Primary Review by: Stephen C. Dapson, Ph.D.
Branch Senior Scientist, Registration Action Branch 3/HED (7509C)

Secondary Review by: William B. Greear, M.P.H., D.A.B.T.
Registration Action Branch 3/HED (7509C)

11/29/1999

DATA EVALUATION RECORD

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

EPA ID No.s: EPA MRID No. 45118327
EPA Pesticide Chemical Code
EPA DP Barcode D
EPA Submission No. S

Test Material: BAS 500 F
Synonyms:

Citation: Schilling, K., Gembardt, Chr., Hildebrand, B. (1999): BAS 500 F - Two Generation Reproduction Toxicity Study in Wistar Rats Continuous Dietary Administration; Department of Toxicology of BASF Aktiengesellschaft for BASF CORPORATION, Agricultural Products, Laboratory Project Identification No. 70R0494/96172, BASF Registration Document No. 1999/11869, November 29, 1999 (Unpublished). EPA MRID Number 45118327.

Executive Summary: In a multigeneration reproduction study (MRID# 45118327), groups of male and female Wistar rats (Chbb = THOM (SPF) from Boehringer Ingelheim, Pharma KG, Biberach/Riss, FRG received 0, 25, 75 or 300 ppm BAS 500 F (Purity: 98.7%; Batch No.: J.-No. 27882/199/b) in the diet for two successive generations [Mean intakes for the 25, 75 and 300 ppm dose groups in mg/kg/day were 2.5 for males and 2.6 for females, 7.4 for males and 7.8 for females, and 29.0 for males and 30.4 for females, respectively, in the F0 generation; from 2.8 for males and 3.0 for females, 8.6 for males and 9.0 for females and 35.0 for males and 36.0 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, pup survival and sexual maturation landmarks, and gross necropsy (macroscopic pathological 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.

No substance related mortality or clinical signs of toxicity was noted in any of the F0 or F1

parental animals. Further, there was no treatment related effects noted in the body weights, body weight gains, food consumption, reproductive performance (all measured parameters), sexual maturation landmarks, necropsy results, both gross and microscopic including assessment of differential ovarian follicle counts and organ weights of the parental animals and the pups.

The Parental (Paternal/Maternal) Systemic Toxicity NOAEL ≥ 300 ppm (29.0-35.0 mg/kg/day for males and 30.4-36.0 mg/kg/day for females) and the Parental (Paternal/Maternal) Systemic Toxicity LOAEL > 300 ppm (29.0-35.0 mg/kg/day for males and 30.4-36.0 mg/kg/day for females)

The Offspring Systemic/Developmental Toxicity NOAEL ≥ 300 ppm (29.0-35.0 mg/kg/day for males and 30.4-36.0 mg/kg/day for females) and the Offspring Systemic/Developmental Toxicity LOAEL > 300 ppm (29.0-35.0 mg/kg/day for males and 30.4-36.0 mg/kg/day for females)

The Reproductive Toxicity NOAEL ≥ 300 ppm (29.0-35.0 mg/kg/day for males and 30.4-36.0 mg/kg/day for females) and the Reproductive Toxicity LOAEL > 300 ppm (29.0-35.0 mg/kg/day for males and 30.4-36.0 mg/kg/day for females)

This study is classified as Unacceptable-Guideline and does not satisfy the guideline requirements (OPPTS 870.3800, OPP §83-4) for a multigeneration reproduction study in rats. This study may be upgraded if a justification for the dose levels is provided that will prove to the Agencies satisfaction that higher levels would not affect reproduction or developmental parameters or would induce significant paternal toxicity with no effects on reproductive or developmental parameters.

Compliance: A signed and dated STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS, a GOOD LABORATORY PRACTICES STATEMENT along with a GLP STATEMENT for OECD, an EPA Flagging Statement (the study neither met nor exceeded applicable criteria), and a STATEMENT OF THE QUALITY ASSURANCE UNIT were provided.

TEST GUIDELINES

EC Commission Directive 87/302/EEC of November 18, 1987; Part B: Methods for the determination of toxicity: Two-generation reproduction toxicity test; Official Journal of the European Communities, No. L 133, pp. 47 - 50 (1988)

OECD Guidelines for Testing of Chemicals; Method No. 416: Two-Generation Reproduction Toxicity Study (Draft April 1996)

U.S. EPA, Health Effects Test Guidelines; OPPTS 870.3800: Reproduction and Fertility Effects (Aug. 1998)(When the study protocol was created, U.S. EPA. 40 CFR Part 799; Toxic Substances Control Act Test Guidelines; Final Rule § 799.9380 TSCA Reproduction and Fertility Effects (August 1997) was available as the latest finalized test guideline. Meanwhile, the final version of EPA Health Effects Test Guidelines; OPPTS 870.3800 was issued, which is substantially similar to the guideline previously referenced.)

Japan/MAFF: Testing Guidelines for Toxicology Studies: Reproduction Study, pp. 45 - 48 (1985)

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: BAS 500 F
Purity: 98.7% (certificate PCP04220)
Description: red-brown, clear solid
Batch No.: J.-No. 27882/199/b (ToxIII/part 2)
Storage: Refrigerator, after grinding at -20°C

Test Animal(s): Species: Male and female rats
Strain: Wistar rats (Chbb = THOM (SPF))
Source: Boehringer Ingelheim, Pharma KG, Biberach/Riss, FRG
Age: weeks at start of dosing
Body Weight: 129.0 g for males, 118.4 g for females on study week 0.
Additional information: According to the investigators: This strain was selected since extensive historical control data was available on Wistar rats and the rat is the preferred animal species for reproduction studies according to the different test guidelines. The animals were received on May 26, 1998. *The animals* were free from any clinical signs of disease... The females were nulliparous and non-pregnant at the beginning of the study. According to a written statement from the breeder, male and female animals were derived from different litters. This was necessary to rule out the possibility of sibling mating. These animals were taken to form the FO generation parental animals. All other animals used in this study (F1 and F2 generation pups and the F1 generation parental rats [raised F1 pups]) were derived from these animals.

AIM OF THE STUDY [scanned from page 30 of the study report]

The objective of this study was to determine the possible adverse effects of BAS 500 F on the integrity and performance of the male and female reproductive systems, including gonadal function, estrous cycle, mating behavior, conception, gestation, parturition, lactation and weaning, and on growth and development of offspring from two successive generations of Wistar rats continuously administered to the test substance in the diet. The study should also provide information about the effects of BAS 500 F on neonatal morbidity, mortality, possible target organs in the offspring and data on prenatal and postnatal developmental toxicity.

SELECTION OF DOSES/CONCENTRATION

The following concentrations in the diet were selected **[no rationale was provided]**:

25 ppm: as the expected "no observed adverse effect level"

75 ppm: as intermediate dose level

300 ppm: as highest dose level

Husbandry (scanned from page 33 of the study report)

During the study period, the rats were housed individually in type DK III stainless steel wire mesh cages supplied by BECKER & CO., Castrop-Rauxel, FRG (floor area of about 800 CM^2), with the following exceptions: from day 18 of gestation until day 14 after birth, the pregnant animals and their litters were also housed in Makrolon type M III cages. The M III cages were again supplied by BECKER & CO.. Pregnant females were provided with nesting material (cellulose wadding) toward the end of gestation.

The cages with the test animals were arranged on the racks in such a way that uniform experimental conditions (ventilation and light) were ensured.

The animals were accommodated in fully air- conditioned rooms (floor area about 22 M^2) in which central air conditioning guaranteed a range of temperature of 20 - 24°C and a range of relative humidity of 30 - 70%. There were no or only minimal deviations from these limits.

The day/night rhythm was 12 hours (12 hours light from 6.00 a.m. to 6.00 p.m. and 12 hours darkness from 6.00 p.m. to 6.00 a.m.) in general.

Before use each room was completely disinfected using a disinfector ("AUTEX" fully automatic, formalin-ammonia-based terminal disinfection). Usually, each week the walls and the floor were cleaned with, water containing about 0.5% Mikro-Quat (supplied by ECOSAN GmbH, FRG).

The food used was ground Kliba maintenance diet rat/ mouse/hamster, 343 meal, supplied by KLINGENTALMOHLE AG, Kaiseraugst, Switzerland, which was available to the animals ad libitum throughout the study (from the day of supply to the day of or the day before necropsy). Drinking water was supplied from water bottles (ad libitum).

The bedding used throughout the study was SSNIFF (type 3/4) supplied by SSNIFF SPEZIALMATEN GmbH, Soest, FRG.

B. Study Design

According to the investigators (from pages 37-40 of the study report):

F0 generation parental animals and their progeny

The 112 male and 112 female rats were 28 (± 1) days old when they arrived from the breeding facilities. During an acclimatization period of about 8 days, animals with lowest and highest body weights were eliminated, used for other purposes and finally sacrificed. The 100 male and 100 female animals required for the study were 35 (± 1) days old at the beginning of treatment, and their mean weights and weight ranges were:

male animals: 129,0 (108,2 - 162,3) g
female animals: 118,3(107,0 - 144,6)g

The assignment of the animals to the different test groups was carried out using a randomization program (NIJENHUIS, A. and WILF, H.S.; 1978), according to their weight one day before the beginning of the administration period (day -1).

After the acclimatization period, the F0 generation parental animals continuously received the test substance at the appropriate concentrations in the diet until or up to about 16 hours before they were sacrificed.

At least 74 days after the beginning of treatment, males and females from the same dose group were mated at a ratio of 1 : 1.

The females were allowed to litter and rear their pups (F1 generation pups) until day 4 (standardization) or 21 after parturition.

After weaning of F1 pups the F0 generation parental animals were sacrificed.

F1 generation parental animals and their progeny

After weaning, 25 males and 25 females (each litter was taken into account) of the F1 pups of test groups 00, 01, 02 and 03 (0; 25; 75 or 300 ppm) were taken per group as the basis of the F1 generation parental animals. These animals were chosen by lot during rearing; it was attempted to take each litter into account. If fewer than 25 litters in these groups were available for selection or if one sex was missing in a litter, more animals were taken from different litters from the relevant test group to give the full number.

All selected animals were exposed continuously to the test substance at the same dose level as their parents from their growth into adulthood until or up to about 16 hours before they were sacrificed.

At least 74 days after assignment of the F1 generation parental animals, the males and females were mated at a ratio of 1 : 1 in general. The partners were randomly assigned to one another. Matings between siblings were, however, avoided.

The females were allowed to litter and rear their pups (F2 generation pups) until day 4 (standardization) or 21 after parturition.

Some weeks after the F2 generation pups had been weaned, the F1 generation parental animals were sacrificed.

