14-20, respectively, and 12% ($p<0.001$) less than controls averaged over the entire gestation period. Feed consumption was slightly decreased by 6% during the GD 14-20 interval only. F_0 females fed the 1500-ppm diet also had food efficiency values 19% lower than the controls during GD 0-7 and 10% ($p<0.01$) lower for the entire gestation period.

F_1 females fed the 1500-ppm diet had mean body weights 8-11% ($p<0.001$ or <0.05) lower than controls throughout the gestation period. Body weight gain was decreased by 25% ($p<0.05$), 17% ($p<0.01$), and 18% ($p<0.01$) for GD 0-7, GD 14-20, and the entire gestation period, respectively. The 12% decrease during GD 7-14 did not achieve statistical significance. F_1 females fed the 1500-ppm diet also consumed 7-10% less feed during the gestation period; statistical significance was achieved only at the GD 14-20 interval (-10%, $p<0.01$). Food efficiency was not significantly affected at any time during gestation, although a 19% decrease was reported for GD 0-7 and an 11% decrease for the entire gestation period at the 1500-ppm level.

During the lactation period, 1500-ppm group F_0 females had mean absolute body weights significantly ($p<0.01$ or <0.05) decreased by 4-7% at PND 0, 4, 7, and 21. Body weight gain by 1500-ppm group F_0 females fluctuated between greater than or less than that of controls throughout the lactation period, and statistical significance was not attained at any time.

F_1 females fed the 1500-ppm diet weighed significantly ($p<0.01$or 0.001) less than controls throughout the lactation period. Mean body weights were 9% less than controls at PND 0, 14% less at PND 4, and only 7% less at PND 21. Weight gain was markedly decreased by 94% ($p<0.01$) compared with that of controls during the first 4 days of lactation. From PND 4-7, 1500-ppm group F_1 females gained weight whereas the controls lost weight; between PND 7 and 21, the treated females either gained more weight than controls or lost less weight than controls. Consequently, weight gain averaged over the entire lactation period exceeded that of controls by 86% (N.S.). F_1 females in the 75- and 300-ppm groups also gained less weight than controls between PND 0 and 4 and either gained more weight or lost less weight for the remainder of the lactation period. F_1 females fed the 1500-ppm diet consumed 15-19% ($p<0.001$) less feed than controls during each interval as well for the entire lactation period. Food efficiency was markedly decreased in 1500-ppm group F_1 females during PND 0-4, but exceeded that of controls after PND 4. Food efficiency was significantly decreased in 75-ppm F_1 females for the PND 0-4 interval.

No treatment-related changes were observed on body weights, weight gain, feed consumption, or food efficiency for F_0 of F_1 females fed the 75- or 300-ppm diets during gestation or lactation. Although, sporadic statistically significant changes were observed for some parameters in F_1 females, they were not considered treatment related because of the lack of dose-related trends or

the lack of consistent time-related trends. Generally, differences between effect at 75 and 300 ppm and the controls were not of a magnitude considered toxicologically significant.

TABLE 5. Body weight, body weight gain, feed consumption, and food efficiency values for pregnant and lactating F_0 and F_1 rats fed carbaryl

Observation/ Gestation or Lactation Day	Dietary concentration (ppm)			
	0	75	300	1500
F_0 Generation				
Mean body weight (g)				
Gestation day 0	295.6 ± 4.6	291.7 ± 4.8	289.6 ± 4.7	285.9 ± 4.5
Gestation day 20	433.8 ± 5.4	421.9 ± 6.4	421.6 ± 5.9	406.9 ± 5.7** (94)[a]
Lactation day 0	332.8 ± 3.9	325.0 ± 5.0	321.5 ± 4.3	312.6 ± 5.3** (94)
Lactation day 4	344.8 ± 3.9	337.8 ± 4.5	336 ± 2 4.7	320.5 ± 4.9***(93)
Lactation day 7	346.9 ± 3.2	337.8 ± 5.0	333.8 ± 4.9	323.9 ± 5.1** (93)
Lactation day 21	342.2 ± 4.0	328.3 ± 3.9	334.1 ± 4.4	327.0 ± 3.7* (96)
Body weight gain (g)				
Gestation days 0-7	34.5 ± 2.3	34.4 ± 1.8	30.5 ± 1.3	27.8 ± 1.2* (81)
Gestation days 14-20	77.8 ± 2.1	72.6 ± 2.4	75.2 ± 1.9	68.1 ± 1.7** (88)
Gestation days 0-20	138.1 ± 3.3	130.2 ± 3.6	132.0 ± 2.8	121.0 ± 3.2*** (88)
Lactation days 0-21	9.3 ± 3.3	3.3 ± 5.1	12.6 ± 3.2	14.4 ± 2.9 (155)
Feed consumption (g/rat/day)				
Gestation days 0-21	63.0 ± 0.8	64.5 ± 1.0	63.9 ± 0.9	64.1 ± 0.9
Lactation days 0-21	56.3 ± 0.6	56.2 ± 1.2	57.0 ± 0.9	55.2 ± 1.0
Food efficiency[b]				
Gestation days 0-7	23.7 ± 1.7	22.6 ± 0.9	20.8 ± 0.8	19.8 ± 0.8** (81)
Gestation days 0-20	31.1 ± 0.9	29.1 ± 0.6	29.9 ± 0.5	28.1 ± 0.6** (90)
Lactation days 0-21	0.9 ± 0.3	0.2 ± 0.4	1.1 ± 0.3	1.4 ± 0.3
F_1 Generation				
Mean body weight (g)				
Gestation day 0	301.0 ± 5.3	301.5 ± 6.7	295.1 ± 6.2	276.8 ± 6.2* (92)
Gestation day 20	441.1 ± 7.2	438.7 ± 7.3	428.8 ± 8.4	392.1 ± 6.8*** (89)
Lactation day 0	339.3 ± 5.7	346.7 ± 6.0	326.9 ± 5.5	308.4 ± 5.4*** (91)
Lactation day 4	358.1 ± 5.2	355.0 ± 4.2	339.8 ± 5.5	309.5 ± 4.6*** (86)
Lactation day 21	347.7 ± 6.0	352.4 ± 4.1	335.6 ± 7.7	324.1 ± 4.1** (93)
Body weight gain (g)				
Gestation days 0-7	32.2 ± 3.8	31.7 ± 1.8	29.3 ± 1.5	24.0 ± 1.5* (75)
Gestation days 7-14	32.9 ± 1.8	34.2 ± 1.3	32.5 ± 1.5	29.0 ± 1.0 (88)
Gestation days 14-20	74.9 ± 2.0	71.3 ± 2.7	73.3 ± 2.7	62.3 ± 1.9*** (83)
Gestation days 0-20	140.1 ± 5.3	137.2 ± 4.1	134.3 ± 3.2	115.3 ± 2.6*** (82)
Lactation days 0-4	18.8 ± 2.5	8.4 ± 2.9** (45)	12.9 ± 2.0	1.1 ± 2.2** (6)
Lactation days 0-21	8.5 ± 3.7	5.7 ± 4.8	10.8 ± 6.9	15.8 ± 3.8 (186)
Feed consumption (g/rat/day)				
Gestation days 14-20	25.7 ± 0.9	25.9 ± 0.6	25.4 ± 0.7	23.1 ± 0.3** (90)
Gestation days 0-20	24.3 ± 0.9	24.9 ± 0.6	24.6 ± 0.7	22.2 ± 0.4 (91)
Lactation day 0-4	37.0 ± 1.3	34.6 ± 1.5	33.0 ± 1.2	29.8 ± 1.2*** (81)
Lactation days 0-21	61.1 ± 1.0	59.6 ± 1.5	57.4 ± 2.2	51.4 ± 1.4*** (84)
Food efficiency[b]				
Gestation days 0-7	20.1 ± 2.4	19.1 ± 0.9	18.2 ± 1.1	16.2 ± 1.0 (81)
Gestation days 0-20	29.3 ± 1.1	27.6 ± 0.8	27.5 ± 0.6	26.2 ± 0.6 (89)
Lactation days 0-4	12.4 ± 1.6	4.7 ± 2.2* (38)	9.3 ± 1.3	-0.0 ± 2.3***
Lactation days 0-21	0.7 ± 0.3	0.4 ± 0.4	0.6 ± 0.8	1.6 ± 0.4

