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TRIFLOXYSTROBIN Reproductive Toxicity-Rat OPPTS 870.3800; OPP §83-4

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DATA EVALUATION RECORD

Study Type: Multigeneration Reproductive Toxicity
Species: Rat; Guideline: OPPTS 870.3800; OPP §83-4

EPA ID No.s: EPA MRID No. 44496710
EPA Pesticide Chemical Code 129112
EPA DP Barcode D243979
EPA Submission No. S538757

Test Material: CGA 279202 Technical
Synonyms: Trifloxystrobin

Citation: Khalil S. (1997): CGA-279202 Tech., Rat Dietary Two-Generation Reproduction Study, EPA Guideline No. 83-4, Toxicology/Experimental Toxicology Laboratory, Novartis Crop Protection, AG for Novartis Crop Protection, Inc, Test No. 943045, Novartis Nexus Number 706-97, October 20, 1997 (Unpublished Study); EPA MRID Number 44496710.

Executive Summary: In a multigeneration reproduction study (MRID# 44496710), groups of Tif: RAI f (SPF) (hybrids of RII/1 x RII/2) rats (30 per sex, per dose) from Animal Production, WST-455 of CIBA-GEIGY Limited, received 0, 50, 750 or 1500 ppm CGA 279202 Technical (Trifloxystrobin; Purity: 96.4%; Batch No.: P.405009) in the diet for two successive generations (2 litters in the first generation) [Mean intakes for the 50, 750 and 1500 ppm dose groups in mg/kg/day were 3.8 for males and 4.1 for females, 55.3 for males and 58.0 for females, and 110.6 for males and 123.1 for females, respectively, in the F0 generation; from 4.2 for males and 4.4 for females, 65.5 for males and 67.0 for females and 143.0 for males and 146.0 for females, respectively, in the F1 generation]. Maternal and paternal recordings and measurements included daily clinical observations, weekly body weights (individual pup weights on days 0, 4, 7, 14, and 21 postpartum), weekly feed consumption, mating, gestation and delivery parameters (number of viable and stillborn pups on day 0 postpartum; external sex of viable pups), pup survival and physical and behavioral developmental landmarks (righting reflex and eye opening), and gross necropsy (macroscopic pathological

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examination) and histopathological observations in organs of parental animals showing gross pathological changes, as well as representative organs from all control and high dose F0 and F1 animals.

Systemic toxicity to the parental animals included in the F0 male high dose group, statistically significant reductions from control in body weights (90% of control, $p<0.01$), body weight gains (76-89% of control, $p<0.01$ for days 1-134) and food consumption (87-93% of control, $p<0.01$) and in the F1 male mid high dose group as statistically significant reductions from control in body weights (70-93% of control, $p<0.01$) and there were reductions in the mid and high dose for body weight gains (92-96% of control) and food consumption (80-98% of control). No significant clinical signs of toxicity were noted in F0 or F1 males.

Systemic toxicity to the maternal animals during the premating period was noted in the F0 female high dose group as statistically significant reductions from control in body weights (91-96% of control, $p<0.01$). There were also reduced body weight gains in the mid and high dose groups, for the 1-68 day period (83% of control, $p<0.01$, other time periods 81-91% of control). The F1 female mid and high dose groups had statistically significant reductions from control in body weights (71-91% of control, $p<0.01$). Body weight gains were also slightly reduced for the mid and high dose groups ($p<0.05$ for overall mid dose group). Food consumption was statistically significantly reduced in the mid and high dose groups (except for the last 2 weeks, 79-93% of control, $p< 0.01$).

Systemic toxicity to the maternal animals during the gestation period was noted in the F0 female mid and high dose groups as statistically significant reductions from control in body weights (88-95% of control, $p<0.05$-0.01) (both gestation periods). Body weight gains were slightly reduced in the mid and high dose groups for the 1st gestation period and were more affected in the 2nd gestation period (89-90% of control, $p<0.01$ for the high dose). Food consumption was reduced in the high dose group for the 2nd gestation period (91-95% of control, $p<0.05$-0.01). The F1 female mid and high dose groups had statistically significant reductions from control in body weights (83-92% of control, $p<0.01$). Body weight gains for the mid and high dose groups were also reduced (81-98% of control; for the 0-21 day period, $p<0.05$-0.01). Food consumption for the mid and high dose groups was reduced (86-99% of control, $p<0.05$-0.01).

Systemic toxicity to the maternal animals during the lactation period was noted in the F0 female high dose group as statistically significant reductions from control in body weights (87-93% of control, p< 0.05-0.01) (both lactation periods). Food consumption values were reduced in the high dose group. The F1 female mid and high dose groups had statistically significant reductions from control in body weights (82-95% of control, p<0.01) for the lactation period. Food consumption values were slightly affected in the in the high dose group (85-90% of control).

Other systemic toxicity to the parental animals included a minimal to moderate hypertrophy of centrilobular hepatocytes in F0 animals of both sexes in the 1500 ppm dose group (males,10/30 and females, 5/30) and in F1 animals of both sexes in the 750 (males, 14/30 and females, 7/30) and 1500 ppm (males, 24/30 and females, 9/30) dose groups. There was also a slightly increased incidences of a minimal pigmentation of renal tubules in 750 ppm F0 males (7/30) and F0 animals of both sexes in the 1500 ppm dose group (males, 3/30 and females, 4/30). There were also decreased incidences of splenic hemosiderosis in F0 and F1 animals both sexes of the 750 (F0 males, 12/30 and F0 females 15/30; F1 males, 5/30 and F1 females, 17/30) and 1500 ppm dose groups (F0 males, 9/30 and F0 females, 8/30; F1 males, 2/30 and F1 females, 14/30). There were no treatment related findings in the reproductive system of animals of either sex. **The Parental (Paternal/Maternal) Systemic Toxicity NOAEL was 50 ppm (3.8-4.2 mg/kg/day for males and 4.1-4.4 mg/kg/day for females) and the Parental (Paternal/Maternal) Systemic Toxicity LOAEL was 750 ppm (55.3-65.5 mg/kg/day for males and 58.0-67.0 mg/kg/day for females) based on reduced body weights, body weight gains, reduced food consumption and liver, renal and spleen histopathological observations.**

Systemic/developmental toxicity was noted as decreased F1a, F1b and F2 pup body weights in the mid and high dose pups at lactation days 7, 14 and 21. There was a slight increase in time to eye opening in the high dose group in both F1a, F1b and F2 litters. **The Offspring Systemic/Developmental Toxicity NOAEL was 50 ppm (3.8-4.2 mg/kg/day for males and 4.1-4.4 mg/kg/day for females) and the Offspring Systemic/Developmental Toxicity LOAEL was 750 ppm (55.3-65.5 mg/kg/day for males and 58.0-67.0 mg/kg/day for females) based on decreased pup body weights during lactation.**

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No effects were noted on reproductive parameters. **The Reproductive Toxicity NOAEL is equal to or greater than 1500 ppm and the Reproductive Toxicity LOAEL is greater than 1500 ppm.**

This study is classified as Acceptable-Guideline and satisfies the guideline requirements (OPPTS 870.3800, OPP §83-4) For a multigeneration reproduction study in rats.

Compliance: A signed and dated Statement of No Data Confidentiality Claims, a Certification of Good Laboratory Practices along with a certification of GLP and Verification of the Report and Statement of Compliance with Good Laboratory Practices, an EPA Flagging Statement (the study neither met nor exceeded applicable criteria), and a Quality Assurance Statement were provided.

Basis for the Protocol

OECD Guidelines for Testing of Chemicals, Section 4: Health Effects, No. 416 "Two-Generation Reproduction Toxicity Study", May 26, 1983.

European Communities Commission Directive 87/302/EEC, "Two- Generation Reproduction Toxicity Test", OJ No L133/47, November 18, 1987.

FIFRA Pesticide Assessment Guidelines, Subdivision F, Series 83-4 "Reproductive and fertility effects", November 1984.

Japanese Ministry of Agriculture, Forestry and Fisheries, 59 NohSan No. 4200, "Reproduction Study", January 28, 1985.

The study was conducted according to GLP Principles, as specified in "Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles", Swiss Federal Department of the Interior and Intercantonal Office for the Control of Medicaments, March 1986. The Swiss GLP Regulations are based on the OECD Principles of Good Laboratory Practice (Council Decision 81/30, adopted May 1981), and are compatible with U.S. EPA 40 CFR 160 (FIFRA), August 1989; U.S. EPA 40 CFR 792 (TSCA), August 1989; and Japan MAFF 59 NohSan No. 3850, August 1984.

THIS REVIEW CONTAINS TEXT INFORMATION SCANNED FROM THE STUDY REPORT BY THE REVIEWER INTO ELECTRONIC FORMAT (USED IN MATERIALS AND METHODS, STUDY DESIGN AND CONCLUSIONS-INVESTIGATORS SUMMARY SECTIONS).

A. Materials and Methods

Test Compound: CGA 279202 Technical (Trifloxystrobin)
Purity: 96.4%
Description: light brown powder
Batch No.: P.405009
Stored at room temperature

Test Animal(s):
Species: Rat
Strain: Tif: RAI f (SPF), hybrids of RII/I x RII/2
Source: Animal Production, WST-455
CIBA-GEIGY Limited
4332 Stein, Switzerland
Age: 5 to 6 weeks at start of dosing
Body Weight: 185.5-186.1 g for males, 146.4-147.2 g for females on study day 1.
Additional information: According to the investigators: Rationale for stock selection: This stock is an outbred cross between two genetically stable inbred Sprague-Dawley derived strains. It is used for all study types at this facility and has high fecundity.

Husbandry (Scanned from pages 32-33 of the study report)

The study was conducted under optimal hygiene conditions (OHC). The animals were housed individually (except during mating) in Macrolon cages with wire mesh tops and standardized granulated soft wood bedding material (Societie Parisienne des Sciures Pantin, Paris, France), in the following environment:

Temperature (°C):	22 ± 3
Relative Humidity (%):	50 ± 20
Ventilation:	about 16 air changes/hour
Light Cycle:	12 hours of light per day (6 a.m.-6 p.m.)

Food and Water

Pelleted, certified standard feed (Nafag No. 8900 for GLP; Nafag, Naehr und Futtermittel AG, Gossau, Switzerland) were provided ad libitum. All batches of feed were analyzed for composition and contaminant levels. Tap water was provided ad libitum in plastic bottles. The water quality was routinely checked to standard specifications. See Appendix 2 [of the study report] for quality specifications of feed and water.

Pre-test Acclimation

After delivery from the in-house animal production facility, F0 animals were housed individually in Macrolon Type 3 cages in the animal room for at least seven days before treatment began. Cageside observations were made daily, and body weights recorded four or five days after arrival. Animals which did not

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appear healthy or did not adequately gain weight were not placed on study.

B. Study Design

According to the investigators (from page 27 of the study report):

This study was designed to obtain general information concerning the effects of dietary administration of the test substance on male and female reproductive performance, such as gonadal function, mating behavior, conception, parturition, lactation, weaning, and on the growth and development of the offspring.

Experimental Design Summary (scanned from page 28 of the study report)

Young adult virgin male and female rats (30 animals per sex and dose) were continuously exposed to the test substance in the diet. After 10 weeks exposure, the FO animals were cohabited 1:1 within each dose group for up to 19 days. After weaning of the Fla litters, pairs were remated for up to 19 days to produce F1b litters.