Standardization of litters (F1 and F2 generation pups)

On day 4 p.p., the individual litters were in general standardized in such a way that, where possible, each litter contained 4 male and 4 female pups (always the first 4 pups/sex and litter were taken for further rearing). If it was not possible in single litters to have 4 pups/sex, it was proceeded in such a way that 8 pups per litter were present for further rearing (e.g., 5 male and 3 female pups). Standardization of litters was not performed in litters with ≤ 8 pups.

Pups after standardization/weaning

With the exception of the F1 generation pups, which were chosen as the basis of the F1 generation parental animals, all pups were sacrificed (by means Of CO_2) after standardization or weaning.

These pups, including stillborn pups and those that died during their rearing period, were subjected to a macroscopic (external and visceral) examination. Thereafter, stillborn pups and those that died during the rearing period as well as pups which were culled on day 4 p.p. or "surplus" pups (i.e. those with scheduled sacrifice after weaning), which showed any remarkable findings during rearing or abnormalities in the macroscopic assessment, were treated as described below (see 3.7.2.6. *of the study report*).

Matings of F0 and F1 generation parental animals

In general, each of the male and female animals was mated overnight at a 1 : 1 ratio for a maximum of 3 weeks. Generally, throughout the mating period, each male animal was mated with a predetermined female animal from the same dose group.

Matings occurred by placing the female in the cage of the male mating partner from about 4.00 p.m. until 7.00 - 9.00 a.m. of the following morning. Deviations from the specified times were possible on weekends and public holidays and were reported in the raw data.

A vaginal smear was prepared after each mating and examined for sperm. If sperm was detected, pairing of the animals was discontinued. The day on which sperm were detected was denoted "day 0" and the following day "day 1 " p.c. (post coitum).

Time schedule and study sequences

In the following table, the relevant intervals for certain study phases are given:

Time schedule

Phase of study/examination	F0 generation parental animals and progeny	F1 generation parental animals and progeny
Arrival of the animals	May 26,1998	not relevant
Acclimatization period	May 26-June 3, 1998	not relevant
Administration period	June 3-Nov.12, 1998	Oct. 7, 1998-Mar.9, 1999
Mating period for litter	F1: Aug.16-Aug. 20,1998	F2: Dec.20-Dec. 27,1998
Gestation period for litter	F1: Aug 17-Sep.12, 1998	F2: Dec.21, 1998-Jan.17, 1999
Birth of litter	F1: Sep.7-Sep. 13,1998	F2: Jan.11-Jan.18, 1999
Lactation period for litter	F1: Sep.7-Oct.3, 1998	F2: Jan.11-Feb.8, 1999
Sacrifice of litter (after weaning)	F1:Sep.27-Oct.3, 1998	F2: Feb.1-Feb.8, 1999
Sacrifice of parental animals*	Nov.2-Nov.4, Nov.12 and Nov.13, 1998	Mar.2, Mar.3 and Mar.8-Mar.10, 1999

* Before necropsy food was withdrawn for about 16 hours

[NOTE: a graphical depiction of the timeline was presented on page 39 of the study report]

Diet preparation and Analysis: (scanned from pages 35-36 of the study report)

Preparations of the mixtures of food and test substance

The test substance was frozen and mechanically crushed. Then an acetonic solution of the respective concentration was made. These solutions were sprayed on about 3 kg diet in a rotation vaporizer (130chi, Rotavapor R 153) under partial vacuum. Acetone was removed by heating up to about 400 C for about 30 minutes. Thereafter these premixes were adjusted to the desired concentrations with appropriate amounts of food and mixed for about 10 minutes in a Ruberg (EM 100) laboratory mixer.

The mixtures of test substance and food were prepared at intervals considering the different demand during the study periods and the proven stability. Storage was at room temperature.

Analyses

All analyses of the test substance preparations were carried out at the Bioanalytical Laboratory, Department of Toxicology of BASF Aktiengesellschaft, Ludwigshafen, FRG.
Analyses of the stability of the test substance in the diet up to 43 days at room temperature were carried out before the start of the study in a comparable batch (27882/37/a).

The homogeneity of the test substance in the diet was analytically investigated before the start of the study.

In order to check the correctness of the concentrations, samples of each one of the doses were drawn for concentration control analyses at the start of the administration period, thereafter in intervals of about 3-months during the study, and about 2 weeks before study termination.

Analytical methods

The methods used for the analytical investigations of the test substance preparations can be found in Volume III (Supplement of the study report).

Food analyses

The food used in the study was assayed for chemical as well as for microbiological contaminants.

Drinking water analyses

The drinking water is regularly assayed for chemical contaminants by the municipal authorities of Frankenthal and the Technical Services of BASF Aktiengesellschaft as well as for the presence of microorganisms by a contract laboratory.

Bedding analyses

The bedding is regularly assayed for contaminants (chlorinated hydrocarbons and heavy metals).

From the data provided, the stability of the test compound was at least least 43 days. Homogeneity was determined prior to the start of the study in samples of the 25 and 300 ppm concentrations and was found to be adequate. Concentration analysis showed that the target concentrations were within ±10%.

Animal assignment: (scanned from pages 32, 34 of the study report)

The rats of the parental generation (F0 and F1 generations) were identified uniquely by ear tattoo. The unit digit of the animal number was tattooed on the outside of a rat's left ear, the ten digit on the inside of the left ear and the hundred digit was tattooed on the inside of the right ear.

All live pups were identified by skin tattoo on day 1 post partum (p.p.) and with picric acid between days 10 and 15 after birth.

F0 generation parental animals

Test group	Concentration (ppm)	Number of animals		Animal number	
		Male	Female	Male	Female
00	0	25	25	1-25	101-125
01	25	25	25	26-50	126-150
02	75	25	25	51-75	151-175
03	300	25	25	76-100	176-200

F1 generation parental animals

Test group	Concentration (ppm)	Number of animals		Animal number	
		Male	Female	Male	Female
10	0	25	25	201-225	301-325
11	25	25	25	226-250	326-350
12	75	25	25	251-275	351-375
13	300	25	25	276-300	376-400

Observation Schedule

Parental animals: (scanned from pages 41-49, 52-55 of the study report)

CLINICAL EXAMINATIONS AND EXAMINATION OF REPRODUCTIVE PERFORMANCE

Mortality

At least once daily a check was made for dead or moribund animals. If animals were in a moribund state, they were sacrificed and necropsied. The examinations of these animals were carried out according to the methods in the laboratory of pathology.

Clinical observations

All parental animals were checked daily for clinically evident signs of toxicity. For technical reasons, however, the clinical observations recorded during the premating periods were printed out on a weekly basis (the daily observations can be found in the raw data).

The nesting, littering, and lactation behavior of the dams was generally evaluated in the mornings in connection with the daily clinical inspection of the dams. Only special findings (e.g., animal could not litter, umbilical cord not cut) were documented on an individual dam basis.

The littering behavior of the dams was also inspected on weekdays (except holidays) in the afternoons in addition to the evaluations in the mornings.

The day of littering was considered the 24-hour period from about 3.00 p.m. of one day until about 3.00 p.m. of the following day. Deviations from this procedure were possible on saturdays, sundays and on public holidays.

Animals in a moribund state were sacrificed and examined in the laboratory of pathology.

Food consumption

During the premating period of the F0 generation parental animals food consumption was determined once a week (in general for a period of 7 days). During the respective study phase of the second parental generation (171 parents) these measurements were performed again once weekly (in general for a period of 7 days).

After the 10th (F0 generation parental animals) or 28th (F1 generation parental animals) week, food consumption of the females during pregnancy (animals with evidence of sperm) was determined weekly on days 0, 7, 14, 20 p.c..

During the lactation period (animals with litter) food consumption was determined weekly on days 1, 4, 7, 14 p.p..

Food consumption was not determined between days 14 and 21 after parturition as required in the test guidelines cited under 2.3., since during this time pups will begin to consume considerable amounts of solid food offered, and therefore there was no point in such a measurement.

Food consumption of the males was not determined any longer after the 10th (F0 generation parental animals) or 28th (F1 generation parental animals) week through sacrifice. Furthermore, there was no determination of food consumption in the females during the mating periods, in the females without positive evidence of sperm during the programmed gestation phase, or in the females without litters during the lactation phase.

Body weight data

In general, the body weight of the male and female parental animals was determined once a week at the same time of the day (in the morning); if possible, the weighings were carried out until the end of the study.

The body weight change of the animals was calculated from these results.

The following exceptions are notable for the female animals:

a) During each mating period the F0 and the F1 generation parental females were weighed on the day of positive evidence of sperm (day 0 p.c.) and on days 7, 14 and 20 post coitum.

b) Females showing no positive evidence of sperm in vaginal smears were not weighed during the mating interval.

c) Females with litter were weighed on the day after parturition (day 1 p.p.) and on days 4, 7, 14 and 21 post partum.

d) Females without litter were not weighed during the lactation phase.

e) After weaning of the last F1 or F2 pups the female F0 or F1 generation parental animals were weighed again once weekly (parallel to the male FO or F1 generation parental animals) until sacrifice.

Intake of test substance

The intake of test substance was calculated from the amount of food consumed and expressed in mg/kg body weight per day.

The calculation of the group values/day was carried out according to the following formula:

$$IT_x = \frac{FC_x \times D}{BWy}$$

IT_x = intake of test substance on day x in mg/kg body weight/day
FC_x = daily food consumption on day x in grams
D = dose in ppm
Bwy = body weight on day y in grams (last weighing before day x)

The values listed in the Summary Tables are group means determined from the daily intakes of test substance by the individual animals.

Estrous cycle determinations

Estrous cycle length and normality were evaluated daily for all F0 and F1 female parental rats for a minimum of 3 weeks prior to mating and were continued throughout the mating period until the female exhibited evidence of mating. Moreover, at necropsy a vaginal smear was examined to determine the stage of the estrous cycle for each F0 and F1 female with scheduled sacrifice.

Male reproduction data

The mating partners, the number of mating days until vaginal sperm could be detected in the female, and the gestational status of the female were noted for F0 and F1 breeding pairs.

For the males, mating and fertility indices were calculated for F1 and F2 litters according to the following formulas:

$$\text{Male mating index (\%)} = \frac{\text{number of males with confirmed mating*}}{\text{number of males placed with females}} \times 100$$

* defined by a female with vaginal sperm or that gave birth to a litter or with fetuses in utero

$$\text{Male fertility index (\%)} = \frac{\text{number of males proving their fertility*}}{\text{number of males placed with females}} \times 100$$

* defined by a female giving birth to a litter or with pups/fetuses in utero

Sperm parameters

Immediately after necropsy and organ weight determination the right testis and cauda epididymis were taken from the males of all dose groups.

The following parameters were determined:

- sperm motility
- sperm morphology
- sperm head count (cauda epididymis)
- sperm head count (testis)

Sperm morphology and sperm head count (cauda epididymis and testis) were evaluated for the control and highest dose group, only.