Data taken from pages 127-130. 134-137. 215-219. and 223-226. MRID 45448101.

[a] Numbers in parentheses are the percent of control calculated by the reviewer.

[b](Body weight gain (g) / feed consumption (g)) × 100

*p<0.05. **p<0.01. ***p<0.001 statistically different from control

3. **Test Substance Intake:** Test substance intake values were calculated based on food consumption and body weight data. Doses as mean mg test substance/kg body weight/day for the premating, gestation, lactation, and postmating periods are presented in Table 6 The high intake for the lactation period is due to consumption of food by the dams and pups during the late phase of lactation. The study authors did not state whether nominal or analytical dietary concentrations were used in the calculations. In the Discussion section (page 65) of the study report, averaged carbaryl intake is given as 5-6 mg/kg/day at 75 ppm, 21-36 mg/kg/day at 300 ppm and 92-136 mg/kg/day at 1500 ppm for F_0 and F_1 parental animals.

TABLE 6. Mean test substance intake during premating (mg/kg body weight/day)

Generation	Male			Female		
	75	300	1500	75	300	1500
F_0 Generation						
Premating	4.67 ± 0.04	31.34 ± 0.28	92.43 ± 0.73	5.56 ± 0.07	36.32 ± 0.50	110.78 ± 1.46
Gestation	–	–	–	4.84 ± 0.07	31.96 ± 0.45	96.11 ± 1.30
Lactation	–	–	–	12.63 ± 0.32	85.60 ± 1.57	258.44 ± 6.37
Postmating[a]	3.54	24.03	71.00	–	–	–
F_1 Generation						
Premating	5.79 ± 0.06	23.49 ± 0.22	124.33 ± 2.05	6.41 ± 0.06	26.91 ± 0.39	135.54 ± 1.51
Gestation	–	–	–	5.19 ± 0.10	21.04 ± 0.41	102.56 ± 1.55
Lactation	–	–	–	12.64 ± 0.39	51.03 ± 1.75	244.14 ± 7.02
Postmating[a]	3.94	15.90	81.84	–	–	–

Data obtained from pages 102. 118. 130. 138. 158. 192. 208. 218. 227. and 250 in the study report. MRID 45448101.
[a] Calculated from the mean of test substance intake for days 77-105.

4. **Reproductive function:**

a. **Estrous cycle length and periodicity:** No treatment-related effects were observed on the estrous cycle length, number of females cycling, or the number of females with abnormal cycles in F_0 or F_1 females fed any dose of the test substance. The average estrous cycle length ranged from 4.35-4.77 days in treated animals to 4.44-4.59 days in control animals.

b. **Sperm measures:** No treatment-related effects were observed on the percent motile sperm, percent progressively motile sperm, epididymal sperm count, spermatid head count, daily sperm production, or efficiency of sperm production in F_0 of F_1 males fed any dose of the test substance. In the F_0 males, there was a dose-related increase in the percent abnormal sperm (1.74±0.14, 1.88±0.26, 2.56±0.78, and 5.14±3.28 at 0, 75, 300 and 1500 ppm, respectively); however the increase was not statistically significant. There was no effect in F_1 males.

5. **Reproductive performance:** Results for reproductive performance of parental animals are summarized in Table 7a (F_0) and 7b (F_1). No treatment-related effects were observed on any parameter of reproductive performance including mating and fertility indexes for both sexes, gestation index, pregnancy index, precoital duration, gestation length, or the number of females producing live litters. In general, the reproductive parameters were within range of historical control values reported by the study authors, except for the pregnancy index, which was below that of historical controls for almost all groups, including concurrent controls.