Selected Fla young (30 animals per sex and dose) were continuously exposed to the test substance in the diet as in the FO generation. After 10 weeks exposure, these Fla animals were cohabited 1:1 with in each dose group for up to 19 days. Resulting (F2) litters are necropsied after weaning.

Clinical observations, body weights, food consumption, mating, gestation and delivery parameters, pup survival and physical developmental landmarks, and gross necropsy and histopathological observations in sex and target organs of mating animals, were recorded.

Time Schedules

Dates of all study events are given below in the "Procedure section, under "Chronology". The major milestones of the study were as follows:

Arrival of Animals:	January 30, 1995
Start Acclimation:	February 13, 1995
Start Dosing FO Generation:	February 20, 1995
Start Necropsy FO Parents:	August 25, 1995
Start Necropsy F1 Parents:	October 30, 1995
Experimental End Data:	August 12, 1997

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Diet preparation: (scanned from pages 30-31 of the study report)

Administration Route and Dose Levels

The test substance was applied by admixture to the diet. The following dietary dose concentrations were selected for this study, based on a previous rangefinding rat dietary reproduction study Number 943044: 0, 50, 750 and 1500 ppm (= mg test substance/kg diet)

Preparation and Storage of Feed Admixtures

Ground feed pellets were mixed with the appropriate quantity of test substance, hydrated and repressed to pellets.

Pelleted feed admixtures were stored at room temperature in unopened sacks, or in polyurethane or stainless steel bins with dust-tight lids.

Content Analyses

In order to verify the stability of the test substance in feed mixtures after storage at room temperature for five weeks and content under actual conditions of administration, test substance content, homogeneity and stability were measured in the pellet samples as shown in the next table.

Samples were frozen at approximately -200C and transported in iceboxes with dry ice to the analytical laboratory, together with approximately 500g of untreated diet and 2g of test substance.

The results of these analyses are given in Appendix 1 [of the study report].

Dates of Preparation and Sampling for Analysis

Fresh batches of diet mix were prepared and pelleted on the following dates (format day.month.year):

Batch	Preparation □Date	Feeding Date	Chemical Analysis
1	13.02.95 - 17.02.95	20.02.95 - 20.03.95	C/H/S
2	13.03.95 - 17.03.95	20.03.95 - 17.04.95	C
3	10.04.95 - 13.04.95	17.04.95 - 15.05.95	
4	08.05.95 - 12.05.95	15.05.95 - 12.06.95	C
5	05.06.95 - 09-06.95	12.06.95 - 10.07.95	
6	03.07.95 - 07.07.95	10.07.95 - 14.08.95	C
7	07.08.95 - 11-08.95	14.08.95 - 04.09.95	
8	28.08.95 - 01-09.95	04.09.95 - 02.10.95	C
9	25.09.95 - 29-09.95	02.10.95 - 30.10.95	
10	23.10.95 - 27.10.95	30.10.95 - 17.11.95	C

C = Analysis of content
C/H/S = Analysis of content, homogeneity and stability
Analyses:
- content (C): analyses of B pellets (all doses)
- homogeneity (H): content analyses of A, B and C pellets (200g, all doses from the first batch)
- storage stability (S): content analysis of B pellets after

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storage for 5 weeks at room temperature (200g, from all dose groups from the first batch)

From Appendix 1, pages 278 of the study report, the analysis of the pellets showed that the test chemical was stable at room temperature for 35 days in the rat feed and the homogeneity ranged from -3% to +3% of the mean concentrations. The overall mean concentrations were 91.8, 93.8 and 96.8% for the 50, 750 and 1500 ppm dose groups, respectively.

Mating and Mating Schedule: (scanned from pages 37 and 35 of the study report)

Mating Procedure

Each female was placed with a single male from the same dose group. Sibling matings were avoided. In order to achieve 30 F1 mating pairs from less than 30 F1 litters per dose group, extra males and females were selected from randomly chosen litters (one male or female per litter). See Appendices 43, 50 and 83 for listings of the FO 1st mating, FO 2nd mating and F1 mating pairs, respectively.

Each mating pair was housed in a single macrolon Type 3 cage, separated by an aluminum divider containing a closed sliding door. Pairs were so cohabited from Thursday or Friday until midnight Sunday, when restricted daily mating access was allowed by programmed automatic opening of the door. Beginning at 6.30 a.m. the following morning, females were examined for presence of vaginal plugs or sperm in a vaginal smear (=positive mating). The day positive mating was observed was designated day 0 post coitum (p.c.). Positively mated females were removed from the mating cage and housed individually; for the remaining females, the door was closed to separate the mating pair and the restricted daily mating access procedure repeated up to a maximum of 19 days.

Selection of the F1 Generation

The F1 generation animals were selected from F1a litters. To maintain as large a genetic pool as possible, one male and one female pup were randomly selected from as many different F1 litters as possible. Where less than 30 litters were available, additional animals were selected on random basis, with a maximum of one additional male or female from a litter.

Dosing and Breeding Schedule

Dosing was continuous up to necropsy in all animals. The sequence of events in this study was as follows:

Study Week	FO Generation	F1 generation	F2 generation
1-10	FO premating period		
11-13	FO first mating period, gestation of F1a		
14-16		F1a born; litters culled	

		to 8 pups	
17-19		F1a weaned	
20		F1a selected for mating and remainder necropsied	
21-23	FO second mating period; gestation of F1b		
24-26		F1b born; Litters culled to 8 pups	
27-29	FO male necropsy (week 28)	F1b weaned, necropsy	
30	FO female necropsy		
20-29		F1 premating period	
30-32		F1 mating period; gestation of F2	
33-35			F2 born; litters culled to 8 pups
36-38		F1 male necropsy (week 37)	F2 weaned, necropsy
39		F1 female necropsy	

Animal assignment: (scanned from pages 33-34, and 36 of the study report)

Allocation to Groups and Identification

Healthy FO animals were allocated to treatment groups by a computer-generated randomization schedule with stratification by body weight (animals are ranked by weight and then assigned in blocks of N to groups 1 to N). This procedure ensures random assignment to groups, while keeping body weight differences between groups to a minimum.

After allocation to treatment groups, animals were individually identified by small numbered metal ear tags and were placed one per cage in Macrolon Type 3 cages. Each adult animal on study is assigned an unique number (ear tags) and cage, and group allocation was indicated by a colored label, as follows:

Group	Males Nos.	Female Nos.	Label Color
1: Control	001-030	121-150	blue
2: Low Dose	031-060	151-180	green
3: Mid Dose	061-090	181-210	yellow
4: High Dose	091-120	211-240	red

These animal numbers are prefixed by a five character code (F0-00 or F1-00) indicating the generation, e.g. F1 control female F1-00121.

Each cage label showed the study number, animal number (=cage number), test substance code, dietary dose level (ppm), mating period, expected delivery day, and dates of treatment and necropsy.

Computer data for the three matings are identified by alphabetical suffix to the study number, as follows:

- F0 (1. mating): Study ID Nr. 943045A
- F0 (2. mating): Study ID Nr. 943045B
- F1 (mating): Study ID Nr. 943045C

Chronology

Start Acclimation:	February 13, 1995
Start Dosing F0:	February 13, 1995
Start first Mating F0:	May 1, 1995
Start Delivery F1a Litters:	May 22, 1995
Start Necropsy F1a Litters:	June 28, 1995
Start second Mating F0:	July 10, 1995
Start Delivery F1b Litters:	August 1, 1995
Start Necropsy F1b Litters:	August 22, 1995
Start Necropsy F0 Males:	August 25, 1995
Start Necropsy F0 Females:	September 11, 1995
Start Mating F1:	September 11, 1995
Start Delivery F2 Litters:	October 3, 1995
Start Necropsy F2 Litters:	October 24, 1995
Start Necropsy F1 Males:	October 30, 1995
Start Necropsy F1 Females:	November 13, 1995

Observation Schedule

Parental animals: (scanned from pages 37-42 of the study report)

Mating and Gestation Indices

Indices are defined as follows:

Female mating:	No. of females positively mated as % of No. of females used for mating
Female fertility: (=fecundity)	No. of females pregnant as % of No. of females mated
Male mating:	No. of males with positive mating as % of No. of males used for mating

Male fertility: No. of males inducing pregnancy as % of
No. of males with positive mating

Parturition: No. of females with births as % of
No. of females with confirmed pregnancy

Gestation: No. of females with liveborn as % of
No. of females with confirmed pregnancy

Adult Observations and Measurements

The following were recorded:

Clinical Signs and Mortality: daily (a.m.; repeated p.m. if signs observed)

Body Weight: weekly

Food Consumption: weekly except during cohabitation for mating

Mean daily food consumption per animal was calculated according to the following formula:

$$\frac{\text{food consumption (g) per period}}{\text{days per period}}$$

Mean daily Substance intake per animal (mg/kg body weight/day) was calculated according to the following formula:

$$\frac{\text{daily food consumption (g) X dose level}}{1000}$$

Adult Necropsy

All animals were bled under ether anesthesia and the exsanguinated body weight recorded. Animals were then subjected to a complete macroscopic pathological examination, with special attention directed to the organs of the reproductive system. In females not producing offspring, uteri were stained by the Salewski method [1] to establish whether pregnancy had occurred ("pregnant by stain").

Macropathology

In all adult animals, including non-pregnant animals and those dying before scheduled sacrifice or killed in moribund condition, the following organs, or representative samples thereof, were examined for gross pathological changes and preserved in neutral buffered 4% formalin. Underlined organs were weighed in animals killed at scheduled sacrifice.

- vagina
- Uterus
- Ovaries
- Testes
- Epididymides
- Seminal vesicles
- Prostate
- Pituitary gland
- Skin
- Mammary area

- Spleen
- Mesenteric lymph node
- Axillary lymph node
- Popliteal lymph node
- Sternum with bone marrow
- Femur with joint
- Skeletal muscle
- Trachea
- Lung
- Heart
- Aorta
- Submandibular salivary glands
- Liver
- Pancreas
- Oesophagus
- Stomach
- Small intestine
- Large intestine
- Kidneys
- Urinary bladder
- Adrenals
- Thyroid with parathyroid
- Thymus
- Peripheral nerve
- Brain
- Spinal cord
- Eyes with optic nerves
- Orbital glands
- Extraorbital lacrimal glands
- Zymbal glands
- Muzzle
- Tongue
- All gross lesions

Histopathology

Full histopathological examination was performed on the following organs/tissues, or representative samples thereof, in all control and high dose F0 and F1 animals selected for mating:

- Vagina
- Uterus
- Ovaries
- Testes
- Epididymides
- Seminal vesicles
- Prostate
- Pituitary gland
- Liver
- Pancreas
- All gross lesions

Changes in liver, spleen and kidney weights were noted in males and females of both generations. To clarify these changes these organs were examined histopathologically in males and females (F0 and F1) of control and all treated groups.

Animal Numbering for Macro- and Histopathology

Because of differences in computer requirements in the pathology group, the following differences should be mentioned:

1. the study numbers for pathology data were as follows:

F0 Generation: Pathology Substudy Number 950004

F1 Generation: Pathology Substudy Number 950005

2. Moribund sacrificed recorded for animal no. FO-00107 in pathology report should be read as elected sacrificed. This animal had a palpable mass and was not in a moribund condition.