Sperm motility examinations in F1 males were carried out in a randomized sequence.

The values obtained were transferred to a computer (VAX; DEC, Munich, FRG).

The methods used can be seen from the following table:

Parameter	Unit	Method
Sperm motility	%	microscopic evaluation
Sperm morphology	%	vital staining with eosin; microscopic evaluation
Sperm head count (cauda epididymis)	sperm heads x 10^6/g cauda epididymis.	microscopic evaluation with MAKLER chamber after homogenization
Sperm head count (testis)	sperm heads x 10^6/g testis	microscopic evaluation with MAKLER chamber after homogenization

	References
For Sperm Motility	**Slott, Suarez and Perreault, "Rat sperm motility analysis: Methodological considerations" in: "Reproductive Toxicology", Vol 5, PP. 449-458 (1991)**
For other sperm measures	**M.H. Feuston, K.R. Bodnar, S.L. Kerstetter, C.P. Grink, M.J. Belcak and E.J. Singer, "Reproductive Toxicity of 2-Methoxyethanol Applied Dermally to Occluded and Nonoccluded Sites in Male Rats" in: Toxicology and applied Pharmacology 100, 145-161 (1989) (Laboratory modification)**

Female reproduction and delivery data

The mating partners, the number of mating days until vaginal sperm could be detected, and gestational status were recorded for FO and F1 females.

For the females, mating, fertility and gestation indices were calculated for F1 and F2 litters according to the following formulas:

Female mating index (%) = $\frac{\text{number of females mated*}}{\text{number of females placed with males}} \times 100$

* defined as the number of females with vaginal sperm or that gave birth to a litter or with fetuses in utero

Female fertility index (%) = $\frac{\text{number of females pregnant*}}{\text{number of females mated**}} \times 100$

* defined as the number of females that gave birth to a litter or with pups/fetuses in utero

** defined as the number of females with vaginal sperm or that gave birth to a litter or with fetuses in utero

Gestation index (%) = $\frac{\text{number of females w/live pups on the day of birth}}{\text{number of females pregnant*}} \times 100$

* defined as the number of females that gave birth to a litter or with fetuses in utero

The total number of pups delivered and the number of liveborn and stillborn pups were noted, and the live birth index was calculated for F1 and F2 litters according to the following formula:

Live birth Index (%) = $\frac{\text{number of liveborn pups at birth}}{\text{total number of pups born}} \times 100$

The implantations were counted[1] and the postimplantation loss (in %) was calculated according the following formula:

Postimplantation loss (%) = $\frac{\text{number of implantations-number of pups delivered}}{\text{number of implantations}} \times 100$

[1] = After sacrifice of the female animals, the uteri and ovaries were removed (including the uteri of apparently non- pregnant animals) and transferred to the reproduction laboratory, for further investigations. To determine the number of implantation sites, the uteri were stained for about 5 minutes in 10% ammonium sulfide solution according to the method of SALEWSKI (Salewski, E.; 1964). Then the uteri were rinsed carefully under running water. Thereafter the implantation sites were recorded for calculation of the postimplantation loss.
After these examinations, the uteri were transferred to the pathology laboratory for further investigations.

Litters/pups

Litter data

Pup number and status at delivery

All pups derived from the F0 parents (F1 litter) and the F1 parents (F2 litter) were examined as soon as possible on the day of birth to determine the total number of pups and the number of liveborn and stillborn members of each litter. Pups, which died before the first determination of their status on the day of birth, were designated as stillborn pups.

Pup viability/mortality

In general, a check was made for any dead or moribund pups twice daily on workdays (once in the morning and once in the afternoon) or as a rule, only in the morning on saturdays, sundays or public holidays. Dead pups were evaluated by the methods, which will be described in detail in section 3.7.2.6 [of the study report].

The number and percentage of dead pups on the day of birth (day 0) and of pups dying between days 1- 4, 5-7, 8-14 and 15-21 of the lactation period were determined; however, pups which died accidentally or had to be sacrificed due to maternal death were not included in these calculations. The number of live pups/litter was calculated on the day of birth, and on lactation days 4, 7, 14 and 21. Furthermore, viability and lactation indices were calculated according to the following formulas:

Viability index (%) = $\frac{\text{number of live pups on day 4* after birth}}{\text{number of live pups on the day of birth}} \times 100$

* before standardization of litters (i.e. before culling)

Lactation index (%) = $\frac{\text{number of live pups on day 21 after birth}}{\text{number of live pups on day 4* after birth}} \times 100$

* after standardization of litters (i.e. after culling)

Sex ratio

On the day of birth (day 0) the sex of the pups was determined by observing the distance between the anus and the base of the genital tubercle; normally, the anogenital distance is considerably greater in male than in female pups. Subsequently the sex of the pups was assessed by the external appearance of the anogenital region and/or the mammary line of the animals and was finally confirmed at necropsy.

The sex ratio was calculated at day 0 and day 21 after birth according to the following formula:

$$\text{Sex ratio} = \frac{\text{number of live male or female pups on day 0/21}}{\text{number of live male and female pups on day 0/21}} \times 100$$

Pup body weight data

The pups were weighed on the day after birth (day 1 p.p.) and on days 4 (before standardization), 7, 14 and 21 after birth.

Pups' body weight change was calculated from these results.

The individual weights were always determined at about the same time of the day (in the morning) and on day 4 p.p. immediately before standardization of the litters.

In the relevant summary tables pup body weights and pup body weight gains are listed for males, females and males + females.

Pup clinical observations

All live pups were examined each day for clinical symptoms (including gross-morphological findings).

Sexual maturation

Vaginal opening

All female pups selected to become the F1 parental generation females (25/group) were evaluated daily for vaginal opening with examinations initiating on day 27 p.p.

Preputial separation

All male pups selected to become the F1 parental generation males (25/group) were evaluated daily for preputial separation with examinations initiating on day 35 p.p.

Pup organ weights

After scheduled sacrifice brain, spleen and thymus of 1 pup/sex and litter from the F1 and F2 pups were weighed.

Normally, the first male and first female pup/litter were taken for these determinations. For the calculation of the respective relative organ weights, the pup body weights, determined routinely during the in-life phase on day 21 p.p., were taken.

Pup necropsy observations

All pups with scheduled sacrifice (i.e. pups, which were culled on day 4 p.p., and pups, which were sacrificed on day 21 after birth or subsequent days) were killed by means of CO_2. These pups were examined externally and eviscerated, their organs were assessed macroscopically. The pups with findings (macrophthalmia, anophthalmia) were further processed and examined according to WILSON'S method (Wilson, J.G. and Warkany, J., 1965).

All stillborn pups and all pups that died up to weaning were examined externally, eviscerated, and their organs assessed macroscopically.

All pups without any notable findings or abnormalities were discarded after their macroscopic evaluation. The pups processed and examined according to WILSON'S method were discarded after the confirmation of the clinically noted findings.

PATHOLOGY

Necropsy

The animals were sacrificed by decapitation under CO_2 anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. Animals that died intercurrently were necropsied and assessed as soon as possible after death to minimize postmortem autolysis.

Organ weights

The following weight parameters of all F0 and F1 parental animals sacrificed at scheduled dates were determined:
1. anesthetized animals
2. liver
3. kidneys
4. epididymides (total and cauda)
5. testes
6. uterus (with cervix uteri and oviducts)
7. ovaries
8. seminal vesicles (with coagulating glands and their fluids)
9. prostate gland
10. thymus
11. brain
12. pituitary gland
13. adrenal glands
14. spleen

Histopathology

The following organs or tissues were fixed in 4% formaldehyde solution (Fo) or in BOUIN's solution (B), respectively:

1. vagina (Fo)
2. cervix uteri (Fo)
3. uterus with (Fo)
4. ovaries (B)
5. oviducts (Fo)
6. left testis (B)
7. left epididymis (B)
8. seminal vesicles (Fo)
9. coagulating glands (Fo)
10. prostate gland (Fo)
11. pituitary gland (Fo)
12. liver (Fo)
13. kidneys (Fo)
14. urinary bladder (Fo)
15. thymus (Fo)
16. spleen (Fo)
17. brain (Fo)
18. adrenal glands (Fo)
19. all gross lesions (Fo)

After fixation, processing, the examination by light microscopy and the evaluation was performed according to the following table:

Organs	Test groups			
	00/10	01/11	02/12	03/13
all gross lesions	A2	A2	A2	A2
vagina	A1	A3	A3	A1
cervix uteri	A1	A3	A3	A1
uterus	A1	A3	A3	A1
ovaries*	A1	A3	A3	A1
oviducts	A1	A3	A3	A1
pituitary gland	A1	A3	A3	A1
left testis	A1	A3	A3	A1
left epididymis	A1	A3	A3	A1
prostate gland	A1	A3	A3	A1
seminal vesicles	A1	A3	A3	A1
coagulating glands	A1	A3	A3	A1
liver	A1	A3	A3	A1
kidneys	A1	A1	A1	A1
thymus	A1	A3	A3	A1
adrenal glands	A1	A3	A3	A1

Methods and scope of examination:

A = hematoxylin and eosin stain
1 = all animals per group
2 = all animals affected per group
3 = animals suspected of impaired fertility

Of the animals that died intercurrently or that had to be sacrificed prematurely, the scope of histologic investigation was the same as indicated for the animals of the control groups 00 and 10, respectively.

An attempt was made to correlate the gross lesions with a meaningful microscopic finding.

* = Follicle Count:

After appropriate fixation, both ovaries of each animal were embedded in the same way one upon another in one paraplast block. As an association with right or left ovary was not possible at that time due to technical reasons, the ovaries were indicated as "Ovary 1 " and "Ovary 2" in the Result tables. With the slide label to the right, "Ovary 1 " refers to the upper ovary on the slide, and "Ovary 2" indicates the lower ovary on the same slide, respectively. Hematoxylin and eosin (H.E.) stained serial sections of about 3 microns thickness were prepared, with a distance of about 50 microns of dismissed ovarian tissue between each consecutive slide.