TABLE 7a. Reproductive Performance – F_0 - Litter A

Observation	Dietary Concentration (ppm)			
	0	75	300	1500
Mean (±SD) precoital interval (days)	2.1 ± 0.2	1.5 ± 0.2	2.2 ± 0.2	2.4 ± 0.3
MALES				
Number at study initiation	30	30	30	30
Number paired with females	30	30	28[a]	30
Number that mated	30	30	28	30
Number siring a litter	29	24	25	30
Intercurrent deaths	0	0	1	0
FEMALES				
Number at study initiation	30	30	30	30
Number paired with males	30	30	30[a]	30
Number that mated	30	30	30	30
Number pregnant	29	24	27	30
Number delivering live litters	29	24	27	30
Intercurrent deaths	0	0	0	0
Mean (±SD) gestation interval (days)	21.8 ± 0.1	22.2 ± 0.1	22.0 ± 0.1	22.1 ± 0.1
Number of litters produced	29	24	27	30
INDICES				
Mating index (male)	100.0	100.0	100.0	100.0
Mating index (female)	100.0	100.0	100.0	100.0
Fertility index (male)	96.7	80.0	89.3	100.0
Fertility index (female)	96.7	80.0	90.0	100.0
Gestation index	100.0	100.0	100.0	100.0
Pregnancy index	96.7	80.0	96.4	100.0

Data obtained from page 143-144 in the study report. MRID 45448101.

[a] Two males each were mated with two females. because one male escaped from the cage and was removed from the study and another died during premating.

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TABLE 7b. Reproductive Performance – F_1 - Litter A

Observation	Dietary Concentration (ppm)			
	0	75	300	1500
Mean (± SD) precoital interval (days)	3.3 ± 0.7	3.2 ± 0.4	3.3 ± 0.3	3.1 ± 0.5
MALES				
Number at study initiation	30	30	30	30
Number paired with females	30	30	30	29[a]
Number that mated	30	29	28	29
Number siring a litter	29	25	26	26
Intercurrent deaths	0	0	0	1
FEMALES				
Number at study initiation	30	30	30	30
Number paired with males	30	30	30	30[a]
Number that mated	30	29	28	30
Number pregnant	29	25	26	27
Number delivering live litters	29	25	26	27
Intercurrent deaths	0	0	0	0
Mean (±SD) gestation interval (days)	22.0 ± 0.1	22.1 ± 0.1	22.0 ± 0.2	21.9 ± 0.1
Number of litters produced	29	25	26	27
INDICES				
Mating index (male)	100.0	96.7	93.3	100.0
Mating index (female)	100.0	96.7	93.3	100.0
Fertility index (male)	96.7	86.2	92.9	89.7
Fertility index (female)	96.7	86.2	92.9	90.0
Gestation index	100.0	100.0	100.0	100.0
Pregnancy index	96.7	86.2	92.9	93.1

Data obtained from page 232-233 in the study report. MRID 45448101.

[a] One male was mated with two females, because one male died during the holding period before premating treatment began.

6. Parental postmortem results:

a. Organ weights: The absolute and relative (to total body weight) weights of the following organs in F_0 and F_1 male rats were reported in the study: liver, kidneys, adrenals, spleen, brain, pituitary, testes, prostate, and seminal vesicles with coagulating gland. The mean terminal body weight of F_0 males fed the 1500-ppm diet was 5% lower than that of controls, but not significantly. The absolute weights of all organs in each treated group were not statistically different from those of controls; however, the absolute weight of the spleen of F_0 males showed clear dose-related increases at all doses. The relative kidney weight of 1500-ppm group F_0 males was 7% ($p<0.01$) greater than that of the controls. In F_1 males, the terminal body weight was 9% ($p<0.001$) less than that of controls, and the absolute weights of the brain (-4%, $p<0.01$), pituitary (-9%, $p<0.05$), and epididymides (-5%, $p<0.05$) were

less than those of the controls at the 1500-ppm dietary level, and the relative weights of the liver, kidneys, and brain were significantly ($p<0.01$ or <0.05) less than those of the controls. The relative liver weight was slightly increased in F_1 males at 300 ppm, otherwise, no statistically significant changes were observed in males at 75 or 300 ppm.

The absolute and relative weights of the following organs were reported for F_0 and F_1 females: liver, kidney, adrenals, spleen, brain, pituitary, ovaries, and uterus. The mean terminal body weight of F_0 females fed the 1500-ppm diet was lower than that of the controls, but not significantly. Also in 1500-ppm group F_0 females, the absolute and relative liver weights were elevated by 10% ($p<0.01$) and 14%, respectively, the absolute adrenal weight was decreased by 11% ($p<0.05$), and the relative kidney weight was elevated by 6% ($p<0.05$) compared with those of controls. In F_1 females, the mean terminal body weight was decreased by 8% ($p<0.01$) and the absolute brain weight was decreased by 4% ($p<0.05$) at the 1500-ppm dietary level. Relative organ weights of F_1 females fed the test substance were not significantly different from those of the control rats. No treatment-related effects occurred at 75 or 300 ppm.

Paired ovarian follicle counts on control and high dose F_0 and F_1 females showed no treatment-related differences.

b. Pathology

1. **Macroscopic examination:** No treatment-related gross lesions were observed in F_0 or F_1 male or female rats administered any dose of the test substance.

2. **Microscopic examination:** No treatment-related microscopic lesions were observed in F_0 or F_1 male or female rats fed any dose of the test material. A common finding in males was chronic inflammation of the prostate occurring at a high incidence in all groups of both generations particularly the F_0 males.

B. OFFSPRING

1. **Viability and clinical signs:** In F_1 pups, no milk was observed in the stomach of one pup on PND 0 and two pups in one litter on day 7 at the 1500-ppm dose level. One pup in the 300-ppm group also had no milk in its stomach on day 7.