Observation of Delivery

Any signs of difficult or prolonged parturition were recorded. Day 0 post partum is defined as the day on which all viable pups have suckled. If labor commences on a given day, but not all viable pups present have suckled by 3 p.m. on that day, then day 0 post partum is taken as the next day. In this case, the number of live and/or dead pups already delivered and any abnormal signs are recorded on the day delivery commences.

Offspring Identification

Suckling-young were identified by maternal cage-card, and skin marked by sex*on day 4 postpartum.

Offspring Survival, Clinical Signs, Body Weights

Litter Size & Sex:	number of viable and stillborn pups on day 0 post partum; external sex of viable pups
Mortality:	daily from day 0 post partum
Clinical Signs:	daily (a.m.; repeated p.m. if signs observed)
Body Weight:	individual pup weights on days 0, 4, 7, 14, and 21 post partum

Behavioral Reflex and Developmental Landmarks

The following were tested in all litters (i.e. F1a, F1b, F2):

Righting Reflex:	Number of pups per litter with all four feet on the ground within 30 seconds of being placed on the back; tested once daily from day 2 post partum to 100% occurrence
Eye Opening:	Number of pups per litter with both eyes open; examined once daily from day 14 post partum to 100% occurrence

Standardization of Litter Sizes

On day 4 post partum, both F1a and F1b litters of F0-generation and F2 litters with nine or more pups were culled by random selection to yield, as nearly as possible, four males and four females per litter. Culled pups were killed by decapitation and subjected to gross necropsy.

Litter Indices

Live Birth:	Mean no. of pups born alive per litter as % of mean no. of pups born per litter
Viability:	Mean no. of pups alive on day 4 (pre-culling) per litter as % of mean no. of pups born alive per litter
Lactation:	Mean no. of pups alive on day 21 per litter as % of mean no. of pups alive on day 4 (post-culling) per litter

Offspring Necropsy

F1a pups not selected for mating, F1b pups and F2 pups were killed on or shortly after weaning of the last litter of that mating and subjected to gross necropsy.

Gross necropsy was conducted on all these pups, and on all pups culled, found dead or killed moribund. Culled pups were killed by decapitation. The gross necropsy examination consisted of a macroscopic examination of the body, limbs, and organs of the thoracic and abdominal cavities, with special attention to the organs of the reproductive system.

Statistical Analyses (scanned from pages 42-43 of the study report)

Statistical Analyses and Data Collection

Statistical analysis of continuous data (e.g. body weight, food consumption) was performed using the Analysis of Variance Procedure (ANOVA) [2] (reference supplied) followed by Dunnett's t-Test [3] (reference supplied) in case of a significant result in the ANOVA.

Categorical data (e.g. malformation counts) were analyzed using Chi-Square test [4] (reference supplied) followed by Fisher's Exact test [5] (reference supplied) in case of a significant result in the Chi-Square test.

Non-parametric data (e.g. mean percent affected fetuses/litter) were analyzed using the Kruskal-Wallis nonparametric analysis of variance test [6] (reference supplied) followed by Mann-Whitney U-test [7] (reference supplied).

In all summary tables with statistics, the p value for the blocking test (ANOVA, Chi-square or Kruskal-Wallis) is given in the control column. P values for subsequent comparisons against controls (Dunnett's, Fisher's Exact or Mann-Whitney U) are given in the appropriate group column, if the blocking test is significant.

The statistics used are indicated by footnotes in the tables. No statistics were performed when the number of observations was insufficient (normally n<2).

Data were collected by hand and on a Digital Equipment Corporation (DEC) VAX computer with SCC Reprotoxicology System software (Scientific Computer Consultants Inc., Ringwood, NJ 07456, USA; customized for CIBA-GEIGY Reproduction Toxicology Stein by SCC). Validation Certification of the SCC Reprotoxicology System was issued by Weinberg Associates Inc., Boothwyn, PA 19061, U.S.A. (Project Code 91041, December 1991).

The SCC Reprotoxicology System is protocol driven and allows authorized personnel to create a study protocol, including related work schedules, diets and dosages. The system prompts for appropriate data input. Weight data are input directly from balances to the on-line database.

This report, consisting of text, figures, and formatted SCC tables, was produced with LEX-WP and LEX-GRAPH software (Ace Microsystems Ltd., London W5 4EH, England) running on a DEC VAX computer.

Censoring of Data

Only pregnant animals with a defined day of mating are included in the gestation summary tables (clinical observations, body weights, food consumption). Pregnant animals without a defined day of mating are excluded from gestation summary tables, but are included in lactation summary tables. only animals with a defined date of giving birth are included in lactation data tables.

Calculation of Pup Developmental Landmark Indices

This is the average of the day on which each pup in the litter reached

criterion: pups which did not reach criterion are not included in the analysis.

e.g. 11 of 17 pups performed the righting reflex test on days 2 and 3; one pup died after testing on day 3, and 14 of the 16 remaining pups performed the test on day 4, which was the last day of testing. The average age to achievement of this developmental landmark for this litter is 2.43 days, and it was achieved by 82.4% of pups in the litter.

Additional Information: (scanned from page 44 and 62 of the study report)

Deviations from the Protocol

The following deviations from the protocol were not covered by protocol amendments:

The company Novartis Crop Protection AG has resulted from the merger of the companies Ciba-Geigy Ltd. and Sandoz Ltd. and is partial successor in business from above-named companies. This applies also to all aspects concerned with the requirements of Good Laboratory Practice.

The name of the test facility was changed from "Short/Long-term Toxicology" to "Toxicology/Experimental Toxicology"

Dr. W. Gfeller assumed responsibilities as facility manager by January 1997.

Dr. E. Puri, cited in the protocol as sponsor. As from January 1, 1996, Dr. Th. Hertner took over the responsibility for studies being conducted with CGA 279202.

Protocol page 5 ("Personnel' I), designated M. Rauch as responsible for Macropathology; she left the facility on December 31, 1996. Ch. Wurmlin is responsible for necropsy.

Protocol page 1 ("Sponsor") describes the Division as "Crop Protection Division"; the title is now "Crop Protection, Human Safety Assessment", as shown in this report.

Due to technical errors during necropsy, heart weights were not recorded for both F0 and F1 generations.

Protocol page 4 dates the FIFRA guideline as November 1982; this should read November 1984.

Protocol Page 9 "Feed and Water" incorrectly gave the Nafag No. as 890; the correct Nafag No. is 8900 as shown in the method section of this Report (page 29, Feed and Water).

For logistical reasons, start necropsy of F0 generation was in fact on August 25, 1995 and not on August 28, 1995 as specified in study protocol.

There deviations were not considered to have affected the integrity of the study. **[The reviewer agrees with this conclusion]**

References

[1] Salewski E., Naunyn-Schmiedebergs Arch. Ex. Path. Pharmakol. 247, 367, 1964.

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[5] Dixon W.J., Fisher's Exact Probability, in: BMDP Statistical Software, University of CaliFornia Press, 1981, p. 663

[6] Kruskal W.H. and Wallis W.A., J. Am. Stat. Assoc. 47, 583-621, 1952

[7] Mann H.B. and Whitney D.R., Ann. Math. Stat. 18, 50-60, 1947

NOTE FROM THE REVIEWER: THE PROTOCOL DESCRIBED ABOVE IN THE MATERIALS AND METHODS SECTION IS ACCEPTABLE TO FULFILL THE INFORMATION SUGGESTED BY THE GUIDELINE OPPTS 870.3800; OPP §83-4.

C. REPORTED RESULTS

Parental animals

Mortality and clinical signs:

One F0 50 ppm female was sacrificed moribund on the day of birthing. This animal had 6 stillborn pups and 9 live pups which she did not nurse. Also, one F0 1500 ppm male was sacrificed on study day 82 due to a palpable mass noted in the abdomen and one F1 750 ppm male was sacrificed moribund on study day 85. No other F0 and no F1 mortalities were reported. No other clinical signs related to treatment were noted in F0 or F1 animals.

Body weight and Food consumption:

The following Table I (from Tables 15-16, 23-24, and 30-31; pages 115-118, 126-129 and 136-138 of the study report) presents selected body weight and food consumption data for F0 males (all values means ± standard deviation if available):

Table I: Body Weights, Body Weight Gains and Food Consumption

Dose:	Control	50 ppm	750 ppm	1500 ppm	
		Body Weights (g)			
Day 1	185.6±17.4	185.6±17.6		186.1±16.9	
185.5±18.1(100)[2]					
Day 43	402.9±30.4	406.0±32.5		395.2±32.7	(98)
378.5**±31.8(94)					
Day 85	464.4±38.2	466.7±39.4		458.2±39.0	(99)
424.1**±38.1(91)					
Day 127	514.7±46.9	517.1±48.3		503.6±45.2	(98)
464.7**±42.0(90)					
Day 190	544.4±46.0	561.6±57.6		545.9±51.3	
497.6**±45.8(91)					
		Body Weight Gains (g)			
Days					
1-43[1]	217.3	220.4		209.1(96)	193.0(89)
1-85[1]	278.8	281.1		272.1 (98)	
238.6(86)					
1-127[1]	329.1	331.5		321.2	
281.8(86)					
1-134	331.9±41.3	335.7±38.8		321.2±37.9	(97)
282.7**±30.8(85)					
1-190[1]	358.8	376.0		359.8	

312.1(87)				
141-183	33.0±12.1	32.4±12.1	31.8±11.6(96)	25.2*±10.8(76)
		Food Consumption (g/animal/day)		
Days				
1-8	20.5±1.8	20.8±2.2	18.7**±1.9(91)	17.9**±2.1(87)
36-43	26.9±2.5	27.6±2.6	25.7±2.2(96)	24.9**±2.1(93)
92-99	23.9±2.5	24.3±2.7	22.8±2.1(95)	22.1**±2.1(93)
120-127	23.2±2.4	23.0±2.5	23.1±2.2	22.4±2.3(97)
183-190	23.2±2.7	24.6±2.6	24.7±1.9	23.6±2.6

1 = calculated by the reviewer from body weight mean values; ² = percent of control; * = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.

The F0 male high dose group had statistically significant reductions from control in body weights (90% of control, p<0.01), body weight gains (76-89% of control, p<0.01 for days 1-134) and food consumption (87-93% of control, p<0.01).