The first cut was taken, when both ovaries showed a reasonable amount of tissue, i.e. both organs revealed parts of cortex and medulla. This slide was labeled "No. 1 ". The last slide was taken, when the medulla faded away in one or both organs at gross appearance. Depending on the size of the ovaries, 10 to 20 serial sections were obtained, which were numbered from No. 1 to the highest number. Evaluation was performed on 10 slides, which showed both cortex and medulla. The inner part of the medulla (located in the mid part of the slide series - comprising a distance of about 200 microns and consisting of about three to four slides) was excluded from the assessment where possible (more than 10 slides available). Depending on the number of serial sections obtained at the microtome, the 10 slides selected for assessment were chosen as follows:

No. of serial ovarian sections per animal	slide Nos. assessed dorsal of the ovarian core	slide Nos. assessed ventral of the ovarian core
10	1-5	6-10
11	1-5	7-11
12	1-5	8-12
13	1-5	9-13
14	2-6	10-14
15	2-6	10-14
16	3-7	11-15
17	3-7	11-15
18	4-8	12-16
19	4-8	13-17
20	4-8	13-17

On each of the ten selected slides, follicle count was performed on "primordial follicles" (comprising types 1, 2, 3a, and 3b) , "growing follicles" (comprising types 4, 5a, and 5b) and "antral follicles" (comprising types 7 and 8) according to the definitions given by Plowchalk et al. (PLOWCHALK, D. R., B. J. SMITH, and D. R. MATTISON: Assessment of Toxicity to the Ovary Using Follicle Quantitation and Morphometrics. In: Methods in Toxicology, Vol. 3, Part B: Female Reproductive Toxicology (J. J. HEINDEL and R. E. CHAPIN, Editors), p. 57-68, 1993, Academic Press). Type 6 of the antral type of follicles was counted as growing follicles, whenever the cumulus of granulosa cells was equal or larger than 6 cells one upon another at the smallest edge of the follicle.

To prevent multiple counting especially of the growing and antrai follicles, only follicles, which had an oocyte with visible chromatin on the slide, were counted.

On the 5th slide of the first series (e. g. slide No. 7 out of a series of 16 slides), all corpora lutea (CL) were counted.

In general, on this 5th slide, the other microscopic findings associated with the ovaries were assessed as well. However, if a specific 5th finding was noted in any of the other slide levels that could not be detected on the slide, this finding was also recorded.

Using EXCEL tables for the reporting of the results, the incidence of each type of follicle was recorded individually for ovary 1 and ovary 2 for every animal and for all slides evaluated. Then a total was determined for each animal (combining both ovaries) for the incidence of each type of follicle evaluated (in the ten levels of slides) and the number of CL (counted on one slide level). Finally, the results of all types of follicles and CL are summarized for all 25 animals per group in dose groups 10 and 13 (F1 generation parental animals) and 00 and 03 (170 generation parental animals), respectively. As primordial follicles continuously develop into growing follicles, the assessment of the follicles was extended to the combined incidence of primordial plus growing follicles. The numbers are not indicated in the tables as they are the simple result of addition of two numbers already present in the tables.

Statistical Analyses (scanned from pages 49-50 and 54 of the study report)

Statistics of the clinical examinations*

Statistical analyses were performed according to following tables:

Parameter	Statistical test	Markers In the tables	References
Food consumption (parental animals), body weight and body weight change (parental animals and pups; for the pup weights, the litter means were used), estrous cycle duration, number of mating days, duration of gestation, number of pups delivered per litter, duration of sexual maturation (days to preputial separation, days to vaginal opening)	Simultaneous comparison of all dose groups with the control group using the DUNNETT-test (two-sided) for the hypothesis of equal means	* for $p \leq 0.05$ ** for $p \leq 0.01$	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA Vol. 50 1096-1121 DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482 - 491
Male and female mating index, male and female fertility index, gestation index, females with liveborn pups, females with stillborn pups, females with all stillborn pups, live birth index, pups stillborn, pups died, pups cannibalized, pups sacrificed moribund, viability index, lactation index, number of litters with affected pups at necropsy, sexual maturation data (preputial separation, vaginal opening)	Pairwise comparison of each dose group with the control group using FISHER'S EXACT test for the hypothesis of equal proportions	* for $p \leq 0.05$ ** for $p \leq 0.01$	Siegel, S. (1956): Non parametric statistics for behavioral sciences. McGraw-Hill New York
Proportions of affected pups per litter with necropsy observations, sperm parameters (total spermatids/g testis, total sperm/g cauda epididymides, % normal and abnormal sperm, % motility	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	Nijenhuis, A.; Wilf H.S. (1978): Combinatorial Algorithms. Academic Press New York, 32-33 Hettmansperger, T.P. (1984); Statistical Inference based on Ranks. John Wiley & Sons New York, 132-142

Pup organ weights (absolute and relative)	**Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided).If the resulting p-value was** **equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using the WILCOXON-test (two-** **sided) for the equal medians**	*** for $p \leq 0.05$** **** for $p \leq 0.01$**	**Miller, R.G. (1981): Simultaneous Statistical Inference. Springer Verlag New York Inc., 165-167** **International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042 3020, USA** **Nijenhuis, A.; Wilf H. S. (1978): Combinatorial Algorithms, Academic Press New York, 32-33** **Hettmansperger, T. P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132-142**

* Note: For the parameter food consumption the "mean of means" was calculated and can be found in the relevant summary tables. The "mean of means" values allow a rough estimation of the total food consumption during the different time intervals (premating, gestation and/or lactation); they are not exactly precise values, because the size of the intervals taken for calculation may differ (especially during gestation and lactation periods). For the "mean of means" values no statistical analysis was performed.

Statistics of pathology

Means and standard deviations of each test group were calculated for the variables of terminal body weight and of absolute and relative organ weights (related to terminal body weight) of the animals in each test group. Further statistical analyses were performed according to following tables:

Parameters	Statistical test	Markers in the tables	References
Weight parameters	Non-parametric. one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians.	* for $p \leq 0.05$ ** for $p \leq 0.01$	HETTMANNSPERGER, T. P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132- 142. International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1 - nakl-3. MILLER, R. G. (1981): Simultaneous Statistical Inference Springer-Verlag New York Inc., 165-167. NIJENHUIS, A. and WILF, H.S. (1978): Combinatorial Algorithms, Academic Press, New York, 32-33.
Follicles: primordial, growing, primordial + growing, antral and corpora lutea	Pairwise comparison of the high dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians.	* for $p \leq 0.05$ ** for $p \leq 0.01$	SIEGEL, S. (1956): Non-parametric statistics for behavioral sciences. Mc.Graw-Hill, New York.

REFERENCES [from page 101 of the study report]

NIJENHUIS, A. and WILF, H.S.
Random permutation of n letters
Combinatorial Algorithms, Academic Press, New York, San Francisco, London, 62 - 64 (1978)

SALEWSKI, E.
Farbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte
Naunyn-Schmiedeberg's Arch. exp. Pathol.
Pharmakol. 247, 367 (1964)

WILSON, J.G. and WARKANY, J.
Teratology: Principles and Techniques
The University of Chicago Press.
Chicago and London (1965)

HOOD, R.D.
Handbook of Developmental Toxicology
CRC Press
Boca Raton, New York, London, Tokyo (1997)

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:

No substance related mortality was noted in any of the F0 or F1 parental animals; however, one F0 high dose female died on the first day of lactation and was assumed to be spontaneous in origin. No clinical signs of toxicity were note in the F0 or F1 parental animals during the premating periods. For the F0 females during the gestation period, 2 dams, 1 each in the 25 and 75 ppm dose groups, exhibited insufficient nesting behavior and one 75 ppm sperm positive female did not deliver. For the F1 females, 2 sperm positive females, 1 each in the 25 and 75 ppm dose groups, did not deliver. For the F0 females during lactation, 1 high dose female was found dead on day 0 p.p.. 1 control and 3 low dose females did not nurse their pups correctly and the pups were cannibalized, and the only pup of a 75 ppm dose dam died on day 5 p.p. For the F1 females during lactation, one mid dose female did not nurse her pups correctly and therefore all pups were cannibalized by day 7 p.p. and the only pup of a mid dose dam was cannibalized on day 3 p.p. Since no dose response was noted, these observations were not considered to be related to treatment.

Body weight and Food consumption:

The following Table I (from Tables IA- 007 and 011-014; pages 112, 116-119 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	25 ppm	75 ppm	300 ppm
		Body Weights (g)		
Week 0	129.5±14.5	128.8±13.6	128.8±14.5	128.7±14.4(99)[1]
Week 5	333.9±23.5	337.2±25.8	341.7±21.1	327.5±25.1(98)
Week 10	416.8±27.3	421.1±35.7	424.9±24.7	402.9±31.0(97)
Week 15	456.0±27.7	465.9±42.8	467.1±31.6	442.1±34.3(97)
Week 21	487.3±31.2	505.1±48.7	501.4±37.9	474.1±38.1(97)
		Body Weight Gains (g)		
Wks 0-21	357.7±29.0	376.3±47.8	372.6±34.3	345.3±35.0(97)
		Food Consumption (g/animal/day)		
Wks 0-10	26.0±1.2	26.5±1.1	26.6±1.2	25.3±1.3(97)

[1] = percent of control

The F0 male high dose group had very slight decreases in body weight, body weight gain and food consumption; however the biological relevance is unclear.

The following Table II (from Tables IA- 050 and 054-057; pages 155, and 159-162 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	25 ppm	75 ppm	300 ppm
		Body Weights (g)		
Week 0	85.7±14.9	92.4±9.5	89.6±10.7	78.1±9.0(91)[1]
Week 5	318.7±34.8	320.4±14.9	334.6±27.0	297.4*±20.8(93)
Week 10	412.4±36.4	410.1±26.2	439.6*±37.5	388.4±31.9(94)
Week 15	461.7±44.8	459.0±30.0	494.8**±42.8	440.1±31.9(95)
Week 20	499.6±47.7	501.4±38.8	540.7**±47.5	482.2±36.5(97)
		Body Weight Gains (g)		
Wks 0-20	413.9±42.7	409.0±38.0	451.1**±43.1	404.1±31.7(98)
		Food Consumption (g/animal/day)		
Wks 0-10	26.6±2.9	26.8±2.7	27.9±3.3	25.4±3.4(96)

[1] = percent of control; * = $p < 0.05$; ** = $p < 0.01$ by 2 sided Dunnett-test.

The F1 male high dose group had very slight decreases in body weight, body weight gain and food consumption and the mid dose had increases in the same parameters; however the biological relevance is unclear.

The following Table III (from Tables IA- 008 and 015-016; pages 113, and 120-121 of the study report) presents selected body weight and food consumption data for F0 females for the premating period (all values means ± standard deviation if available):

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

Dose:	Control	25 ppm	75 ppm	300 ppm
		Body Weights (g)		
Week 0	118.5±5.9	118.8±8.6	117.9±6.1	118.2±6.4
5	217.8±9.9	218.6±19.1	219.8±16.0	211.7±12.6(97)
10	263.1±12.5	263.9±21.5	265.0±19.9	252.3±14.2(96)
		Body Weight Gains (g)		
Wks 0-5[1]	99.3	99.8	101.9	93.5(94)
0-10	144.6±11.8	145.0±19.8	147.1±16.7	134.1±11.9(93)
		Food Consumption (g/animal/day)		
Wks 0-10	19.9±0.6	19.9±0.6	20.0±0.5	18.8±0.5(95)

1 = percent of control

The F0 female high dose group had very slight decreases in body weight, body weight gain and food consumption during the premating period; however the biological relevance is unclear.