In F_2 pups, brown crustiness in the nose occurred in 3 pups in 2 litters from the control group, 20 pups in 2 litters from the 75-ppm group, 9 pups in 1 litter from the 300-ppm group, and 30 pups in 3 litters from the 1500-ppm group. The brown crustiness was observed on day 7 for all groups. Five female pups in one litter in the 300-ppm group were dehydrated on day 14. Seven pups in the one litter of the 1500-ppm group were cyanotic, lethargic, and had no milk band on day 4.

Viability data for F_1 and F_2 generation pups are summarized in Table 8. In F_1 generation litters, the mean numbers of implantations/litter, live pups born/litter, dead pups born/litter,

mean live litter size throughout lactation, and the survival (live birth, viability, and lactation) indices were not significantly affected by treatment with the test substance. In the 300-ppm group, 20 F_1 pups died (including those found dead, cannibalized, missing and presumed dead, or killed moribund) between birth and PND 4 compared with 11 controls and 15 in the 1500-ppm group. From PND 5 to the end of lactation, 16 pups in the 1500-ppm group died compared with only 2 controls. A total of 13, 7, 25, and 31 F_1 pups in the 0, 75-, 300-, and 1500-ppm groups died during lactation. The sex ratios were similar in treated and control groups throughout the lactation period.

In F_2 generation pups, the mean numbers of implantations (93% of controls, N.S.), live pups born/litter (90%, N.S.), and the total number of live pups born (91% of control, N.S.) were slightly less in the 1500- ppm group than in the control group. The anogenital distance was measured in F_2 pups because of the delays in developmental milestones in F_1 offspring. The anogenital distance in 1500-ppm group F_2 male pups (-5%, $p<0.01$) was significantly less than that of the controls, but was not different when adjusted for weight of the pups at birth. No significant effect was observed for female pups at any dose level. The sex ratios were similar in treated and control groups throughout the lactation period. The number of F_2 pups in the 300- and 1500-ppm groups that died during lactation was increased 3.6- and 4.5-fold, respectively, compared with controls from PND 0-4 and 3.5- and 3.8-fold, respectively, from PND 0-21. For F_2 pups, total of 16, 15, 57, and 69 pups in the 0, 75-, 300-, and 1500-ppm groups died during lactation. One whole litter in the 1500-ppm group was lost before PND 4, and two whole litters in the 300-ppm group were lost, one before PND 4 and one about PND 7. The increased numbers of dead pups caused the 18 and 19% ($p<0.05$) reductions in live litter size on PND 4 and the reduction in the viability index by 6 and 10 percentage points (N.S.) in the 300- and 1500-ppm groups, respectively, compared with the control group. The live litter sizes on PND 7, 14, and 21 were comparable with those of the control group. The lactation index for 300- and 1500-ppm litters was about 4 and 8 percentage points less than that of controls, but statistical significance was not achieved.

TABLE 8. Litter parameters for F_1 and F_2 generation pups

Observation/Lactation Day (PND)	Dietary Concentration (ppm)			
	0	75	300	1500
F_1 Generation				
Mean implantation sites/litter	15.07 ± 0.41[a]	14.25 ± 0.59	15.59 ± 0.43	15.53 ± 0.35
Number live pups born/litter	14.2 ± 0.5	13.4 ± 0.5	14.7 ± 0.4	14.5 ± 0.4
Number dead pups born/litter	0.1 ± 0.1	0.2 ± 0.2	0.0 ± 0.0*	0.2 ± 0.1
Sex Ratio, Day 0 (% ♂)	51.0 ± 2.2	50.2 ± 3.0	50.8 ± 2.4	46.7 ± 2.6
Number Deaths[b], Days 0-4	11	7	20	15
Number Deaths, Days 5-21	2	0	5	16
Mean live litter size				
Day 0	14.2 ± 0.5	13.4 ± 0.5	14.7 ± 0.4	14.5 ± 0.4
Day 4[c]	13.9 ± 0.5	13.3 ± 0.5	13.9 ± 0.5	14.3 ± 0.4
Day 7	9.9 ± 0.1	9.8 ± 0.2	9.8 ± 0.1	9.9 ± 0.0
Day 14	9.8 ± 0.1	9.8 ± 0.2	9.7 ± 0.2	9.5 ± 0.3
Day 21	9.8 ± 0.1	9.8 ± 0.2	9.6 ± 0.2	9.5 ± 0.3
SURVIVAL INDICES				
Live birth index	99.0 ± 0.5	98.9 ± 0.5	100.0 ± 0.0	98.3 ± 0.6
Viability index	98.4 ± 1.1	99.1 ± 0.5	95.0 ± 1.9	98.1 ± 0.8
Lactation index	99.3 ± 0.5	100.0 ± 0.0	98.1 ± 1.2	94.7 ± 2.8
F_2 Generation				
Mean implantation sites	15.86 ± 0.36	15.44 ± 0.45	15.15 ± 0.65	14.78 ± 0.48
Anogenital distance (mm), PND 0				
Male pups, unadjusted	2.11 ± 0.02	2.17 ± 0.03	2.12 ± 0.03	2.00 ± 0.02*
Male pups, weight adjusted	2.12 ± 0.02	2.14 ± 0.02	2.08 ± 0.02	2.05 ± 0.02
Female pups, unadjusted	0.96 ± 0.01	1.01 ± 0.02	1.00 ± 0.02	0.96 ± 0.01
Female pups, weight adjusted	0.96 ± 0.02	1.00 ± 0.02	0.98 ± 0.02	0.99 ± 0.02
Number live pups born/litter	15.7 ± 0.4	14.1 ± 0.7	14.0 ± 0.7	14.1 ± 0.5
Number dead pups born/litter	0.1 ± 0.1	0.4 ± 0.2	0.4 ± 0.1	0.1 ± 0.1
Sex Ratio, Day 0 (% ♂)	52.1 ± 2.1	47.1 ± 2.8	48.4 ± 3.4	51.7 ± 2.5
Number Deaths[b], Days 0-4	12	12	43	54
Number Deaths[b], Days 5-21	4	3	14	15
Mean live litter size				
Day 0	15.7 ± 0.4	14.1 ± 0.7	14.0 ± 0.7	14.1 ± 0.5
Day 4[c]	15.4 ± 0.4	13.9 ± 0.7	12.7 ± 0.9* (82)	12.5 ± 0.6** (81)
Day 7	10.0 ± 0.0	9.5 ± 0.3	8.9 ± 0.5	9.0 ± 0.4
Day 14	9.9 ± 0.1	9.5 ± 0.3	8.8 ± 0.6	9.0 ± 0.3
Day 21	9.9 ± 0.1	9.4 ± 0.3	9.1 ± 0.4	9.0 ± 0.3
SURVIVAL INDICES				
Live birth index	99.2 ± 0.7	97.8 ± 0.9	95.9 ± 2.0	99.1 ± 0.4
Viability index	98.3 ± 0.7	98.7 ± 0.5	92.0 ± 4.5 (94)	88.9 ± 3.5 (90)
Lactation index	98.6 ± 0.8	98.8 ± 0.7	94.4 ± 4.1 (96)	90.3 ± 4.3 (92)