The following Table II (from Tables 67, 71 and 75; pages 212-214, 219-221 and 226-227 of the study report) presents selected body weight and food consumption data for F1 males (all values means ± standard deviation if available):

Table II: Body Weights, Body Weight Gains and Food Consumption

Dose:Control	**50 ppm**	**750 ppm**	**1500 ppm**
		Body Weights (g)	
Day 1	160.3±23.5	161.6±29.7	143.2**±19.6(89)²
111.8**±30.6(70)			
29	351.2±26.8	354.1±31.2	327.1**±30.3(93)
287.8**±40.2(82)			
57	437.2±33.7	443.3±31.2	406.6**±36.2(93)
369.6**±36.5(85)			
85	473.9±37.0	478.4±34.6	437.2**±42.2(92)
404.9**±36.2(85)			
120	525.1±48.5	531.3±37.5	489.9*±45.7(93)
448.5**±42.0(85)			
		Body Weight Gains (g)	
Days			
1-29¹	190.9	192.5	183.9(96) 176.0(92)
1-57¹	276.9	281.7	263.4(95) 257.8(93)
1-85¹	313.6	316.8	294.0(94) 293.1(93)
1-120	364.8±51.2	369.7±37.6	347.2±40.1(95)
336.7±39.2(92)			
		Food Consumption (g/animal/day)	
Days			

1-8	23.0±2.3	23.8±2.9	21.6±2.2(94)	18.3**±3.9(80)
22-29	26.7±2.4	27.1±1.8	25.6±2.2(96)	24.1*±2.4(90)
50-57	26.2±2.8	26.4±1.8	25.0±2.4(95)	24.9±2.0(95)
113-120	25.6±2.9	26.1±2.2	25.1±2.5(98)	24.4±2.1(95)

1 = calculated by the reviewer from body weight mean values; [2] = percent of control; * = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.

The F1 male mid and high dose groups had statistically significant reductions from control in body weights (70-93% of control, $p<0.01$). There were reduction in the mid and high dose groups for body weight gains (92-96% of control) and food consumption (80-98% of control).

The following Table III (from Tables 17, 20, 25 and 32; pages 119-120, 123, 130-131 and 139-140 of the study report) presents selected body weight and food consumption data for F0 females for the 2 premating periods (all values means ± standard deviation if available):

Table III: Body Weights, Body Weight Gains and Food Consumption

Dose:	Control	50 ppm	750 ppm	1500 ppm
		Body Weights (g)		
		1st Premating		
Day				
1	147.1±11.3	146.7±11.9	147.2±11.2	146.4±11.1(100)[2]
22	215.0±15.4	213.9±16.8	209.0±14.1(97)	205.3*±12.8(96)
36	240.3±18.3	239.8±19.7	231.3±16.5(96)	226.2**±14.0(94)
57	263.5±19.7	262.9±24.1	252.0±18.0(96)	245.5**±13.5(93)
71	274.3±21.2	273.7±25.2	258.8*±17.0(94)	249.0**±13.0(91)
		2nd Premating		
141	313.1±21.7	310.8±29.9	291.6**±20.3(93)	277.4**±15.3(89)
		Body Weight Gains (g)		
Days				
		1st Premating		
1-22[1]	67.9	67.2	61.8(91)	58.9(87)
1-36[1]	93.2	93.1	84.1(90)	79.8(86)
1-57[1]	116.4	116.2	104.8(90)	99.1(85)
1-68	124.8±14.7	122.9±18.4	109.3**±14.8(88)	103.0**±9.7(83)
1-71[1]	127.2	127.0	111.6(88)	102.6(81)
		Overall		
1-141[1]	166.0	122.9±18.4	144.4(87)	131.0(79)
		Food Consumption (g/animal/day)		
Days				
		1st Premating		
1-8	15.3±1.4	15.2±1.6	13.0**±1.2(85)	13.3**±1.4(87)
15-22	17.7±1.5	17.7±1.8	16.4**±1.4(93)	17.2±1.5(97)
50-57	17.1±1.3	17.2±1.8	16.9±1.7(99)	17.6±1.7
64-68	17.5±1.1	16.4*±1.7	15.9**±1.2(91)	16.8±1.8(96)

1 = calculated by the reviewer from body weight mean values; [2] = percent of control; * = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.

The F0 female high dose group had statistically significant reductions from control in body weights (91-96% of control, $p<0.01$) There were also reduced body weight gains in the mid and high dose groups, for the 1-68 day period (83% of control, $p<0.01$, other time periods 81-91% of control).

The following Table IV (from Tables 18, 21, 26, 28, 33 and 35; pages 121, 124, 132, 134, 141 and 143 of the study report) presents selected body weight and food consumption data for F0 females for the 2 gestation periods (all values means ± standard deviation if available):

Table IV: Body Weights, Body Weight Gains and Food Consumption

Dose: Control	50 ppm	750 ppm	1500 ppm
		Body Weights (g)	
		1st Gestation	
Day 0 273.7±20.1	247.5**±15.1(90)	270.8±25.5	258.2*±18.9(94)[2]
Day 7 292.7±20.9	269.9**±15.6(92)	290.2±26.1	277.7*±21.0(95)
Day 14 321.7±20.7	295.0**±15.7(92)	318.6±29.0	305.6*±22.2(95)
Day 21 408.7±27.6	371.5**±24.7(91)	400.7±39.3	381.8**±29.7(93)
		2nd Gestation	
Day 0 311.2±23.3	273.1**±17.5(88)	303.5±23.9	286.8**±19.8(92)
Day 7 329.9±25.2	293.3**±15.2(89)	319.6±25.4	306.1**±21.6(93)
Day 14 357.0±25.7	316.5**±18.0(89)	348.7±26.8	330.4**±22.6(93)
Day 21 451.3±34.5	397.2**±27.7(88)	438.6±36.8	413.4**±34.8(92)
		Body Weight Gains (g)	
Days			
		1st Gestation	
0-7 19.0±4.3		19.4±6.8	19.5±7.8 / 22.4±5.8
0-14[1] 48.0		47.8	47.4(99) / 47.5(99)
0-21 135.0±13.8	124.0±±15.6(92)	129.9±22.9	123.6±19.9(92)
		2nd Gestation	
0-7 18.7±5.9		16.1±5.8	19.3±7.1 / 20.2±6.9
0-14[1] 45.8		45.2	43.6(95) / 43.4(95)
0-21 140.1±20.0	124.1**±±16.6(89)	133.9±17.8	126.6±24.7(90)

continued

Table IV: Body Weights, Body Weight Gains and Food Consumption continued

Dose:	Control	50 ppm	750 ppm	1500 ppm
		Food Consumption (g/animal/day)		
Days				
		1st Gestation		
0-7	18.4±1.3	18.4±1.9	18.1±1.6(98)	18.5±1.5
7-14	21.1±1.2	21.0±2.2	20.4±1.7(97)	20.4±1.5(97)
14-21	22.2±1.4	22.3±2.2	21.4±2.3(96)	21.5±1.9(97)
		2nd Gestation		
0-7	17.3±1.8	16.8±2.0	16.3±1.6(94)	15.8**±1.2(91)
7-14	22.2±2.2	21.8±1.9	21.2±1.7(96)	21.0*±1.1(95)
14-21	22.9±2.1	22.3±1.9	21.7±2.4(95)	21.0*±1.3(92)

1 = calculated by the reviewer from body weight mean values; ² = percent of control; * = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.

The F0 female mid and high dose groups had statistically significant reductions from control in body weights (88-95% of control, p<0.05-0.01) for both gestation periods. Body weight gains were slightly reduced in the mid and high dose groups for the 1st gestation period and were more affected in the 2nd gestation period (89-90% of control, p<0.01 for the high dose). Food consumption was reduced in the high dose group for the 2hd gestation period (91-95% of control, p<0.05-0.01).

The following Table V (from Tables 19, 22, 27, 29, 34 and 36; pages 122, 125, 133, 135, 142 and 144 of the study report) presents selected body weight and food consumption data for F0 females for the 2 lactation periods (all values means ± standard deviation if available):

Table V: Body Weights, Body Weight Gains and Food Consumption

Dose:	Control	50 ppm	750 ppm	1500 ppm
		Body Weights (g)		
		1st Lactation		
Day 0	302.8±20.8	302.2±30.7	291.6±24.0(96)[2]	277.5**±19.3(92)
7	311.5±19.0	310.6±29.1	301.7±20.8(97)	286.4**±19.0(92)
14	326.2±19.1	322.5±27.5	312.4±24.3(96)	297.9**±19.3(91)
21	316.7±18.1	315.7±22.4	310.7±20.8(98)	294.0**±17.8(93)
		2nd Lactation		
0	346.0±28.4	340.9±38.3	322.2**±25.3(93)	302.1**±18.4(87)
7	347.1±26.0	345.4±35.2	334.7±24.0(96)	314.0**±19.7(91)
14	356.6±24.3	352.6±28.6	342.1±23.8(96)	324.8**±21.3(91)
21	353.4±21.2	347.5±28.2	343.7±20.0(97)	328.4**±18.3(93)
		Body Weight Gains (g)		
Days				
		1st Lactation		
0-7	8.7±11.6	7.0±9.9	10.2±12.8	8.9±13.5
0-14[1]	23.4	20.3	20.8(89)	20.4(87)
0-21	14.0±13.9	12.0±14.7	19.2±15.2	16.5±15.9
		2nd Lactation		
0-7	1.2±12.9	4.5±13.6	12.5**±11.4	11.9**±12.3
0-14[1]	10.6	11.7	19.9	22.7
0-21	7.4±15.6	6.6±17.4	21.5**±18.0	26.3**±11.9
		Food Consumption (g/animal/day)		
Days				
		1st Lactation		
0-7	30.6±4.1	31.0±5.0	31.2±4.9	29.1±5.2(95)
7-14	50.6±4.3	49.3±7.3	48.0±7.1(95)	45.8±6.3(91)
14-21	62.5±7.9	62.2±9.3	58.1±9.6(93)	52.9**±7.6(85)

2nd Lactation

0-7	31.3±4.5	32.9±6.3	32.9±5.2	34.7±7.3
7-14	52.0±3.4	51.7±5.0	49.6±8.8(95)	47.0**±6.0(90)
14-21	68.3±4.7	64.2±11.1	61.0*±12.4(89)	57.6**±7.2(84)

1 = calculated by the reviewer from body weight mean values; ² = percent of control; * = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.

The F0 female high dose group had statistically significant reductions from control in body weights (87-93% of control, p< 0.05-0.01) for both of the lactation periods. Food consumption values were reduced in the high dose group.
The following Table VI (from Tables 68, 72 and 76; pages 215-216, 222-223 and 228-229 of the study report) presents selected body weight and food consumption data for F1 females for the premating period (all values means ± standard deviation if available):

Table VI: Body Weights, Body Weight Gains and Food Consumption

Dose:Control	**50 ppm**	**750 ppm**	**1500 ppm**
		Body Weights (g)	
Day			
1 137.7±18.5		136.6±23.0	122.7**±18.1(89)²
97.5**±23.9(71)			
22 217.0±18.1		214.6±22.4	197.0**±18.8(91)
174.5**±19.9(80)			
36 246.9±19.3		245.9±24.1	224.6**±19.3(91)
203.4**±17.8(82)			
57 274.2±21.9		271.6±24.7	248.8**±20.4(91)
231.2**±18.2(84)			
71 285.7±23.1		283.4±27.0	258.2**±21.3(90)
237.6**±18.4(83)			
		Body Weight Gains (g)	
Days			
1-22¹ 79.3		78.0	74.3(94) 77.0(97)
1-36¹ 109.2		109.3	101.9(93) 105.9(97)
1-57¹ 136.5		135.5	126.1(92) 133.7(98)
1-71 148.0±18.8		146.8±24.1	135.5*±15.3(92)
140.1±19.5(95)			
		Food Consumption (g/animal/day)	
Days			
1-8 18.9±1.4		18.5±2.1	17.2**±1.5 (91) 15.0**±2.5(79)
15-22 20.7±1.9		20.3±1.8	19.0**±1.6 (92) 17.7**±1.6(86)
50-57 18.7±1.8		18.1±1.3	17.4*±1.6(93) 17.1**±1.6(91)
64-68 16.1±1.7		15.9±1.5	15.4±2.1(96) 16.1±1.8
174.5** (91)			

1 = calculated by the reviewer from body weight mean values; [2] = percent of control; * = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.