The following Table IV (from Tables IA- 009 and 017-018; pages 114, and 122-123 of the study report) presents selected body weight and food consumption data for F0 females for the gestation period (all values means ± standard deviation if available):

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

Dose:	Control	25 ppm	75 ppm	300 ppm
		Body Weights (g)		
Day 0	266.0±12.1	263.7±23.1	266.0±20.4	252.4*±15.0(95)
Day 7	293.5±12.5	290.8±25.2	292.5±21.3	280.7±15.4(96)
Day 14	324.2±13.2	323.6±26.9	323.9±23.5	310.9±18.4(96)
Day 20	396.6±22.5	395.8±30.5	391.0±28.4	378.4*±22.6(95)
		Body Weight Gains (g)		
Days				
0-7	27.5±5.5	27.1±6.5	26.5±5.0	28.34±5.3
0-14[1]	58.2	59.9	57.9	58.5
0-20	130.6±17.6	132.1±17.5	125.0±17.3	125.9±14.5(96)
		Food Consumption (g/animal/day)		
Days				
0-7	24.4±1.4	24.6±2.0	25.0±2.1	23.6±1.6(97)
7-14	25.4±1.6	25.9±2.1	26.2±2.0	24.4±1.5(96)
14-20	26.1±1.3	26.5±2.6	26.9±2.0	25.6±1.5(98)
0-20	25.3±0.8	25.6±1.0	26.0±1.0	24.5±1.0(97)

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

The F0 female high dose group had very slight decreases in body weight, body weight gain and food consumption during the gestation period; however the biological relevance is unclear.

Historical Control Body Weights (g)

Day 0	Day 7	Day 14	Day 20
283.5	310.1	340.8	413.4

The following Table V (from Tables IA- 010, and 019-020; pages 115, and 124-125 of the study report) presents selected body weight and food consumption data for F0 females for the lactation period (all values means ± standard deviation if available):

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

Dose:	Control	25 ppm	75 ppm	300 ppm
		Body Weights (g)		
Day 1	300.9±17.0	300.6±30.1	303.4±24.3	286.7±18.7(95)[2]
Day 4	315.3±16.6	310.5±29.0	314.3±25.1	299.8±20.3(95)
Day 7	320.8±16.7	319.3±27.6	319.7±23.0	311.3±21.6(97)
Day 14	334.5±18.4	334.7±23.5	333.3±24.3	318.1*±21.3(95)
Day 21	325.2±17.2	321.3±24.4	324.5±21.7	310.8±19.5(96)
		Body Weight Gains (g)		
Days				
1-4	14.4±8.0	9.8±10.3	10.8±9.3	13.1±10.5(91)
1-7[1]	19.9	18.7	16.3	24.6
1-14[1]	33.6	34.1	29.9	31.4(93)
4-7	5.6±8.1	8.8±9.8	5.5±6.7	11.5*±6.2
7-14	13.6±8.4	15.4±8.5	13.5±11.0	6.8*±7.9(50)
14-21	-9.2±10.8	-13.3±13.8	-8.8±8.4	-7.3±8.9
1-21	24.3±15.9	20.7±17.7	21.0±15.8	24.1±13.5(99)
		Food Consumption (g/animal/day)		
Days				
1-4	37.4±6.6	35.5±6.3	35.6±7.5	36.7±4.4(98)
4-7	44.2±6.3	43.7±8.0	43.4±6.5	45.2±4.3
7-14	57.5±9.5	57.4±11.7	56.1±9.9	55.7±3.7(97)
1-14	46.3±10.2	45.6±11.1	45.0±10.3	45.9±9.5(99)

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

The F0 female high dose group had very slight decreases in body weight, body weight gain and food consumption during the lactation period; however the biological relevance is unclear.

Historical Control Body Weights (g)

Day 0	Day 1	Day 4	Day 7	Day 14	Day 21
325.5	323.3	333.8	338.7	349.3	337.6

The following Table VI (from Table IA- 021; page 126 of the study report) presents selected body weight data for F0 females after weaning (all values means ± standard deviation if available):

Table VI: Body Weights

Dose:	Control	25 ppm	75 ppm	300 ppm
		Body Weights (g)		
Week 18	297.5±12.4	297.9±24.6	298.4±20.9	277.6**±15.5(93)[1]
Week 19	304.5±14.6	305.2±26.5	307.2±23.5	287.4*±17.6(94)
Week 20	303.9±14.2	303.7±25.9	307.0±23.0	286.6*±18.8(94)
Week 21	303.2±13.5	304.0±27.9	308.4±22.2	288.1*±18.1(95)

[1] = percent of control

The F0 female high dose group had very slight decreases in body weights after weaning; however the biological relevance is unclear.

The following Table VII (from Tables IA- 051 and 058; pages 156 and 163-164 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 VII: Body Weights, Body Weight Gains and Food Consumption

Dose:	Control	25 ppm	75 ppm	300 ppm
		Body Weights (g)		
Week 0	80.9±7.5	85.7±10.6	82.6±9.5	73.4*±7.7(91)
Week 5	201.3±12.6	208.0±14.4	210.4±14.2	195.5±13.9(97)
Week 10	250.1±18.2	259.9±17.8	262.0±17.3	246.5±17.0(99)
		Body Weight Gains (g)		
Wks 0-10	169.2±17.9	174.2±18.8	179.5±16.6	173.1±15.2
		Food Consumption (g/animal/day)		
Wks 0-10	20.2±1.4	20.6±1.3	20.5±1.3	19.1±1.5(95)

[1] = percent of control; * = $p < 0.05$ by 2-sided Dunnett-test.

The F1 female high dose group had very slight decreases in body weights during the premating period; however the biological relevance is unclear. Food consumption was statistically significantly reduced in the high dose group at weeks 0-1, 5-6, 6-7, 8-9 and 9-10 and were reduced at other time points (not s.s.) and for overall 0-10 weeks.

The following Table VIII (from Tables IA- 052, 060, and 061; pages 157, 165, and 166 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 VIII: Body Weights, Body Weight Gains and Food Consumption

Dose:	Control	25 ppm	75 ppm	300 ppm
		Body Weights (g)		
Day 0	252.9±16.2	262.4±16.4	263.7±19.4	248.3±15.4(98)
Day 7	282.1±17.5	289.1±16.8	291.7±19.8	278.4±16.7(99)
Day 14	316.5±19.3	322.5±15.5	322.2±18.4	308.2±18.0(97)
Day 20	381.5±23.2	384.6±27.4	378.7±29.0	373.6±24.7(98)
		Body Weight Gains (g)		
Days				
0-7	29.2±7.8	26.8±6.0	28.0±7.8	30.0±6.1
0-14[1]	63.6	60.1	58.5	59.9(94)
0-20	128.6±18.3	122.2±25.6	115.0±29.6	125.3±14.9(97)
7-14	34.4±6.7	33.4±5.4(97)	30.5*±4.9(89)	29.9*±4.2(87)
14-20	65.0±14.3	62.0±21.9	56.5±26.4	65.4±11.8
		Food Consumption (g/animal/day)		
Days				
0-7	23.7±1.7	23.7±1.7	23.9±1.7	23.3±1.5(98)
7-14	26.4±1.5	26.5±1.3	26.5±2.0	24.5**±1.5(93)
14-20	27.7±2.3	27.0±2.1	26.8±2.6	26.1*±1.7(94)
0-20	25.9±2.0	25.8±1.8	25.7±1.6	24.6±1.4(95)

[1] = calculated by the reviewer from body weight mean values; * = $p < 0.05$; ** = $p < 0.01$ by 2-sided Dunnett-test.

The F1 female high dose group had very slight decreases in body weights for the gestation period with statistically significant differences in body weight gain days 7-14; however the absolute weights were not that different (1-3%) and there was statistically significantly lower food consumption at that time point. This was not seen in the F0 females during the same time period; therefore the biological relevance is unclear.

The following Table IX (from Tables IA- 053, and 062-063; pages 158 and 167-168 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 IX: Body Weights, Body Weight Gains and Food Consumption

Dose:	Control	25 ppm	75 ppm	300 ppm
		Body Weights (g)		
Day 1	298.6±20.8	302.5±21.8	294.1±24.6	288.3±17.4(97)
Day 4	301.4±20.3	306.7±19.8	301.9±22.0	291.3±20.9(97)
Day 7	308.3±22.5	310.6±18.5	312.5±21.7	304.1±22.3(97)
Day 14	324.5±22.1	322.7±18.6	323.4±24.1	314.6±22.1(97)
Day 21	322.4±23.6	322.6±15.8	321.8±20.5	315.2±18.1(98)
		Body Weight Gains (g)		
Days				
1-4	2.8±9.7	4.2±8.9	7.9±8.9	3.0±10.3
4-7	6.9±7.0	3.9±7.5	10.6±9.9	12.8*±6.0
7-14	16.2±10.5	12.1±11.4(75)	10.9±12.6(67)	10.5±9.6(65)
14-21	-2.1±9.5	-0.1±12.2	-1.7±12.6	0.6±9.3
1-7[1]	9.7	8.1	18.4	15.8
1-14[1]	25.9	20.2	29.3	26.3
1-21	23.8±12.0	20.1±12.9	27.7±15.1	27.0±13.7
		Food Consumption (g/animal/day)		
Days				
1-4	34.2±5.8	34.6±5.5	33.2±7.3	34.6±5.3
4-7	42.3±5.1	43.2±4.2	40.7±8.7	42.1±3.7
7-14	59.2±7.2	58.7±4.8	53.6±14.4	56.9±4.9(96)
1-14	45.2±12.8	45.5±12.2	42.5±10.3	44.5±11.3(99)

[1] = calculated by the reviewer from body weight mean values; * = $p < 0.05$ by 2-sided Dunnett-test.

The F1 female high dose group had very slight decreases in body weights for the lactation period with statistically significant differences in body weight gain days 7-14; however the absolute weights were not that different (3%) and there was lower food consumption at that time point. Changes in body weight occur during lacation due to a variety of effects, including the nurign of the pups; therefore the biological relevance is unclear, this is not considered a toxicological effect.

The following Table XI (from Table IA- 064; page 169 of the study report) presents selected body weight data for F1 females after weaning (all values means ± standard deviation if available):

Table XI: Body Weights

Dose:	Control	25 ppm	75 ppm	300 ppm
		Body Weights (g)		
Week 18	295.9±21.7	299.1±15.2	298.8±18.5	280.5*±20.5(95)[1]
Week 19	302.4±19.6	302.8±17.9	304.4±20.4	283.3**±19.9(94)
Week 20	305.3±20.1	306.8±16.3	306.9±20.0	288.4**±17.6(95)
Week 21	304.8±20.8	309.1±16.5	309.0±20.2	289.0*±19.5(95)

The F1 female high dose group had very slight decreases in body weights after weaning; however the biological relevance is unclear.