Data taken from pages 144-150 and 233-240 in the study report. MRID 45448101.

[a]Mean ± standard deviation

[b]Number found dead, cannibalized, missing and presumed dead, or killed moribund.

[c]Precull or postcull was not specified, precull assumed.

* $p<0.05$. ** $p<0.01$, statistically different from control
(percent of control value)

2. **Body weight:** Mean body weights and weight gain are presented in Table 9a for F_1 litters and 9b for F_2 litters. F_1 and F_2 pups in the 1500-ppm group weighed significantly ($p<0.001$) less than the controls from PND 4 to the end of the lactation period. In F_1 litters, mean weights of 1500-ppm group male pups, female pups, and both sexes combined were slightly reduced by 4% compared with that of controls at birth and reduced by 11-15% at PND 4, 7, 14, and 21. In F_2 litters, mean pup weights at the 1500-ppm dose level were slightly reduced by 4-5% (N.S.) for males, females and the combined sexes at birth compared with the control weights. At 1500 ppm, F_2 pup weights were reduced by 14-23% ($p<0.01$or 0.001) at PND 4, 7, 14, and 21 compared with control weights. F_1 pups in the 1500-ppm group gained 21-23% less weight than control from PND 0-4 and 4-7, 12-13% less from PND 7-14 and 3-8% for PND 14-21, and 12-13% less for the entire lactation period. F_2 pups in the 1500-ppm group gained 26-32% ($p<0.01$) less weight than controls from PND 0-4, 4-7, and 7-14; 13-19% ($p<0.01$) less from PND 14-21; and 22-24% less for the entire lactation period.

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TABLE 9a. Mean F_1 pup weight per litter and weight gain during lactation.

Lactation Day (PND)	Dietary Concentration (ppm) 0	75	300	1500
F_1 Litters				
Mean (± S.D.) pup weight/litter (g)				
0	6.34 ± 0.10	6.68 ± 0.12	6.41 ± 0.07	6.09 ± 0.10 (96)
4	10.30 ± 0.24	10.85 ± 0.27	10.32 ± 0.24	9.14 ± 0.19** (89)
7	16.35 ± 0.32	16.87 ± 0.39	16.23 ± 0.40	13.87 ± 0.34*** (85)
14	32.21 ± 0.48	32.28 ± 0.59	31.90 ± 0.52	27.73 ± 0.50*** (86)
21	48.79 ± 0.83	49.46 ± 1.04	50.73 ± 0.84	43.36 ± 0.85*** (89)
Weight gain[b] (g)				
0-4	3.96	4.17	3.91	3.05 (77)
4-7	6.05	6.02	5.91	4.73 (78)
7-14	15.86	15.41	15.67	13.86 (87)
14-21	16.58	17.18	18.83	15.63 (94)
0-21	42.45	42.78	44.32	37.27 (88)
F_1 Pups - male				
Mean (± S.D.) pup weight/litter (g)				
0	6.51 ± 0.10	6.82 ± 0.13	6.57 ± 0.07	6.27 ± 0.11 (96)
4	10.53 ± 0.25	11.08 ± 0.29	10.53 ± 0.23	9.37 ± 0.21** (89)
7	16.67 ± 0.35	17.18 ± 0.43	16.60 ± 0.37	14.18 ± 0.40*** (85)
14	32.73 ± 0.53	32.75 ± 0.64	32.48 ± 0.55	28.30 ± 0.55*** (86)
21	50.10 ± 0.92	50.72 ± 1.15	51.87 ± 0.86	44.28 ± 0.91*** (88)
Weight gain[b] (g)				
0-4	4.02	4.26	3.96	3.10 (77)
4-7	6.14	6.10	6.07	4.81 (78)
7-14	16.06	15.57	15.88	14.12 (88)
14-21	17.37	17.97	19.39	15.98 (92)
0-21	43.59	43.90	45.30	38.01 (87)
F_1 Pups - female				
Mean (± S.D.) pup weight/litter (g)				
0	6.17 ± 0.10	6.55 ± 0.11*	6.24 ± 0.08	5.91 ± 0.10 (96)
4	10.04 ± 0.24	10.67 ± 0.27	10.03 ± 0.27	8.97 ± 0.18** (89)
7	16.02 ± 0.32	16.62 ± 0.38	15.75 ± 0.46	13.56 ± 0.32*** (85)
14	31.68 ± 0.48	31.83 ± 0.56	31.20 ± 0.58	27.14 ± 0.54*** (86)
21	47.45 ± 0.81	48.17 ± 0.94	49.43 ± 0.94	42.37 ± 0.86*** (89)
Weight gain[b] (g)				
0-4	3.87	4.12	3.79	3.06 (79)
4-7	5.98	5.95	5.72	4.59 (77)
7-14	15.66	15.21	15.45	13.58 (87)
14-21	15.77	16.34	18.23	15.23 (97)
0-21	41.28	41.62	43.19	36.46 (88)

Data obtained from pages 146-148 in the study report. MRID 45448101.

[a]Numbers in parentheses are the percent of the control values. calculated by the reviewer.