The F1 female mid and high dose groups had statistically significant reductions from control in body weights (71-91% of control, $p<0.01$). Body weight gains were also slightly reduced for the mid and high dose groups ($p<0.05$ for overall mid dose group). Food consumption was statistically significantly reduced in the mid and high dose groups (except for the last 2 weeks, 79-93% of control, $p< 0.01$) for the premating period.

The following Table VII (from Tables 69, 73 and 77; pages 217, 224 and 230 of the study report) presents selected body weight and food consumption data for F1 females for the gestation period (all values means ± standard deviation if available):

Table VII: Body Weights, Body Weight Gains and Food Consumption

Dose:	Control	50 ppm	750 ppm	1500 ppm
		Body Weights (g)		
Day 0	280.9±22.5	283.0±26.4	257.1**±21.0(92)	236.5**±17.9(84)
7	303.3±21.0	301.6±24.9	278.0**±23.3(92)	258.5**±20.1(85)
14	332.7±21.6	330.0±25.8	305.1**±23.5(92)	282.2**±20.3(85)
21	419.3±24.9	412.8±30.9	384.2**2±27.3(92)	348.0**±23.9(83)
		Body Weight Gains (g)		
Days				
0-7	22.4±5.6	18.6±6.5	20.8±6.2(93)	22.0±7.7(98)
0-14[1]	51.8	47.0	48.0(93)	45.7(88)
0-21	138.4±14.5	129.8±18.0	127.0*±16.0(92)	111.5**±13.9(81)
		Food Consumption (g/animal/day)		
Days				
0-7	19.7±1.7	19.2±1.8	18.9±1.6(96)	19.4±1.7(99)
7-14	22.4±1.4	21.9±1.6	21.0*±1.8(94)	20.5**±1.3(92)
14-21	21.9±1.8	21.7±2.0	20.6*±1.8(94)	18.9**±1.9(86)

1 = calculated by the reviewer from body weight mean values; * = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.

The F1 female mid and high dose groups had statistically significant reductions from control in body weights (83-92% of control, p<0.01). Body weight gains for the mid and high dose groups were also reduced (81-98% of control; for the 0-21 day period, p<0.05-0.01). Food consumption for the mid and high dose groups was reduced (86-99% of control, p<0.05-0.01).

female

The following Table VIII (from Tables 70, 74 and 78; pages 218, 225 and 231 of the study report) presents selected body weight and food consumption data for F1 females for the lactation period (all values means ± standard deviation if available):

Table VIII: Body Weights, Body Weight Gains and Food Consumption

Dose:	Control	50 ppm	750 ppm	1500 ppm
		Body Weights (g)		
Day 0	317.6±20.4	313.3±25.8	289.6**±23.3(91)	259.6**±21.6(82)
7	325.3±18.4	325.3±24.4	300.3**±23.6(92)	278.1**±18.2(86)
14	339.8±21.4	339.2±23.3	315.4**±23.4(93)	290.0**±21.7(85)
21	330.2±19.5	331.1±25.1	313.8**±19.5(95)	297.2**±21.7(90)
		Body Weight Gains (g)		
Days				
0-7	7.7±11.0	12.0±8.9	10.7±10.0	18.6**±12.5
0-14[1]	22.2	25.9	25.6	30.4
0-21	12.6±12.4	17.8±10.5	24.2**±12.5	37.7**±13.7
		Food Consumption (g/animal/day)		
Days				
0-7	30.6±5.1	33.2±3.2	31.7±4.6	30.7±3.8
7-14	51.4±8.3	54.0±4.2	50.0±5.9(97)	46.2*±4.1(90)
14-21	68.8±11.5	70.1±5.8	64.2±6.8(93)	58.6**±4.6(85)

1 = calculated by the reviewer from body weight mean values; * = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.

The F1 female mid and high dose groups had statistically significant reductions from control in body weights (82-95% of control, p<0.01) for the lactation period. Food consumption values were slightly affected in the in the high dose group (85-90% of control).

Test Substance Intake:

Based on food consumption, body weight, and dietary analyses results, the doses expressed as mean mg test substance/kg body weight were as follows (Table IX) during the 1st pre-mating period (from Tables 1, 3, 59 and 60, pages 88-89, 91, 193-194 and 195 of the study report):

Table IX: Test Substance Intake

Dose:	50 ppm	750 ppm	1500 ppm
Week			
		F0 Generation	
1	5.6/5.2[1]	75.4/66.4	144.8/137.0
2	5.4/5.4	77.3/77.2	156.5/161.5
3	4.3/4.5	62.0/63.8	126.6/135.6
4	4.1/4.5	59.0/63.7	122.5/135.0
5	3.6/3.8	51.1/53.4	101.8/115.2
6	3.6/3.9	51.4/56.5	103.3/120.7
7	3.2/3.7	47.6/54.0	96.0/115.4
8	2.9/3.3	45.1/51.2	91.3/110.1
9	2.8/3.2	42.0/47.7	79.6/100.1
10	2.7/3.0	41.7/46.5	83.3/100.8
MEAN	**3.8/4.1**	**55.3/58.0**	**110.6/123.1**
		F_1 Generation	
1	7.5/6.9	114.2/106.9	250.4/236.2
2	5.7/5.4	88.2/80.6	188.6/175.8
3	5.3/5.3	82.4/81.3	180.5/174.7
4	4.3/4.4	65.4/67.4	146.9/147.0
5	4.0/4.3	61.5/64.9	133.3/141.6
6	3.7/4.0	56.9/61.1	123.1/131.5
7	3.2/3.5	49.2/53.5	106.1/118.2
8	3.1/3.4	47.9/54.0	105.9/115.4
9	3.1/3.5	48.7/54.7	105.6/117.1
10	2.5/2.9	40.5/45.5	89.7/102.8
MEAN	**4.2/4.4**	**65.5/67.0**	**143.0/146.0**

[1] = males/females

Parental animals and offspring

Reproductive performance:

Results for the first mating of F0 parental animals and the F1a pups are summarized from the report on Table X (from Tables 37-40 and 42, pages 145-151 and 155-157) as follows (mating of 30 animals per sex per dose group):

Table X: Reproductive Performance and Lactation Observations

Dose:	Control	50 ppm	750 ppm	1500 ppm
		F0 Generation		
Observation				
Mean precoital interval (days)	3.6±2.4	3.4±3.0	3.9±2.6	4.8±3.4
		Males		
Mated	30	30	29	29
Mating index(%)	100	100	96.7	96.7
Fertile	30	30	29	27
Fertility index(%)	100	100	100	93.1
		Females		
Mated	30	30	29	29
Mating index(%)	100	100	96.7	96.7
w/o evidence of mating	0	1	2	1
pregnant	0	1	1	0
nonpregnant	0	0	1	1
Fertile	30	30	29	27
Fertility index(%)	100	100	96.7	96.7
Intercurrent deaths	0	1	0	0
Nonpregnant	0	0	1	3
Number of litters	30	30	29	27
Gestation index(%)	100	100	100	100
Parturition index(%)	100	100	100	100
Mean gestation interval (days)	22.2±0.5	22.3±0.5	22.3±0.5	22.2±0.4
Total litter losses	0	0	0	0
Litters w/stillborn	1(3.3%)	4(13.3%)	2(6.9%)	0

continued

Table X: Reproductive Performance and Lactation Observations

Dose: Observation	Control	50 ppm	750 ppm	1500 ppm
F0 Generation - F1 litters				
Total pups	406	382	349	336
Litter size	13.5±2.4	12.7±3.3	12.0±2.7	12.4±2.1
Pups liveborn	405	371	347	336
Live birth index(%)	99.8	97.1	99.4	100
Pups stillborn	1	11**	1	0
Perinatal loss(%)	0.2	2.9	0.6	0
Pups:				
died/sac.	4	8	3	14*
missing	7	3	1	14
cannibalized	0	0	2	1
elec.sac.	0	9**	0	0
Pups culled day 4	157	127	120	104
Pups - all deaths, sacrifice, cannibalized, accidental				
day 0	0	9**	0	0
days 1-4	10	11	2	18
days 5-7	0	0	1	0
days 8-14	1	0	0	3
days 15-21	0	0	3	1
days 22-42	0	0	0	7*
Pup survival				
days 0-4	395	351	345	318
Viability index(%)	97.5	94.6	99.4	94.6
days 4-21	237	224	221	208
Lactation index(%)	99.6	100	98.2	97.2
Live pups per litter				
day 0	13.5±2.4	12.4±3.2	12.0±2.7	12.4±2.1
day 4 precull	13.2±2.4	12.1±3.1	11.9±2.7	11.8±2.3
day 4 postcull	7.9±0.4	7.7±1.2	7.8±1.1	7.9±0.4
day 7	7.9±0.4	7.7±1.2	7.7±1.1	7.9±0.4
day 14	7.9±0.4	7.7±1.2	7.7±1.1	7.8±0.8
day 21	7.9±0.4	7.7±1.2	7.6±1.2	7.7±0.9
Sex ratio (% males)				
day 0	45.7	46.4	45.0	50.3
day 21	50.2	49.1	45.7	52.9
Pup weights				
day 0	6.1±0.6	6.1±0.5	6.1±0.6	6.2±0.5
day 4 precull	9.2±1.5	9.4±1.2	9.3±1.0	8.6±1.3
day 4 postcull	9.2±1.5	9.5±1.1	9.3±1.0	8.6±1.3
day 7	15.0±2.0	15.2±1.4	14.5±1.3	12.7**±2.2
day 14	30.1±2.6	30.2±2.4	28.3*±2.5	23.7**±3.3

day 21	49.3±4.5	49.0±5.8	44.7**±6.4
			35.3***±5.8

* = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.

For F0 parental animals and F1a pups, the only treatment related effects were decreased body weights in the mid and high dose pups at lactation days 7, 14 and 21. No effects were noted on reproductive parameters.