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 XII) during the pre-mating period (from Tables IA- 023-024, and 066-067; pages 128-129 and 171-172 of the study report):

Table XII: Mean Test Substance Intake

Dose: Weeks	25 ppm	75 ppm	300 ppm
		F0 Generation	
0-10	2.5±1.0/2.6±0.6[1]	7.4±3.0/7.8±2.0	29.0±10.6/30.4±7.1
		F1 Generation	
0-10	2.8±1.3/3.0±1.0[1]	8.6±3.9/9.0±3.1	35.0±14.9/36.0±12.0

[1] = males/females

Parental animals and offspring

Reproductive performance:

Results for the mating of F0 parental animals and the F1a pups are summarized from the report on Table XIII (from Tables IA- 027-035, pages 132-140) as follows (mating of 25 animals per sex per dose group):

Table XIII: Reproductive Performance and Lactation Observations

Dose:	Control	25 ppm	75 ppm	300 ppm
		F0 Generation		
Observation				
Estrus cycle duration	4.0±0.0	4.1±0.5	4.0±0.1	4.0±0.0
Mean precoital interval (days)	2.8±0.8	2.0**±0.8	2.2*±0.9	2.5±1.0
		Males		
Mated	25	25	25	25
Mating index(%)	100	100	100	100
Fertile	25	25	24	25
Fertility index(%)	100	100	96	100
Total spermatids/g testis	105±9.7			108±10.9
Total spermatids/g cauda epididymis	472±82.3			485±77.7
% normal sperm	98.2±1.4			98.6±0.9
% abnormal sperm	1.8±1.4			1.4±0.9
% motility	90±5.9	89±5.5	90±6.6	89±6.6
		Females		
Mated	25	25	25	25
Mating index(%)	100	100	100	100
Fertile	25	25	24	25
Fertility index(%)	100	100	96	100
Number of litters	25	25	24	25
Gestation index(%)	100	100	100	100
Mean gestation interval (days)	21.9±0.3	22.0±0.5	21.9±0.3	22.0±0.0
Total litter losses	0	0	0	0
Litters w/stillborn	5(20%)	10(40%)	4(17%)	5(20%)
Total pups	363	351	301	328

continued

Table XIII: Reproductive Performance and Lactation Observations continued

Dose:	Control	25 ppm	75 ppm	300 ppm
		F0 Generation		
Observation				
Litter size	14.5±3.7	14.0±3.1	12.5±4.3	13.1±2.7
Pups liveborn	356	336	295	322
Live birth index(%)	98	96	98	98
Pups stillborn	7	15	6	6
Perinatal loss(%)	1.9	4.3	2.0	1.8
Pups:				
died	31	40	1	8
sacrificed	0	0	0	0
cannibalized	1	3	2	4
accidental death	0	0	0	0
sac. (Mat death)	0	0	0	13
Pups culled day 4	136	114	117	110
Pups - all deaths				
day 0	1	5	0	1
days 1-4	23	38	1	9
days 5-7	8	0	1	0
days 8-14	0	0	1	2
days 15-21	0	0	0	0
Pup survival				
days 0-4	332	293**	294	299
Viability index(%)	93	87	100	93
days 4-21	188	179	175	187
Lactation index(%)	96	100	99	99
Live pups per litter				
day 0	14.2±3.7	13.4±3.7	12.3±4.2	12.9±2.6
day 4 precull	13.3±3.5	11.7±4.6	12.3±4.2	12.0±3.5
day 4 postcull	7.8±0.8	7.2±2.2	7.4±1.7	7.6±1.6
day 7	7.5±1.6	7.2±2.2	7.3±1.9	7.6±1.6
day 14	7.5±1.6	7.2±2.2	7.3±1.9	7.5±1.7
day 21	7.5±1.6	7.2±2.2	7.3±1.9	7.5±1.7
Sex ratio (% males)				
day 0	49.7	47.9	51.9	54.7
day 21	48.9	48.6	50.3	51.9
Implantation sites	417	404	348	361
Postimplantation loss (%)	13.3±15.7	12.6±15.6	15.6±18.1	9.1±12.1

continued

Table XIII: Reproductive Performance and Lactation Observations continued

Dose:	Control	25 ppm	75 ppm	300 ppm
		F0 Generation		
Observation				
Pup weights				
day 1	6.4±0.5	6.3±0.7	6.6±0.7	6.6±0.5
day 4 precull	9.2±1.5	9.2±1.4	9.6±1.3	9.5±1.0
day 4 postcull	9.3±1.5	9.2±1.3	9.6±1.3	9.5±1.1
day 7	14.8±2.6	14.7±2.0	15.3±1.7	14.6±1.5(99)[1]
day 14	31.1±5.5	31.9±2.9	31.7±2.4	29.8±2.4(96)
day 21	51.3±8.8	52.5±4.5	51.6±4.0	46.4*±3.5(90)

* = $p < 0.05$; ** = $p < 0.01$ by 2-sided Dunnett-test; [1] = percent of control.

There were no apparent effects on reproductive performance and parameters for the F0 matings and the F1 pups. Although there was slightly smaller litter size for the mid and high dose groups, after culling, there was no effect on litter size through weaning, also the control and low dose litter size was on the high side.

Results for the F1 parental animals and the F2 pups are summarized from the report on Table XIV (from Tables IA- 070-078, pages 175-183) as follows (mating of 25 animals per sex per dose group):

Table XIV: Reproductive Performance and Lactation Observations

Dose:	Control	25 ppm	75 ppm	300 ppm
		F1 Generation		
Observation				
Estrus cycle duration	4.2±0.8	4.1±0.3	4.0±0.0	4.0±0.0
Mean precoital interval (days)	1.9±1.1	2.0±1.1	2.0±1.0	2.8*±1.3
		Males		
Mated	25	25	25	25
Mating index(%)	100	100	100	100
Fertile	25	24	24	25
Fertility index(%)	100	96	96	100
Total spermatids/g testis	98±10.9			97±11.3
Total spermatids/g cauda epididymis	660±95.7			667±153.1

continued

Table XIV: Reproductive Performance and Lactation Observations

Dose:	Control	25 ppm	75 ppm	300 ppm
		F1 Generation		
Observation				
% normal sperm	98.5±1.1			98.8±0.8
% abnormal sperm	1.5±1.1			1.2±0.8
% motility	92±4.7	89±19.1	90±11.4	92±4.0
		Females		
Mated	25	25	25	25
Mating index(%)	100	100	100	100
Fertile	25	24	24	25
Fertility index(%)	100	96	96	100
Number of litters	25	23	23	25
Gestation index(%)	100	96	96	100
Mean gestation interval (days)	22.0±0.2	22.0±0.0	22.0±0.4	22.0±0.2
Total litter losses	0	0	0	0
Litters w/stillborn	5(20%)	3(13%)	3(13%)	1(4%)
Total pups	328	299	287	350
Litter size	13±3.1	13.0±3.4	12.5±4.8	14.0±2.7
Pups liveborn	320	295	283	346
Live birth index(%)	98	99	99	99
Pups stillborn	8	4	4	4
Perinatal loss(%)	2.4	1.3	1.4	1.1
Pups:				
died	7	4	11	8
sacrificed	0	0	0	0
cannibalized	2	3	14**	9*
accidental death	0	0	0	0
sac. (Mat death)	0	0	0	0
Pups culled day 4	118	110	107	134
Pups - all deaths				
day 0	0	0	0	1
days 1-4	9	7	15	15
days 5-7	0	0	9	2
days 8-14	0	0	1	0
days 15-21	0	0	0	0
Pup survival				
days 0-4	311	288	268	331

continued

Table XIV: Reproductive Performance and Lactation Observations continued

Dose:	Control	25 ppm	75 ppm	300 ppm
		F1 Generation		
Observation				
Viability index(%)	97	98	95	96
days 4-21	193	178	151**	195
Lactation index(%)	100	100	94	99
Live pups per litter				
day 0	12.8±3.1	12.8±3.5	12.3±4.8	13.8±2.8
day 4 precull	12.4±3.3	12.5±3.6	11.7±4.8	13.2±2.6
day 4 postcull	7.7±1.2	7.7±0.8	7.0±2.3	7.9±0.6
day 7	7.7±1.2	7.7±0.8	6.6±2.7	7.8±0.7
day 14	7.7±1.2	7.7±0.8	6.6±2.7	7.8±0.7
day 21	7.7±1.2	7.7±0.8	6.6±2.7	7.8±0.7
Sex ratio (% males)				
day 0	49.4	49.8	46.6	53.2
day 21	49.2	50.0	50.3	50.3
Implantation sites	363	330	317	386
Postimplantation loss (%)	10.1±9.5	13.9±20.9	14.3±25.9	9.0±8.4
Pup weights				
day 1	6.4±0.4	6.4±0.4	6.5±0.9	6.3±0.5(98)[1]
day 4 precull	9.2±0.9	9.3±1.1	9.0±1.5	8.7±0.8(95)
day 4 postcull	9.3±0.8	9.3±1.1	9.1±1.5	8.7±0.9(94)
day 7	14.8±1.2	14.8±1.5	14.4±1.8	13.3**±1.3(90)
day 14	31.1±2.4	31.0±2.9	30.7±2.8	28.3*±1.8(91)
day 21	50.8±3.7	51.4±4.4	49.9±4.2	44.2**±2.5(87)

*** = p < 0.05; ** = p < 0.01 by 2-sided Dunnett-test; [1] = percent of control.**

There were no apparent effects on reproductive performance and parameters for the F0 matings and the F1 pups. High dose pup weights were decreased from day 7 on most likely due to the fact that pups start eating food at this time along with potentially receiving test copound or it's metabolites in mother milk.

Clinical observations

Clinical signs of toxicity:

The provided data for pup clinical signs observed during lactation did not reveal any treatment related effects.

Sexual maturation landmarks:

The following Table XV presents the sexual maturation landmarks reported by the investigators for F1 pups only (from Table IA- 038-039; page 143-144) from the study report):

Table XV: Physical/Behavioral Landmarks

Dose (ppm): Observation	Control	25	75	300
		F0 Generation - F1 litters		
Vaginal opening	31.7±2.1	32.1±1.7	32.4±1.8	33.3**±1.8
Preputial separation	43.8±4.4	43.4±1.1	43.6±1.7	43.7±1.7

* = p < 0.05; ** = p < 0.01 by 2-sided Dunnett-test and Fisher's exact test.