[b]Weight gain calculated by the reviewer using mean body weights.

*p<0.05. **p<0.01. ***p<0.001. statistically significant compared with the control group.

TABLE 9b. Mean F_2 pup weight per litter and weight gain during lactation.

	Dietary Concentration (ppm)			
Lactation Day (PND)	**0**	**75**	**300**	**1500**
		F_2 Litters		
Mean (± S.D.) pup weight/litter (g))				
0	6.27 ± 0.09	6.51 ± 0.10	6.58 ± 0.12	6.00 ± 0.11 (96)[a]
4	9.81 ± 0.23	10.46 ± 0.25	10.21 ± 0.33	8.46 ± 0.37** (86)
7	15.93 ± 0.36	16.84 ± 0.34	16.20 ± 0.36	12.86 ± 0.63** (81)
14	33.20 ± 0.44	34.22 ± 0.38	32.72 ± 0.57	25.58 ± 1.07*** (77)
21	50.91 ± 0.72	52.30 ± 0.76	49.68 ± 1.54	40.39 ± 1.33*** (79)
Weight gain[b] (g)				
0-4	3.54	3.95	3.63	2.46 (69)
4-7	6.21	6.38	5.99	4.40 (71)
7-14	17.27	17.38	16.52	12.72 (74)
14-21	17.71	18.08	16.96	14.81 (84)
0-21	44.64	45.79	43.10	34.39 (77)
		F_2 Pups - male		
Mean (± S.D.) pup weight/litter (g)				
0	6.43 ± 0.09	6.72 ± 0.12	6.71 ± 0.12	6.20 ± 0.12 (96)
4	10.03 ± 0.22	10.67 ± 0.27	10.33 ± 0.35	8.64 ± 0.39** (86)
7	16.34 ± 0.36	16.99 ± 0.39	16.63 ± 0.42	13.19 ± 0.66*** (81)
14	33.84 ± 0.47	34.57 ± 0.37	32.84 ± 0.62	25.96 ± 1.14*** (77)
21	52.24 ± 0.84	53.12 ± 0.79	49.93 ± 1.53	40.83 ± 1.46*** (78)
weight gain[b] (g)				
0-4	3.60	3.95	3.62	2.44 (68)
4-7	6.31	6.32	6.30	4.55 (72)
7-14	17.50	17.58	16.21	12.77 (73)
14-21	18.40	18.55	17.09	14.87 (81)
0-21	45.81	46.40	43.22	34.63 (76)
		F_2 Pups - female		
Mean (± S.D.) pup weight/litter (g)				
0	6.09 ± 0.09	6.33 ± 0.10	6.41 ± 0.12	5.81 ± 0.10 (95)
4	9.55 ± 0.26	10.21 ± 0.26	9.98 ± 0.32	8.29 ± 0.35** (87)
7	15.48 ± 0.45	16.56 ± 0.39	15.66 ± 0.36	12.56 ± 0.61*** (81)
14	32.50 ± 0.51	33.71 ± 0.52	32.19 ± 0.57	25.16 ± 1.02*** (77)
21	49.53 ± 0.77	51.39 ± 0.88	48.47 ± 1.50	39.91 ± 1.26*** (81)
Weight gain[b] (g)				
0-4	3.46	3.88	3.57	2.48 (72)
4-7	5.93	6.35	5.68	4.27 (72)
7-14	17.02	17.15	16.53	12.60 (74)
14-21	17.03	17.58	16.28	14.75 (87)
0-21	43.44	45.06	42.06	34.10 (78)

Data obtained from pages 236-238 in the study report. MRID 45448101.

[a]Numbers in parentheses are the percent of the control values. calculated by the reviewer.

[b]Weight gain calculated by the reviewer using mean body weights.

*p<0.05. **p<0.01. ***p<0.001. statistically significant compared with the control group.

3. **Sexual maturation (F_1):** Both male and female offspring in the 1500-ppm group showed delays in acquisition of developmental milestones (preputial separation in males and vaginal patency in females) (see Table 10). Acquisition of preputial separation was delayed by 2.1 days ($p<0.1$) compared with that of controls and acquisition of vaginal patency was delayed by 1.4 days ($p<0.01$). Both sexes also weighed less than controls on the day of acquisition, 7% ($p<0.01$) less for the males and 11% ($p<0.01$) less for females. Even when body weight at acquisition was accounted for, the animals at 1500 ppm still had delays in the developmental landmarks.

TABLE 10. Sexual maturation of F_1 rats

Observation	Dietary Concentration (ppm)			
	0	75	300	1500
Males				
Day of preputial separation	41.6 ± 0.3	41.5 ± 0.3	41.7 ± 0.3	43.7 ± 0.6** (+2.1 days)
Body weight (g) on day of acquisition	213.57 ± 3.13	212.70 ± 2.83	209.28 ± 3.37	198.76 ± 3.88** (93%)
Adjusted day of preputial separation[a]	41.4 ± 0.4	41.4 ± 0.4	41.7 ± 0.4	44.2 ± 0.4*** (+2.8 days)
Females				
Day of vaginal patency	30.6 ± 0.3	31.1 ± 0.4	31.1 ± 0.3	32.0 ± 0.3** (+1.4 days)
Body weight (g) on day of acquisition	104.60 ± 1.76	106.73 ± 2.96	105.65 ± 1.74	93.30 ± 2.19*** (89%)
Adjusted day of vaginal patency[a]	30.3 ± 0.2	30.6 ± 0.2	30.7 ± 0.2	33.0 ± 0.3*** (+2.7 days)

Data obtained from page 181 in the study report. MRID 45448101.
[a]Adjusted for body weight on the day of acquisition.
**p<0.01. statistically significant compared with the control group. Dunnett's test.
***p<0.001. statistically significant compared with the control group. Dunnett's test with body weight on day of acquistion as covariate

4. **Offspring postmortem results:**

A total of 86, 68, 76, and 85 F_1 male weanlings, 87, 72, 68, and 75 F_2 male weanlings, 85, 68, 79, and 83 F_1 female weanlings, and 87, 75, 68, and 75 F_2 female weanlings were necropsied.

a. **Organ weights:** At 1500-ppm, terminal body weights of F_1 weanlings were significantly ($p<0.01$) reduced by 10-11% for males and females. Absolute thymus weight was reduced by 13% ($p<0.01$) and the relative brain weight was increased by 9% ($p<0.01$) in 1500-ppm group male weanlings compared with the control organ weight. In 1500-ppm group female weanlings, the absolute thymus, spleen, and brain weights were significantly reduced by 13% ($p<0.01$), 12% ($p<0.05$), and 3% ($p<0.01$), respectively, whereas the relative brain weight was elevated by 6% ($p<0.05$) compared with that of controls.