Results for the second mating of F0 parental animals and the F1b pups are summarized from the report on Table XI (from Tables 45-48 and 50, pages 166-171 and 174-176) as follows (mating of 30 animals per sex per dose group):

Table XI: Reproductive Performance and Lactation Observations

Dose:	Control	50 ppm	750 ppm	1500 ppm
		F0 Generation		
Observation				
Mean precoital interval (days)	3.1±0.8	3.4±1.6	3.0±1.4	3.7±3.5
		Males		
Mated	30	28	30	29
Mating index(%)	100	96.6	100	100
Fertile	30	28	30	29
Fertility index(%)	93.3	92.9	96.7	93.1
		Females		
Mated	30	29	30	30
Mating index(%)	100	96.6	100	100
w/o evidence of mating	1	5	3	1
pregnant	1	4	3	1
nonpregnant	0	1	0	0
Fertile	28	26	29	28
Fertility index(%)	93.3	92.9	96.7	93.3
Intercurrent deaths	0	1	0	0
Nonpregnant	2	3	1	2
Number of litters	28	26	29	28
Gestation index(%)	100	100	100	100
Parturition index(%)	100	100	100	100
Mean gestation interval (days)	22.0±0.2	21.9±1.2	22.1±0.3	22.0±0.2
Total litter losses	0	0	0	0
Litters w/stillborn	2(7.1%)	2(7.7%)	1(3.4%)	2(7.1%)

continued

Table XI: Reproductive Performance and Lactation Observations

Dose:	Control	50 ppm	750 ppm	1500 ppm
Observation				
	F0 Generation - F1 litters			
Total pups	390	353	383	351
Litter size	13.9±2.9	13.6±2.9	13.2±4.2	12.5±2.3
Prenatal loss(%)	0	0	0	0
Pups liveborn	388	350	382	349
Live birth index(%)	99.5	99.2	99.7	99.4
Pups stillborn	2	3	1	2
Perinatal loss(%)	0.5	0.8	0.3	0.6
Pups:				
died/sac.	3	15**	6	4
missing	2	11	4	12
cannibalized	0	0	0	1
accid.death	0	0	0	0
Pups culled day 4	160	129	157	116
Pups - all deaths, sacrifice, cannibalized, accidental				
day 0	0	0	0	0
days 1-4	5	24**	9	12
days 5-7	0	1	1	1
days 8-14	0	0	0	2
days 15-21	0	1	0	2
Pup survival				
days 0-4	383	326	373	337
Viability index(%)	98.7	93.1	97.6	96.6
days 4-21	223	195	215	216
Lactation index(%)	100	99.0	99.5	97.7
Live pups per litter				
day 0	13.9±2.8	13.5±2.9	13.2±4.2	12.5±2.3
day 4 precull	13.7±2.6	13.0±2.9	12.9±4.2	12.0±2.5
day 4 postcull	8.0±0.2	7.9±0.6	7.4±1.7	7.9±0.6
day 7	8.0±0.2	7.8±0.6	7.4±1.7	7.9±0.6
day 14	8.0±0.2	7.8±0.6	7.4±1.7	7.8±0.6
day 21	8.0±0.2	7.8±0.6	7.4±1.7	7.7±0.7
Sex ratio (% males)				
day 0	46.4	54.0	46.1	54.4
day 21	49.3	51.3	48.8	50.5
Pup weights				
day 0	5.9±0.5	6.2±0.5	6.0±0.6	6.3±0.5
day 4 precull	8.7±1.0	9.1±1.2	8.7±1.2	8.2±1.0
day 4 postcull	8.9±1.0	9.3±1.1	8.8±1.2	8.3±1.0
day 7	14.5±1.4	15.0±1.6	13.7±1.6	12.2**±1.8
day 14	30.2±1.8	30.6±2.5	27.4**±3.0	

23.6**±2.7			
day 21	51.8±3.3	52.0±4.6	46.2**±5.3
37.7***±4.7			

*** = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.**

For the F1b pups, the only treatment related effects were decreased body weights in the mid and high dose pups at lactation days 7, 14 and 21. No effects were noted on reproductive parameters.

Results for the F1 parental animals and the F2 pups are summarized from the report on Table XII (from Tables 79-82 and 84, pages 232-238 and 241-243) as follows (mating of 30 animals per sex per dose group):

Table XII: Reproductive Performance and Lactation Observations

Dose:	Control	50 ppm	750 ppm	1500 ppm
		F1 Generation		
Observation				
Mean precoital interval (days)	3.2±1.6	3.8±3.3	3.8±2.4	3.7±2.5
		Males		
Mated	29	29	28	29
Mating index(%)	96.7	96.7	93.3	96.7
Fertile	29	28	28	29
Fertility index(%)	100	96.6	100	100
		Females		
Mated	29	29	28	29
Mating index(%)	96.7	96.7	93.3	96.7
w/o evidence of mating	4	2	2	2
pregnant	3	1	0	1
nonpregnant	1	1	2	1
Fertile	29	28	28	29
Fertility index(%)	100	100	100	100
Intercurrent deaths	0	0	0	0
Nonpregnant	1	2	2	1
Number of litters	29	28	28	29
Gestation index(%)	100	100	100	100
Parturition index(%)	100	100	100	100
Mean gestation interval (days)	22.0±0.0	22.1± 0.4	22.1±0.4	22.0±0.0
Total litter losses	0	0	0	0
Litters w/stillborn	1(3.4%)	0	2(7.1%)	1(3.4%)
Implantation sites	409(14.1±2.0)	388(13.9±2.2)	364(13.0±2.2)	372(12.8±2.9)

continued

Table XII: Reproductive Performance and Lactation Observations

Dose:	Control	50 ppm	750 ppm	1500 ppm
Observation				
	F1 Generation - F2 litters			
Total pups	371	363	351	347
Litter size	12.8±2.3	13.0±2.9	12.5±2.4	12.0±2.6
Prenatal loss(%)	9.3	6.4	3.6	6.7
Pups liveborn	370	363	349	346
Live birth index(%)	99.7	100	99.4	99.7
Pups stillborn	1	0	2	1
Perinatal loss(%)	0.5	0	0.6	0.3
Pups:				
died/sac.	15	4	1**	9
missing	13	8	3	3
cannibalized	0	0	0	1
elect.sac.	1	0	0	1
Pups culled day 4	120	131	128	104
Pups - all deaths, sacrifice, cannibalized, accidental				
day 0	1	0	0	0
days 1-4	25	11	2**	12
days 5-7	1	1	0	0
days 8-14	1	0	2	1
days 15-21	1	0	0	1
Pup survival				
days 0-4	344	352	347**	334
Viability index(%)	93.0	97.0	99.4	96.5
days 4-21	221	220	217	228
Lactation index(%)	98.7	99.5	99.1	99.1
Live pups per litter				
day 0	12.8±2.3	13.0±2.9	12.5±2.4	11.9±2.6
day 4 precull	11.9±3.2	12.6±2.8	12.4±2.4	11.5±2.4
day 4 postcull	7.7±1.3	7.9±0.6	7.8±0.9	7.9±0.4
day 7	8.0±0.2	7.9±0.6	7.8±0.9	7.9±0.4
day 14	7.9±0.3	7.9±0.6	7.8±1.0	7.9±0.4
day 21	7.9±0.3	7.9±0.6	7.8±1.0	7.9±0.4
Sex ratio (% males)				
day 0	47.8	49.3	43.3	47.4
day 21	48.9	50.9	47.5	50.0
Pup weights				
day 0	6.0±0.5	6.1±0.5	6.0±0.5	6.1±0.4
day 4 precull	9.0±1.3	9.3±1.4	8.7±1.0	8.5±1.1
day 4 postcull	9.1±1.3	9.4±1.4	8.8±1.0	8.5±1.1
day 7	14.9±1.5	15.0±1.7	13.6**±1.4	12.5**±1.2
day 14	29.8±2.8	30.2±2.2	26.6**±1.9	

23.3**±1.8
day 21 51.8±5.0 51.8±4.3 44.5**±3.5
37.3**±3.2 * = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.

For the F2 pups, the only treatment related effects were decreased body weights in the mid and high dose pups at lactation days 7, 14 and 21. No effects were noted on reproductive parameters.

Clinical observations

Clinical signs of toxicity:

The following Table XIII presents the pup clinical signs observed during lactation (from Tables 41, 49 and 83; pages 152-154, 172-173 and 239-240 from the study report):

Table XIII: Clinical Signs of Toxicity

Dose (ppm):	Control	50	750	1500
	F0 Generation - F1a litters			
Observation				
Cannibalized	0/0[1]	0/0	3/3	0/0
Subcutaneous hemorrhage	2/5	1/2	1/2	1/2
Hypothermic	0/0	1/1	0/0	0/0
Pallor	0/0	1/1	0/0	0/0
Not suckled	4/6	0/0	0/0	3/9
Necrosis	0/0	0/0	0/0	1/1
Partly cannibalized-living	0/0	0/0	0/0	1/1
Fur thin	0/0	1/8	1/10	1/6
Swelling	0/0	1/1	0/0	0/0
Bite wound	0/0	1/1	0/0	0/0
Crust/scurf	0/0	1/1	0/0	0/0
Icterus	0/0	1/1	0/0	0/0
	F0 Generation - F1b litters			
Observation				
Cannibalized	0/0	0/0	0/0	1/1
Subcutaneous hemorrhage	7/12	3/4	4/6	2/3
Not suckled	1/1	1/1	1/1	4/7
Bite wound	0/0	0/0	1/4	0/0
Pallor	0/0	0/0	1/4	0/0
Fur thin	0/0	2/12	1/7	2/16
	F1 Generation - F2 litters			
Cannibalized	0/0	0/0	0/0	1/1

Not suckled	4/9	2/4	2/8	2/2
Partly cannibalized-living	1/1	1/1	0/0	1/1
Subcutaneous hemorrhage	1/1	2/6	1/2	5/10
Kinked tail	0/0	1/1	0/0	0/0
Abdomen distended	0/0	0/0	1/1	0/0
Fur thin	0/0	0/0	2/12	0/0
Crust/scurf	2/12	1/3	0/0	0/0
Bite wound	1/1	1/1	0/0	0/0

[1] = number of animals with observation/number of days with observation.

No treatment related clinical signs of toxicity were noted in the above data for either generation.

Physical/behavioral landmarks:

The following Table XIV presents the physical/behavioral landmarks reported by the investigators as clinical signs observed during lactation (from Tables 43, 51 and 85; pages 160, 179 and 246 from the study report):

Table XIV: Physical/Behavioral Landmarks

Dose (ppm): Observation	**Control**	**50**	**750**	**1500**
		F0 Generation - F1a litters		
Eye opening	15.3±0.7	15.2±0.6	15.4±0.6	16.0**±0.9
Surface righting	2.2±0.2	2.3±0.2	2.2±0.2	2.4±0.3
		F0 Generation - F1b litters		
Eye opening	15.6±0.4	15.4±0.5	15.6±0.6	16.2**±0.6
Surface righting	2.2±0.2	2.2±0.2	2.2±0.2	2.3±0.3
		F1 Generation - F2 litters		
Eye opening	15.3±0.5	15.4±0.5	15.5±0.5	16.0**±0.4
Surface righting	2.3±0.4	2.2±0.2	2.3±0.3	2.2±0.2

*** = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.**

There was a slight increase in time to eye opening in the high dose group in both F1a, F1b and F2 litters.