There was an increase in vaginal opening time in the high dose females.

Necropsy results

Necropsy observations (macroscopic):

The following Table XVI presents the necropsy observations for the F0 and F1 parental animals (from Tables IB- 5 and 15; pages 195 and 205 of the study report):

Table XVI: Necropsy Observations

Dose (ppm): Observation	Control	25	75	300
		F0 Generation Parental Animals Males/Females		
Number of Animals	25/21	22/19	22/22	23/25
Animal not preg.	0	0	1	0
Impaired fertility	0/0	0/0	0/0	0/0
Erosion/ulcer - glandular stomach	0/0	0/0	0/1	0/0
Jejunum:				
dilation	0/0	1/0	0/0	0/0
enlarged follicles	0/0	1/0	0/0	0/0
Liver:				
focus	0/0	2/2	1/1	1/0
lobe missing	0/1	0/0	0/0	0/0
mass	0/0	0/0	0/1	0/0
Pancreas - edema	0/0	0/1	0/0	0/0
Kidneys:				
cyst	0/0	0/0	0/0	1/0
retraction	0/0	0/0	0/1	0/0
Ovaries - cyst	0	1	0	0
Uterus - dilation	2	3	0	0
Spleen				
enlarged	0/0	0/0	1/0	0/0
focus	0/0	0/1	0/0	0/0
Liver lymph node - discoloration	0/0	0/0	0/1	0/0

continued

Table XVI: Necropsy Observations continued

Dose (ppm): Observation	Control	25	75	300
	F1 Generation Parental Animals Males/Females			
Number of Animals	23/22	24/23	22/21	24/24
Animal not preg.	0	1	1	0
Impaired fertility	0/0	1/0	1/0	0/0
Liver - focus	0/1	0/1	1/0	1/0
Kidneys:				
cyst	1/0	0/0	0/0	0/0
retraction	0/0	0/0	1/0	0/0
Testis - organ size reduced	1	0	0	0
Left epididymis - organ size reduced	1	0	0	0
Oviducts - cyst	1	0	2	0
Uterus - dilation	0	0	1	0
Eyes - organ size reduced	0/0	0/0	0/0	0/1
Adipose tissue - necrosis	0/1	0/0	0/0	0/0

No treatment related effects were noted in the necropsy observations for the F0 or F1 parental animals.

The following Table XVII presents the necropsy observations for the F1 and F2 pups (from Tables IA- 042-043 and 083-085, pages 147-148 and 188-190 of the study report):

Table XVII: Necropsy Observations - Pups/Litter Incidence

Dose (ppm):	Control	25	75	300

Table XVII: Necropsy Observations continued

Dose (ppm): Observation	Control	25	75	300
		F1 Pups		
Litters evaluated	25	25	24	25
Pups evaluated	312	298	249	274
Live	307	283	243	268
Stillborn	7	15	6	6
Autolysis	12/4[1]	3/3	0/0	2/1
Incisors sloped	1/1	2/1	5/5	6/4
Macrophthalmia	1/1	0/0	0/0	0/0
Anophthalmia	0/0	0/0	0/0	2/1
Dilated renal pelvis	4/3	3/2	0/0	2/2
		F2 Pups		
Litters evaluated	25	23	22	25
Pups evaluated	325	296	272	341
Live	317	292	268	337
Stillborn	8	4	4	4
Autolysis	1/1	1/1	4/4	4/3
Incisors sloped	3/3	5/4	4/4	7/5
Incisors broke off	1/1	0/0	0/0	0/0
Misshapen spleen	0/0	0/0	0/0	2/2
Dilated renal pelvis	2/2	4/2	1/1	1/1
Small testis	1/1	0/0	0/0	0/0

[1] = fetal/litter incidence

No treatment related effects necropsy observations were noted in either F1 or F2 pups.

Organ weights:

The following Table XVIII presents selected organ weight data for the F0 and F1 parental animals (from Tables IB 1-4 and 11-14, pages 191-194 and 201-204 of the study report):

Table XVIII: Organ Weights (g)

Dose (ppm):		Control	25	75	300
			F0 Generation		
			Males		
Liver	**A**	15.115±1.575	15.489±1.865	14.928±1.689	15.029±1.987
	R	3.222±0.204	3.212±0.182	3.133±0.221	3.319±0.283
Kidneys	**A**	3.097±0.262	2.996±0.293	2.966±0.279	3.154±0.318
	R	0.662±0.055	0.623*±0.041	0.624*±0.047	0.699*±0.059
Testes	**A**	3.895±0.260	3.911+±0.301	3.859±0.247	3.809±0.267
	R	0.084±0.067	0:818±0.09	0.814±0.066	0.847±±0.08
Epididy.	**A**	1.383±0.077	1.374±0.092	1.357±0.07	1.358±0.097
	R	0.296±0.026	0.287±0.029	0.286±0.018	0.302±0.025
Cauda epi.	**A**	0.574±0.05	0.574±0.05	0.57±0.047	0.568±0.046
	R	0.123±0.014	0.12±0.015	0.12±0.007	0.126±0.014
Seminal ves.	**A**	1.423±0.248	1.306±0.154	1.329±0.158	1.345±0.157
	R	0.305±0.055	0.272*±0.033	0.280±0.036	0.298±0.031
Prostate	**A**	1.228±0.205	1.286±0.163	1.355±0.2	1.355±0.228
	R	0.263±0.048	0.269±0.044	0.286±0.043	0.301±0.052
Spleen	**A**	0.883±0.13	0.912 ±0.123	0.882 ±0.202	0.835±0.095
	R	0.189±0.026	0.19±0.023	0.186±0.04	0.185±0.017
Thymus(mg)	**A**	205.32±40.04	224.84±49.28	202.8±37.77	213.60±44.87
	R	0.044±0.008 0	.047±0.01	0.043±0.007	0.047±0.009
Brain	**A**	2.121±0.055	2.123±0.104	2.096±0.083	2.069±0.77
	R	0.454±0.03	0.443±0.034	0.442±0.031	0.460±0.032
Adrenals(mg)	**A**	74.08±10.21	81.48*±11.05	79.20±10.05	72.96±11.16
	R	0.016±0.002	0.017±0.003	0.017±0.002	0.016±0.002
Pituitary(mg)	**A**	12.24±2.15	12.08±2.68	12.48±1.94	14.12±9.88
	R	0.003±0.	0.003±0.001	0.003±0.	0.003±0.002
			Females		
Liver	**A**	8.676±0.491	8.509±0.797	8.616±0.754	8.266±0.603
	R	3.09±0.191	3.059±0.149	3.047±0.136	3.102±0.134
Kidneys	**A**	1.948±0.117	1.959±0.135	1.966±0.164	1.942±0.174
	R	0.694±0.049	0.706±0.044	0.696±0.042	0.729±0.059

continued

Table XVII: Organ Weights (g) continued

Dose (ppm):		Control	25	75	300
			F0 Generation		
			Females		
Ovaries(mg)	**A**	136.6±27.171	122.583*±21.415	111.56**±31.018	120.292*±20.155
	R	0.049±0.01	0.044±0.009	0.04**±0.011	0.045±0.008
Uterus	**A**	0.846±0.328	0.827±0.278	0.844±0.214	0.847±0.239
	R	0.302±0.119	0.299±0.105	0.3±0.081	0.317±0.086
Spleen	**A**	0.66±0.082	0.641±0.09	0.612±0.092	0.658±0.069
	R	0.235±0.029	0.231±0.029	0.217±0.034	0.247±0.023
Thymus(mg)	**A**	251.28±51.99	236.76±54.27	241.16±49.30	250.25±41.60
	R	0.089±0.017	0.085±0.018	0.085±0.017	0.094±0.013
Brain	**A**	1.975±0.05	1.972±0.071	1.973±0.068	1.961±0.048
	R	0.704±0.036	0.713±0.056	0.701±0.046	0.739±0.058
Adrenals(mg)	**A**	110.52±9.62	109.76±14.39	109.60±12.87	104.5±11.24
	R	0.039±0.004	0.04±0.005	0.039±0.005	0.039±0.005
Pituitary(mg)	**A**	15.480±3.029	15.280±3.835	14.958±2.010	14.542±2.828
	R	0.006±0.001	0.005±0.001	0.005±0.001	0.005±0.001
			F1 Generation		
			Males		
Liver	**A**	14.842±1.964	14.756±1.98	16.09*±2.148	14.539±1.542
	R	3.156±0.29	3.128±0.337	3.151±0.297	3.207±0.211
Kidneys	**A**	2.905±0.379	2.938±0.269	3.075±0.42	2.99±0.264
	R	0.618±0.056	0.625±0.057	0.604±0.075	0.661**±0.05
Testes	**A**	3.822±0.408	3.978±0.372	3.984±0.303	3.867±0.247
	R	0.816±0.095	0.845±0.067	0.785±0.076	0.858±0.083
Epididy.	**A**	1.319±0.108	1.333±0.154	1.364±0.096	1.358±0.090
	R	0.282±0.027	0.283±0.029	0.269*±0.024	0.301*±0.029
Cauda epi.	**A**	0.533±0.059	0.535±0.074	0.533±0.048	0.552±0.053
	R	0.114±0.015	0.114±0.015	0.105*±0.012	0.123±0.015
Seminal ves.	**A**	1.207±0.167	1.218±0.199	1.23±0.119	1.249±0.166
	R	0.258±0.039	0.259±0.044	0.243±0.033	0.277±0.042
Prostate	**A**	1.158±0.155	1.183±0.157	1.196±0.187	1.223±0.181
	R	0.248±0.039	0.252±0.037	0.237±0.048	0.271±0.041
Spleen	**A**	0.886±0.111	0.912±0.142	0.924±0.112	0.837±0.146
	R	0.189±0.024	0.193±0.025	0.181±0.019	0.184±0.025
Thymus(mg)	**A**	245.84±57.90	255.24±64.05	263.88±55.49	236.08±52.70
	R	0.052±0.011	0.054±0.013	0.052±0.009	0.052±0.011