Terminal body weights of male and female F_2 weanlings in the 1500-ppm group were reduced by 20% ($p<0.01$) and 16% ($p<0.01$), respectively. Absolute thymus, spleen, and brain weights in 1500-ppm male weanlings were significantly ($p<0.01$) reduced by 21, 24, and 5%, respectively, compared with the controls, and the relative brain weight was elevated by 21% ($p<0.01$); relative thymus and spleen weights were not affected in F_2 male weanlings. In 1500-ppm group F_2 female weanlings, the absolute thymus and spleen weights were

reduced by 16% ($p<0.01$) compared with the controls and the relative brain weight was elevated by 18% ($p<0.01$). No treatment-related effects were observed on organ weights of offspring in the 75- and 300-ppm groups.

b. Pathology

1. **Macroscopic examination:** No treatment-related effects were noted in 21-day old F_1 male or female offspring in any dose group. In F_1 offspring dying between PND 0 and 9, milk was not seen in the stomachs of 2 pups/2 litters, 7 pups/2 litters, and 2 pups/1 litter in the 0-, 300-, and 1500-ppm groups, respectively. In F_2 offspring dying between PND 0 and 9, milk was not seen in the stomach of 5 pups/2 litters, 7 pups/6 litters, 23 pups/11 litters and 31 pups/8 litters in the control, 75-, 300-, and 1500-ppm groups, respectively. The only gross findings noted in 21-day old pups was hydronephrosis in two pups each in the 75- and 300-ppm group and displaced spleen in one pup in the 1500-ppm group.

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

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS:

According to the study authors, feeding of carbaryl to male and female rats through two generations, one litter/generation resulted in decreased absolute body weights, body weight gain, and feed consumption in both generations at 1500 ppm and slightly decreased body weights and feed consumption at 300 ppm. F_1 and F_2 offspring toxicity manifested as decreased body weight beginning on PND 4 through the time of puberty at 1500 ppm. F_1 pup mortality at 1500 ppm during lactation, and F_1 and F_2 pup mortality at 300 and 1500 ppm during lactation. Delays in developmental milestones occurred in F_1 offspring at 1500 ppm. Feeding of carbaryl had no effects on any reproductive parameter. The study authors concluded that the no-observed-effect levels were 75 ppm for parental systemic toxicity, 1500 ppm for reproductive toxicity, and 75 ppm for offspring toxicity.

B. REVIEWER COMMENTS:

No treatment-related clinical signs of toxicity or mortality were observed in male and female F_0 and F_1 parental rats fed carbaryl. Body weights, body weight gain, food consumption, and food efficiency were reduced in parental F_0 and F_1 male and female rats fed the 1500-ppm diet. However, body weights were only slightly reduced in F_0 male and females and did not exceed 6% during the premating period and the postmating period for males. In F_1 male and female rats, however, body weights were markedly reduced (19-22%) at the beginning of premating but were reduced by only 9-10% at the end of premating and the end of postmating for males. Body weight gain and feed consumption for both generations showed the greatest reduction during the early phase of the premating period and only a slight effect averaged over the entire premating period. During the 0-7 day period, body weight gain was reduced

23%, 27%, 16% and 9% in the F_0 males, F_0 females, F_1 males and F_1 females, respectively. Food efficiency was significantly reduced for F_0 males and females and F_1 males during the first week of premating. Weight gain and food efficiency were not affected during the postmating period in F_0 or F_1 males at 1500 ppm; feed consumption was significantly reduced in F_0 but not F_1 males. F_0 females fed the 1500-ppm diet weighed less than controls on day 20 of gestation and all except day 14 of lactation; the difference did not exceed 7%. The 1500-ppm group F_1 females weighed significantly less throughout gestation and lactation; the difference was as great as 11% during gestation and 14% during lactation. The 1500-ppm F_0 and F_1 females gained 12% and 18% less weight ($p<0.01$ or $p<0.001$) than controls during gestation, and the F_1 females gained markedly less weight than controls during the first 4 days of lactation, but gained more weight or lost less weight than controls after day 4. Food consumption and food efficiency in F_1 females were significantly reduced during the first 4 days of lactation. No treatment-related effects occurred on body weight, weight gain, food consumption, or food efficiency in 75- and 300-ppm group F_0 or F_1 male or female parental rats at any time during the study.

The study author noted that body weights at 300 ppm were consistently lower in F_0 and F_1 parental animals. The differences between the treated and the control group did not achieve statistical significance in "most periods examined", and statistical significance was achieved on PND 4 for F_1 females. Therefore, the study authors concluded that the lower body weight at 300 ppm was biologically significant. The reviewer concludes that differences between body weights of the 300-ppm group and control parental rats are not related to treatment with the test substance or biologically significant at any time during the study. Only on rare occasions did the difference between treated and control groups exceed 5% and statistical significance was achieved only rarely (F_1 females on PND 4 (-5%, $p<0.05$)). The only statistically significant reduction (9%) in body weight gain occurred during day 0-7 of the premating period in F_1 males.

Statistically significant changes in either absolute or relative organ weights in treated rats compared with controls were due to the body weight differences and are not attributed to a direct effect of the test substance. No treatment-related macroscopic or microscopic lesions were observed in male or female rats at any dose level. Chronic inflammation of the prostate was observed in all groups of male rats in both generations.