Necropsy results

Necropsy observations (macroscopic):

The following Table XV presents the necropsy observations for the F0 and F1 parental animals (from Tables 53-54 and 87-88, pages 184-188 and 251-253 of the study report):

Table XV: Necropsy Observations

Dose (ppm): Observation	Control	50	750	1500
F0 Generation Males (30 sires)				
Skin:				
Scab formation	0	1	2	0
hairloss	0	0	1	0
Chest wall: Scab forma.1		0	0	0
Kidney: Cyst	0	0	0	1
Testis: Small	0	0	1	0
Testis tunica albuinea, contents fluid	0	0	0	1

continued

Table XV: Necropsy Observations continued

Dose (ppm): Observation	Control	50	750	1500
Prostate: Mass	0	0	0	1
Lymph Node: Axillary, large	0	0	1	0
F0 Generation Females (30 dams)				
Hindlimb: Mass	0	0	0	1
Skin:				
Scab formation	0	1	1	0
hairloss	0	0	3	1
Abdominal wall: Mass	0	0	1	0
Chest Wall: Mass	0	1	1	1
Caecum: Cyst	0	0	0	1
Kidney: Cyst cab for	1	0	0	0
Vagina: hemorrhage	0	1	0	0
Thymus: Mottled	1	0	0	0
Esophagus:				
Contents Solid	0	1	0	0
F1 Generation Males (30 sires)				
Back:				
hairloss	1	0	0	0
Scab formation	2	2	0	0
Skin:				
Scab formation	0	1	1	0
hairloss	0	1	0	1

Kidney:							
Cyst	1		0		0		0
Renal pelvic dilation	0		0		1		0
Testis: Small	1		0		3		0
Large	0		0		1		0
Testis tunica albuinea, contents fluid	0		1		0		0
Thymus: Mottled	2		0		0		0
Mass	0		0		1		0
Lymph node: Large	0		0		1		0
Females (30 dams)							
Back: Scab formation	1		0		0		0
Skin: Scab formation	2		0		0		1
hairloss	1		0		0		0
Chest wall: Mass	0		1		0		1
Spleen: Large	0		0		0		1

No treatment related effects were noted in either F0 of F1 parental animals for necropsy observations.

The following Table XVI presents the necropsy observations for the F1a, F1b and F2 pups (from Tables 44, 52 and 86, pages 161-165, 180-183 and 247-250 of the study report):

Table XVI: Necropsy Observations

Dose (ppm):	**Control**	**50**	**750**	**1500**
Observation				
		F1a Pups		
Litters evaluated	30	30	29	27
Pups evaluated	339	319	280	257
Live	338	308	279	257
Stillborn	1	11	1	0
Autolysis	3/2[1]	6/6	2/2	5/4
Icterus	0/0	1/1	0/0	0/0
Cannibalized	0/0	0/0	2/2	0/0
Lungs: Mottled	2/2	0/0	0/0	0/0
Stomach: No milk	2/2	2/2	0/0	2/2
Gas filled stomach	1/1	0/0	0/0	2/2
Testis: Small	2/2	0/0	0/0	0/0
Missing	1/1	0/0	0/0	0/0
Abdominal cavity: Contents fluid	0/0	0/0	0/0	4/1
		F1b Pups		
Litters evaluated	28	26	29	28
Pups evaluated	388	342	379	339
Live	386	339	378	337
Stillborn	2	3	1	2
Autolysis	4/4	6/5	5/5	1/1
Cannibalized	0/0	9**/1	0/0	1/1
Fur thin	0/0	8**/1	0/0	
14**/2				
Stomach: No milk	1/1	0/0	0/0	0/0
Gas filled stomach	0/0	0/0	0/0	1/1
Testis: Small	0/0	0/0	0/0	1/1
		F2 Pups		
Litters evaluated	29	28	28	29
Pups evaluated	358	355	348	344
Live	357	355	346	343
Stillborn	1	0	2	1
Autolysis	13/6	3/3	2*/2	6/4
M conjoint twins with exencephaly	0/0	0/0	1/1	0/0
Cannibalized	0/0	0/0	0/0	2/2
Eyes: hemorrhage	0/0	0/0	0/0	1/1
Stomach: No milk	5/5	0/0	4/4	2/1

Testis: hemorrhagic	0/0	0/0	1/1	0/0
Limbs: hemorrhage	0/0	0/0	0/0	1/1

[1] = fetal/litter incidence; * = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.

No treatment related effects were noted in either F1a, F1b or F1 pups for necropsy observations.

Organ weights

The following Table XVII presents selected organ weight data for the F0 and F1 parental animals (from Tables 55-58 and 89-92, pages 189-192 and 254-257 of the study report):

Table XVII: Organ Weights (g)

Dose (ppm):		**Control**	**50**	**750**	**1500**
			F0 Generation		
		Males (30 sires, except high dose 29 sires)			
Spleen	A	0.806±0.104	0.782±0.108	0.760±0.086	0.698**±0.098
	R	0.1503±0.0185	0.1445±0.0189	0.1435±0.0165	0.1457±0.0180
Liver	A	19.836±2.883	20.385±3.340	20.669±2.195	20.297±2.817
	R	3.6747±0.2502	3.7431±0.3767	3.8845**±0.2091	4.2222**±0.3696
Kidneys	A	3.249±0.321	3.324±0.329	3.390±0.265	3.352±0.414
	R	0.6052±0.0427	0.6140±0.0454	0.6396*±0.0466	0.6988**±0.0644
Testis	A	4.183±0.331	4.039±0.354	4.130±0.496	4.140±0.412
	R	0.7819±0.0720	0.7501±0.0929	0.7779±0.0823	0.8654**±0.0773
Adrenals	A	0.072±0.009	0.070±0.011	0.070±0.010	0.067±0.010
	R	0.0135±0.0025	0.0129±0.0017	0.0132±0.0017	0.0139±0.0018
Thymus	A	0.341±0.074	0.412*±0.110	0.327±0.097	0.315±0.075
	R	0.0638±0.0153	0.0765*±0.0226	0.0612±0.0161	0.0658±0.0155
Brain	A	2.364±0.093	2.392±0.089	2.352±0.089	2.342±0.105
	R	0.4426±0.0380	0.4442±0.396	0.4452±0.0381	0.4912**±0.0420
		Females (30 dams, except low dose 29 dams)			
Spleen	A	0.609±0.084	0.600±0.096	0.567±0.083	0.559±0.086
	R	0.1902±0.0241	0.1904±0.0247	0.1907±0.0247	0.1986±0.0278
Liver	A	12.781±1.199	12.167±1.342	12.549±1.434	12.470±1.310
	R	3.9857±0.1760	3.8661±0.2927	4.2127**±0.3186	4.4298**±0.3479
Kidneys	A	2.234±0.204	2.206±0.249	2.132±0.205	2.150±0.165
	R	0.6979±0.0492	0.7017±0.0654	0.7171±0.0533	0.7657**±0.0610
Ovaries	A	0.207±0.037	0.207±0.041	0.217±0.040	0.209±0.033
	R	0.0650±0.0124	0.0658±0.0111	0.0731*±0.0130	0.0744**±0.0109

Adrenals	A	0.088±0.008	0.086±0.010	0.085±0.010	0.081**±0.009
	R	0.0276±0.0029	0.0275±0.0035	0.0288±0.0033	0.0287±0.0035
Thymus	A	0.236±0.059	0.236±0.151	0.224±0.054	0.207±0.058
	R	0.0737±0.0177	0.0753±0.0151	0.0753±0.0162	0.0738±0.0205
Brain	A	2.246±0.107	2.234±0.103	2.173*±0.104	2.138**±0.094
	R	0.7032±0.0465	0.7141±0.0617	0.7339±0.0623	0.7621**±0.0518

continued

Table XV: Organ Weights (g) continued

Dose (ppm):		Control	50	750	1500
			F1 Generation		
		Males (30 sires, except mid dose 29 sires)			
Spleen	A	0.825±0.119	0.862±0.104	0.765±0.097	0.730**±0.092
	R	0.1626±0.170	0.1688±0.0185	0.1628±0.0154	0.1698±0.0197
Liver	A	20.887±3.474	20.677±3.357	19.798±2.383	19.860±2.355
	R	4.0942±0.3204	4.0344±0.5365	4.2163±0.4020	4.6035**±0.2830
Kidneys	A	3.283±0.381	3.222±0.335	3.090±0.352	3.101±0.297
	R	0.6479±0.0542	0.6305±0.0560	0.6580±0.0556	0.7208*±0.0532
Testis	A	4.240±0.362	4.309±0.331	4.180±0.597	4.199±0.344
	R	0.8402±0.0823	0.8447±0.0708	0.8912±0.1139	0.9802**±0.1030
Adrenals	A	0.068±0.010	0.067±0.010	0.068±0.008	0.069±0.010
	R	0.0135±0.0019	0.0132±0.0020	0.0146±0.0017	0.0160**±0.0022
Thymus	A	0.488±0.117	0.534±0.079	0.488±0.107	0.459±0.092
	R	0.0969±0.0244	0.1050±0.0181	0.1036±0.0201	0.1071±0.0226
Brain	A	2.382±0.084	2.353±0.099	2.285**±0.097	2.246**±0.100
	R	0.4732±0.0433	0.4621±0.0371	0.4892±0.0405	0.5245**±0.0451
		Females (24 dams control & mid dose, 25 dams low dose, 18 dams high dose)			
Spleen	A	0.612±0.073	0.592±0.104	0.560±0.089	0.582±0.190
	R	0.1958±0.0206	0.1957±0.0307	0.1994±0.0348	0.2234±0.0685
Liver	A	13.247±1.435	12.840±1.557	11.817**±1.292	12.432±1.193
	R	4.2259±0.2572	4.2457±0.4010	4.1863±0.3410	4.7667**±0.2829
Kidneys	A	2.266±0.248	2.180±0.196	2.059**±0.192	2.060**±0.228
	R	0.7234±0.0550	0.7225±0.0569	0.7301±0.0566	0.7896**±0.0570
Ovaries	A	0.212±0.030	0.232±0.118	0.239±0.030	0.205±0.035
	R	0.0678±0.0094	0.0767±0.0364	0.0849**±0.0113	0.0788**±0.0120
Adrenals	A	0.085±0.010	0.086±0.015	0.105±0.010	0.085±0.009
	R	0.0271±0.0036	0.0285±0.0054	0.0370±0.0361	0.0326±0.0039
Thymus	A	0.320±0.043	0.347±0.076	0.360*±0.068	0.319±0.059
	R	0.1025±0.0146	0.1146*±0.0223	0.1276**±0.0239	0.1228**±0.0236
Brain	A	2.188±0.100	2.179±0.118	2.132±0.094	2.112**±0.079
	R	0.7019±0.0520	0.7235±0.0521	0.7586**±0.0589	0.8127**±0.0462

A = Absolute organ weights in grams; R = Relative organ to body weight ratio expressed as % of body weight; * = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett-test.

No treatment related effects were noted in the above data.

Pathology

Microscopic examination:

From the data provided there were no treatment related macroscopical changes observed in the F0 and F1 parent animals.

The histopathological data showed a minimal to moderate hypertrophy of centrilobular hepatocytes in F0 animals of both sexes in the 1500 ppm dose group (males,10/30 and females, 5/30) and in F1 animals of both sexes in the 750 (males, 14/30 and females, 7/30) and 1500 ppm (males, 24/30 and females, 9/30) dose groups. There was also a slightly increased incidences of a minimal pigmentation of renal tubules in 750 ppm F0 males (7/30) and F0 animals of both sexes in the 1500 ppm dose group (males, 3/30 and females, 4/30). There were also decreased incidences of splenic hemosiderosis in F0 and F1 animals both sexes of the 750 (F0 males, 12/30 and F0 females 15/30; F1 males, 5/30 and F1 females, 17/30) and 1500 ppm dose groups (F0 males, 9/30 and F0 females, 8/30; F1 males, 2/30 and F1 females, 14/30). The investigators stated that: "The amount of splenic hemosiderosis is known to correlate with turnover of red blood cells. Therefore, a decreased incidence of splenic hemosiderosis may indicate a decrease of red blood cell turnover. This phenomenon was not observed in any previous toxicity study with CGA 279202 tech. Although this effect may could be treatment- related, it has to be considered not to be adverse."

There were no treatment related findings in the reproductive system of animals of either sex.

III. DISCUSSION

A. Investigators' Conclusions: (scanned from pages 23-226 of the study report)

Purpose

This study was designed to investigate the effects of continuous dietary administration of CGA 279202 Technical to the rat on gonadal function, mating behavior, conception, parturition, lactation, weaning, and the growth and development of the offspring through the production of two litters in the first and one litter in the second generation.

Design

In two successive generations (F0 and F1), male and female rats were

continuously exposed to the test substance admixed to feed at nominal concentrations of 0, 50, 750 and 1500 ppm.

After 10 weeks premating dietary exposure to the test substance animals were paired 1:1 within each dose group (30 animals per sex and dose) until there was evidence of positive mating or for 19 days, whichever occurred first. Dams were allowed to litter and suckle their pups naturally. Litters were culled to 4 male and 4 female pups, where possible, on day 4 post partum. After weaning of the F1 pups, the F0 parent animals were remated to produce second litters. The F1 generation was selected from the first litters of the F0 generation.

Clinical signs, body weights, food consumption, mating, gestation and delivery parameters, pup survival and development were recorded. A gross necropsy examination was performed on all pups not selected for mating. Parent animals were necropsied after weaning of the second (F0 parents) or first (F1 parents) litters and subjected to macroscopic examination, with histopathology of the reproductive and target organs.

Results

F0 Generation

Initially, mean test substance intake for the males was 0.0, 5.6, 75.4 and 144.8 mg/kg body weight/day in the 0, 50, 750 and 1500 ppm dose groups, respectively. By the end of the treatment period, substance intake had been reduced by approximately half these initial values. Substance intake for the females was similar to that of the males during the premating period. During the gestation periods, test substance intake remained similar to that at the end of the premating period and then during the lactation periods, there was a marked increase as a result of increased food intake. For example at the end of the first lactation period, values, in ascending group order, were 0.0, 9.7, 139.4 and 267.0 mg/kg body weight/day.

There were no treatment-related clinical signs or treatment-related deaths among the F0 parent animals.

At 1500 ppm, food consumption was reduced and body weight gain was retarded from the start of the dosing period, as a result, body weights in this group were significantly lower than those of the control group.

At 750 ppm, food consumption was slightly reduced and body weights were slightly lower than those of the control group, but in general, differences from the control value did not attain statistical significance. Generally speaking, these effects were more pronounced in the females than in the males.

Male and female mating and fertility indices, maternal gestation and parturition indices and the duration of gestation were unaffected by treatment at either the first or the second mating.
For both the first and second matings, mean pup weight at birth was similar in all groups. At 750 and 1500 ppm, for both the first and the second matings, weight gain of the pups was retarded during the lactation period and body weights were reduced at weaning. At 1500 ppm, retardation in the achievement of the physical/behavioral landmarks by the pups eye opening (both the first and the second mating) was observed.

For all test groups, for both matings, no treatment-related effects were noted

in F1 offspring in terms of litter size, post-natal loss, sex ratios, clinical signs, or the macroscopic findings noted at necropsy.

For the F0 parent animals, there were no treatment-related findings at terminal necropsy or in the organ weights. No treatment-related changes were observed at histopathological examination of the reproductive organs of the control and high dose (1500 ppm) groups.

Microscopic examination of the liver showed an increased incidence of males and females at 1500 ppm with minimal hypertrophy of centrilobular hepatocytes. Microscopic examination of the kidneys and spleen showed an increased incidence of males and females at 1500 ppm and of males at 750 ppm with minimal pigmentation of renal tubules.

F1 Generation

As in the F0 generation, mean test substance intake reduced as body weight increased. Initially, values were approximately 0.0, 7.5, 114.2 and 250.4 mg/kg body weight/day for males and 0.0, 6.9, 106.9 and 236.2 mg/kg body weight/day for the females, in ascending group order. By the end of the premating period, substance intake had reduced by between ca. 55-65%. There was a very slight increase in substance intake during the gestation period, but a marked increase during the lactation period - values were 0.0, 10.4, 153.4 and 301.0 mg/kg/day at the end of the lactation period.

There were no treatment-related clinical signs or treatment-related deaths among the F1 parent animals.

At 1500 ppm, body weights of the selected F1 animals were significantly lower than those of the control group. Food consumption and body weights in this group were significantly lower than controls throughout most of the F1 generation. A slight reduction in body weights (both sexes) and food consumption (females only) were also observed at 750 ppm.

Male and female mating and fertility indices, maternal gestation and parturition indices and the duration of gestation were unaffected by treatment.

As in the F0 generation, there was a retardation of body weight gain during the lactation period in the pups at 750 and 1500 ppm; consequently, mean pup body weights were lower than those of controls at weaning on day 21 post partum. At 1500 ppm, retardation in the achievement of the physical/behavioral landmarks by the pups -eye opening- was observed.

For all test groups, for both matings, no treatment-related effects were noted in F2 offspring in terms of litter size, post-natal loss, sex ratios, clinical signs, or the macroscopic findings noted at necropsy.

For the F1 parent animals, there were no treatment-related findings at terminal necropsy or in the organ weights. No treatment-related changes were observed at microscopic examination of the reproductive organs of the control and high dose (1500 ppm) groups.

Microscopic examination of the liver showed an increased incidence of males and females at 750 and 1500 ppm with minimal to moderate hypertrophy of centrilobular hepatocytes.

Conclusion

In this study, CGA 279202 Technical was administered in the diet to rats at dosages of 0, 50, 750 and 1500 ppm. over two generations, with two mating periods in the first and one mating period in the second generation.

Dosages of 750 and 1500 ppm were associated with reduced food consumption and retarded body weight gain in the parent F0 and F1 animals. Additionally body weight gain of the pups was retarded during the lactation period (F1, both A and B litters and F2 animals). At 1500 ppm retardation in achievement of the physical/behavioral landmarks by the pups - eye opening - during both lactation periods in the F1 generation and F2 generation was observed.

None of the reproductive parameters - gonadal function, mating behavior, conception, parturition, lactation and weaning - were affected by the administration of CGA 279202 Technical to the parent animals.

There was an increased incidence of minimal to moderate hypertrophy of centrilobular hepatocytes in males and females (both generations at 1500 ppm, and F1 generation at 750 ppm); increased incidence of F0 males and females with minimal pigmentation of renal tubules in the 1500 ppm group and in F0 males in the 750 ppm. group.

On the basis of the results obtained in this study, the no observed effect level (NOEL) for CGA 279202 Technical was 50 ppm (approximately 2.2 to 10.4 mg/kg body weight/day).

B. Reviewer's conclusions:

Systemic toxicity to the paternal animals was noted in the F0 male high dose group as statistically significant reductions from control in body weights (90% of control, $p<0.01$), body weight gains (76-89% of control, $p<0.01$ for days 1-134) and food consumption (87-93% of control, $p<0.01$) and in the F1 male mid and high dose groups as statistically significant reductions from control in body weights (70-93% of control, $p<0.01$) and there were reductions in the mid and high dose groups for body weight gains (92-96% of control) and food consumption (80-98% of control). No significant clinical signs of toxicity were noted in F0 or F1 males.

Systemic toxicity to the maternal animals during the premating period was noted in the F0 female high dose group as statistically significant reductions from control in body weights (91-96% of control, $p<0.01$). There were also reduced body weight gains in the mid and high dose groups, for the 1-68 day period (83% of control, $p<0.01$, other time periods 81-91% of control). The F1 female mid and high dose groups had statistically significant reductions from control in body weights (71-91% of control, $p<0.01$). Body weight gains were also slightly reduced for the mid and high dose groups ($p<0.05$ for overall mid dose group). Food consumption was statistically significantly reduced in the mid and high dose groups (except for the last 2 weeks, 79-93% of control, $p< 0.01$).

Systemic toxicity to the maternal animals during the gestation period was noted in the F0 female mid and high dose groups as statistically significant reductions from control in body weights (88-95% of control, $p<0.05$-0.01) (both gestation periods). Body weight gains were slightly reduced in the mid and high dose groups for the 1st gestation period and were more affected in the 2nd gestation period (89-90% of control, $p<0.01$ for the high dose). Food consumption was reduced in the high dose group for the 2nd gestation period (91-95% of control, $p<0.05$-0.01). The F1 female mid and high dose groups had statistically significant reductions from control in body weights (83-92% of control, $p<0.01$). Body weight gains for the mid and high dose groups were also reduced (81-98% of control; for the 0-21 day period, $p<0.05$-0.01). Food consumption for the mid and high dose groups was reduced (86-99% of control, $p<0.05$-0.01).

Systemic toxicity to the maternal animals during the lactation period was noted in the F0 female high dose group as

statistically significant reductions from control in body weights (87-93% of control, p< 0.05-0.01) (both lactation periods). Food consumption values were reduced in the high dose group. The F1 female mid and high dose groups had statistically significant reductions from control in body weights (82-95% of control, p<0.01) for the lactation period. Food consumption values were slightly affected in the in the high dose group (85-90% of control).

Other systemic toxicity to the parental animals included a minimal to moderate hypertrophy of centrilobular hepatocytes in F0 animals of both sexes in the 1500 ppm dose group (males,10/30 and females, 5/30) and in F1 animals of both sexes in the 750 (males, 14/30 and females, 7/30) and 1500 ppm (males, 24/30 and females, 9/30) dose groups. There was also a slightly increased incidences of a minimal pigmentation of renal tubules in 750 ppm F0 males (7/30) and F0 animals of both sexes in the 1500 ppm dose group (males, 3/30 and females, 4/30). There were also decreased incidences of splenic hemosiderosis in F0 and F1 animals both sexes of the 750 (F0 males, 12/30 and F0 females 15/30; F1 males, 5/30 and F1 females, 17/30) and 1500 ppm dose groups (F0 males, 9/30 and F0 females, 8/30; F1 males, 2/30 and F1 females, 14/30). There were no treatment related findings in the reproductive system of animals of either sex.

For the pups, systemic/developmental toxicity was noted as decreased F1a, F1b and F2 pup body weights in the mid and high dose pups at lactation days 7, 14 and 21. There was a slight increase in time to eye opening in the high dose group in both F1a, F1b and F2 litters.

No effects were noted on reproductive parameters.

Parental (Paternal/Maternal) Systemic Toxicity NOAEL = 50 ppm (3.8-4.2 mg/kg/day for males and 4.1-4.4 mg/kg/day for females)

Parental (Paternal/Maternal) Systemic Toxicity LOAEL = 750 ppm (55.3-65.5 mg/kg/day for males and 58.0-67.0 mg/kg/day for females)

Offspring Systemic/Developmental Toxicity NOAEL = 50 ppm (3.8-4.2 mg/kg/day for males and 4.1-4.4 mg/kg/day for females)

Offspring Systemic/Developmental Toxicity LOAEL = 750 ppm (55.3-65.5 mg/kg/day for males and 58.0-67.0 mg/kg/day for females)

Reproductive Toxicity NOAEL ≥ 1500 ppm
Reproductive Toxicity LOAEL > 1500 ppm

TRIFLOXYSTROBIN Reproductive Toxicity-Rat OPPTS 870.3800; OPP §83-4

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