In conclusion, **the lowest-observed-effect level (LOAEL) for parental systemic toxicity is 1500 ppm (92-124 mg/kg/day for males and 111-136 mg/kg/day for females) based on decreased body weight, weight gain, and feed consumption. The no-observed-adverse-effect level is 300 ppm (23-31 mg/kg/day for males and 27-36 mg/kg/day for females).**

Assessment of reproductive function showed no treatment-related effects on the estrous cycle of either F_0 or F_1 females at any dose level or on percent motile sperm, sperm count, percent progressively motile sperm, epididymal sperm count, spermatid head count, daily sperm production, or efficiency of daily sperm production in F_0 or F_1 males at any dose level. There wasa dose-related increase in the percentage of abnormal sperm; however, there were no statisically significant increases. Gross and microscopic examination of the reproductive

organs in treated animals revealed no treatment-related findings. No treatment-related effects were observed on any parameter of reproductive performance including, mating and fertility indexes, gestation, index, pregnancy index, precoital duration, gestation length, or number of females producing live litters.

In conclusion, **the LOAEL for reproductive toxicity could not be established because no effects were observed at any dose level; therefore, the NOAEL is ≥1500 ppm.**

No treatment-related clinical signs were observed in F_1 or F_2 pups. A large number of pups, but only three litters, had a brown crustiness in the nose at 1500 ppm compared with two litters and three pups affected in the control group. This effect is not considered treatment related. According to the viability data, feeding of the test substance had no effect on the number of implantation sites/litter, number of live pups born/litter, the number of dead pups born/litter, or the sex ratio at birth. However, the number of pups that died from PND 0-4 and from PND 5-21 was higher at 300 and 1500 ppm than in the control group. This effect on F_2 litters was much greater than on F_1 litters at both dose levels. The increased number of deaths did not affect the F_1 litter size on day 4, but it had a statistically significant effect on F_2 live litter size at 300 and 1500 ppm. The survival indexes for 1500-ppm group F_1 pups were comparable with the controls, but the viability and lactation indexes for F_2 pups were noticeably lower than those of controls. The increased numbers of pup deaths, reduced live litter size on PND 4, and the lower survival indexes indicate a treatment-related effect on pup survival.

In pups that died during lactation, the most notable gross finding was the lack of milk in the stomach (indicates lack of nursing) of F_2 pups in the 300- and 1500-ppm groups between PND 0 and 9. The lack of nursing most likely contributed to the death of these pups. The study report speculates that the increased F_2 pup losses during lactation could be related to the SDAV infection which was present in F_1 parental animals (see Study Deficiencies). This explanation does not account for the increased losses only at the mid- and high-dose levels. F_1 and F_2 pups in 1500-ppm group sacrificed on PND 21 had decreased (not all significantly) absolute thymus, spleen, and brain weights; the decreases were due to decreased absolute body weights in the weanlings and not to treatment with the test substance. F_1 and F_2 weanlings also had no treatment-related gross findings. A microscopic examination was not conducted on organs and tissues from weanlings.

Except for PND 0 (day of birth) pup weight/litter was significantly decreased for the 1500-ppm group throughout the lactation period of both generations compared with that of control. Weight gain also was significantly decreased throughout lactation, with the greatest effect occurring from PND 0-7 for F_1 litters and from PND 0-14 for F_2 pups. There was no noticeable difference between male and female pups. No treatment-related effect occurred on body weight or weight gain in F_1 or F_2 pups at 75 or 300 ppm.

Assessment of developmental milestones to determine if feeding of the test substance caused delayed sexual maturation of offspring showed that acquisition of balanopreputial separation in male and vaginal patency in female F_1 offspring was delayed. It should be noted that

growth retardation was most severe during the first week of lactation for F_1 pups and at the time of sexual maturation, the 1500-ppm group offspring were still lagging behind the controls. Even when the body weight at acquistion was acounted for, the animals at 1500 ppm still had delays in developmental landmarks. As a result in the delayed maturation in F_1 offspring, anogenital distance was measured on the day of birth for F_2 pups. A very small decrease (5%, $p<0.05$) in male pups in the 1500-ppm group was noted; however when the distance was adjusted for pup weight no effect was observed.

In conclusion **the LOAEL for offspring toxicity was 300 ppm based on increased numbers of F_2 pups with no milk in the stomach and decreased pup survival. The NOAEL is 75 ppm.**

C. STUDY DEFICIENCIES:

During the quarantine period, 4 rats/sex were designated as sentinels and were maintained under comparable conditions to animals in the study. At the time of necropsy of F_0 and F_1 parental animals, 2 sentinel animals/sex were terminated. A viral screen of serum at the time of necropsy of F_0 parental animals was negative. However, at the time of necropsy of F_1 parental animals, all 4 sentinels (2/sex) were positive for rat coronavirus/sialoacryoadenitis (RCV/SDA). At the time of this discovery, 4 remaining F_1 females had not been necropsied. Blood was taken from these animals for viral testing. Three (2 control and 1 high dose) animals were negative and one high dose female was positive. All 4 females produced large, live litters and did not show signs of RCV/SDA infection. The study report postulates that the viral translocation was from a group of rats housed in a different room which exhibited signs of SDA infection. It is likely that the SDA infection in the F_1 animals in the carbaryl study occurred around the beginning of the prebreed period and resolved prior to mating.

DATA FOR ENTRY INTO ISIS

Reproductive Study - rats (870.3800)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range ppm	Doses tested ppm	NOAEL ppm	LOAEL ppm	Target organ(s)	Comments
056801	45448101	reproductive	rats	2-generat.	oral	diet	75-1500	0, 75, 300, 1500	300	1500	decr. body wt	Parental/ systemic
056801	45448101	reproductive	rats	2-generat.	oral	diet	75-1500	0, 75, 300, 1500	75	300	decr. pup survival	Offspring
056801	45448101	reproductive	rats	2-generat.	oral	diet	75-1500	0, 75, 300, 1500	>1500	none estab.	no effects	Reproductive