continued

Table XVII: Organ Weights (g) continued

Dose (ppm):		Control	25	75	300
			F1 Generation		
			Males		
Brain	A	2.11±0.112	2.092±0.085	2.149±0.085	2.078±0.075
	R	0.452±0.037	0.446±0.032	0.424**±0.031	0.461±0.034
Adrenals(mg)	A	77.84±8.10	75.92±10.49	78.80±8.60	74.64±10.65
	R	0.017±0.002	0.016±0.002	0.016±0.002	0.016±0.002
Pituitary(mg)	A	11.36±2.43	11.32±2.12	12.28±2.46	11.56±2.65
	R	0.002±0.001	0.002±0.000	0.002±0.001	0.003±0.001
			Females		
Liver	A	8.908±0.906	8.812±0.572	8.880±0.78	8.554±0.85
	R	3.137±0.201	3.092±0.157	3.120±0.172	3.186±0.281
Kidneys	A	1.965±0.188	2.007±0.125	2.007±0.147	1.983±0.114
	R	0.693±0.046	0.704±0.037	0.706±0.039	0.739**±0.04
Ovaries(mg)	A	123.72±26.27	126.56±22.03	121.96±27.25	117.84±19.51
	R	0.043±0.007	0.044±0.008	0.043±0.009	0.044±0.007
Uterus	A	0.827±0.243	0.91±0.304	0.85±0.321	0.865±0.316
	R	0.294±0.095	0.32±0.108	0.298±0.106	0.324±0.121
Spleen	A	0.593±0.089	0.642±0.087	0.618±0.083	0.656±0.087
	R	0.209±0.029	0.226±0.03	0.217±0.026	0.244**±0.03
Thymus(mg)	A	234.88±47.71	232.12±34.15	230.48±53.19	233.96±41.26
	R	0.083±0.017	0.082 ±0.013	0.081±0.017	0.087±0.017
Brain	A	1.974±0.091	2.008±0.068	1.984±0.09	1.976±0.078
	R	0.698±0.039	0.706±0.035	0.699±0.033	0.737**±0.042
Adrenals(mg)	A	99.2±13.47	102.52±8.80	104.16±12.83	95.32±10.02
	R	0.035±0.004	0.036±0.003	0.037±0.004	0.036±0.004
Pituitary(mg)	A	14.88±2.09	14.8±2.08	15.6±1.92	15.80±1.78
	R	0.005±0.001	0.005±0.001	0.005±0.001	0.006**±0.001

A = Absolute organ weights in grams; R = Relative organ to body weight ratio expressed as % of body weight at day 21 p.p.; * = p < 0.05 ** = p < 0.01 by Kruskal-Wallis + 2-sided Wilcoxon-test.

No treatment related effects were noted in the F0 and F1 parental organ weights.

The following Table XVIII presents selected organ weight data for the F1 and F2 pups (from Tables IA- 040-041 and 081-082, pages 145-146 and 186-187 of the study report):

Table XVIII: Organ Weights (g)

Dose (ppm):		Control	25	75	300
			F1 Males		
Brain	**A**	1.495±0.065	1.505±0.067	1.485±0.041	1.469±0.059
	R	2.725±0.276	2.793±0.195	2.846*±0.241	3.136**±0.286
Thymus	**A**	0.192±0.030	0.184±0.032	0.172*±0.028	0.157*±0.029
	R	0.346±0.041	0.340±0.044	0.327±0.032	0.332±0.047
Spleen	**A**	0.259±0.053	0.257±0.061	0.248±0.054	0.211**±0.043
	R	0.464±0.070	0.471±0.089	0.469±0.083	0.445±0.067
			F1 Females		
Brain	**A**	1.435±0.053	1.444±0.058	1.461±0.068	1.420±0.055
	R	2.846±0.250	2.875±0.225	2.869±0.202	3.142**±0.181
Thymus	**A**	0.193±0.036	0.179±0.029	0.182±0.025	0.160*±0.031
	R	0.378±0.048	0.354±0.044	0.355±0.031	0.352±0.060
Spleen	**A**	0.241±0.055	0.239±0.045	0.243±0.052	0.210*±0.048
	R	0.470±0.078	0.470±0.070	0.471±0.081	0.459±0.082
			F2 Males		
Brain	**A**	1.501±0.056	1.492±0.058	1.478±0.048	1.444**±0.039
	R	2.876±0.225	2.842±0.291	2.884±0.267	3.200**±0.196
Thymus	**A**	0.174±0.028	0.177±0.030	0.171±0.033	0.143**±0.024
	R	0.332±0.042	0.333±0.043	0.331±0.057	0.314±0.044
Spleen	**A**	0.234±0.058	0.243±0.057	0.237±0.048	0.203±0.034
	R	0.442±0.083	0.454±0.080	0.458±0.080	0.447±0.061
			F2 Females		
Brain	**A**	1.451±0.052	1.440±0.063	1.425±0.059	1.389**±0.058
	R	2.920±0.239	2.850±0.194	2.911±0.177	3.258**±0.275
Thymus	**A**	0.176±0.024	0.180±0.026	0.174±0.024	0.150**±0.029
	R	0.352±0.041	0.355±0.048	0.354±0.042	0.348±0.057
Spleen	**A**	0.229±0.046	0.238±0.052	0.229±0.039	0.183**±0.035
	R	0.457±0.078	0.467±0.081	0.465±0.066	0.425±0.064

A = Absolute organ weights in grams; R = Relative organ to body weight ratio expressed as % of body weight at day 21 p.p.; * = p < 0.0
**** = p < 0.01 by Kruskal-Wallis + 2-sided Wilcoxon-test.**

No treatment related effects on the F1 or F2 pup organ weights were noted in the above data.

Pathology

Assessment of the differential ovarian follicle count

No statistically significant differences were noted in the follicles primordial, follicles growing, follicles primordial plus growing, follicles antral and corpora lutea counts in the F0 and F1 generation parental females.

Microscopic examination:

No treatment related effects were noted in the microscopic findings data provided by the registrant in either F0 or F1 generation parental animals.

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

III. DISCUSSION

A. Investigators' Summary and Conclusions: (scanned from pages 25-29 of the study report)

METHODS

BAS 500 F was administered to groups of 25 male and 25 female healthy young Wistar rats (F0 parental generation) as a constant homogeneous addition to the food in different concentrations (0; 25; 75 or 300 ppm). At least 74 days after the beginning of treatment, FO animals were mated to produce a lifter (F1). Mating pairs were from the same dose group and F1 animals selected for breeding were continued in the same dosing group as their parents. Groups of 25 males and 25 females selected from F1 pups as F1 parental generation were offered diets containing 0; 25; 75 and 300 ppm of the test substance post weaning, and the breeding program was repeated to produce F2 litter. The study was terminated with the terminal sacrifice of the F2 weanlings and F1 adult animals. Test diets containing BAS 500 F were offered continuously throughout the study.

The parents' and the pups' state of health was checked each day, and parental animals were examined for their mating and reproductive performances.

Food consumption of the F0 and F1 parents was determined regularly during premating (once weekly over a period of 7 days each), and weekly during gestation (days 0, 7, 14, 20) and lactation periods (days 1, 4, 7, 14).

In general, body weights of FO and F1 parents were determined once weekly. However, during gestation and lactation F0/F1 females were weighed on days 0, 7, 14 and 20 of gestation, and on days 1, 4, 7, 14 and 21 after birth.

Estrous cycle data were evaluated for FO and F1 generation females over a three week period prior to mating until evidence of mating occurred. Moreover, the estrous stage of each female was determined on the day of scheduled sacrifice.

Various sperm parameters (motility, sperm head count, morphology) were assessed in all F0 and F1 generation males at scheduled sacrifice or shortly thereafter.

The F1 and F2 pups were sexed and weighed on the day after birth and on days 4, 7, 14 and 21 post partum. Their viability was recorded. All pups were examined macroscopically at necropsy (including weight determinations of brain, spleen and thymus in one pup/sex/lifter). Sexual maturation (day of preputial separation/vaginal opening) of all pups selected to become F1 parental generation animals was determined.

All F0 and F1 parental animals were assessed by gross pathology (including weight determinations of several organs) and subjected to an extensive histopathological examination, special attention being paid to the organs of the reproductive system. A quantitative assessment of primordial follicles, growing follicle and antral follicles in the ovaries was performed for all control and high dose FO and F1 parental females.

The mean dose of BAS 500 F administered (during premating (170 and F1 parental animals)) was approx. 2,7 mg/kg body weight/day in the 25 ppm group, approx. 8,2 mg/kg body weight/day in the 75 ppm group and approx. 32,6 mg/kg body weight/day in the 300 ppm group.

RESULTS

The following substance-related findings were obtained:

300 ppm (about 32.6 mg/kg body weight/day)

FO parental animals

CLINICAL EXAMINATIONS

- statistically significantly decreased food consumption (up to 5%) in the males during the first weeks of the premating phase and in the females during the entire premating period (up to 8%).
- statistically significantly reduced body weight gain in the FO males during premating weeks 9 -10 (about 25%).
- statistically significantly reduced mean body weights (BW)/body weight gains (BWC) in the females during premating (BWC: up to 12%), gestation (BW: up to 5%) and lactation (BW: up to 5%/BWC: up to 50%) period.

REPRODUCTIVE PERFORMANCE/ ORGAN WEIGHTS/ GROSS AND HISTOPATHOLOGICAL FINDINGS

- no substance-related adverse effects

F1 pups

CLINICAL EXAMINATIONS/ SEXUAL MATURATION

- statistically significantly lower mean body weights in F1 pups until weaning (about 10% on day 21 p.p., both sexes combined) and statistically significantly impaired body weight gains in these pups from day 4 p.p. up to weaning (about 12%, both sexes combined).

- delay in vaginal opening in the selected F1 female animals due to delay in physical development as substantiated by reduced body weight.

PUP ORGAN WEIGHTS/ GROSS FINDINGS

- statistically significantly lower mean absolute weights of thymus (about 18% below controls) and spleen (about 16% below controls) in the F1 pups. statistically significantly increased mean relative weights of brain (about 13% above controls) in Fl.

F1 parental animals

CLINICAL EXAMINATIONS

- statistically significantly decreased food consumption (up to 12%) in the males during the first weeks of the premating phase and in the females during the premating (up to 9%) and gestation (up to 7%) period.
- statistically significantly reduced mean body weights in the males during main phases of treatment period (up to 8%, week 1; up to 7%, weeks 4 - 11, week 14) and in the females during the first weeks of premating (up to 9%) and after weaning (up to 6%).

REPRODUCTIVE PERFORMANCE/ ORGAN WEIGHTS/ GROSS AND HISTOPATHOLOGICAL FINDINGS

- no substance-related adverse effects

F2 pups

CLINICAL EXAMINATIONS

- statistically significantly lower mean body weights in F2 pups until weaning (about 13% on day 21 p.p., both sexes combined) and
- statistically significantly impaired body weight gains in these pups from day 4 p.p. up to weaning (about 15%, both sexes combined).

PUP ORGAN WEIGHTS/ GROSS FINDINGS

- statistically significantly lower mean absolute weights of brain (about 4% below control), thymus (about 17% below controls) and spleen (about 17% below controls) in the F2 pups
- statistically significantly increased mean relative weights of brain (about 11 % above controls) in F2

75 ppm (about 8.2 mg/kg body weight/day)
FO and F1 parental animals

CLINICAL EXAMINATIONS/ REPRODUCTIVE PERFORMANCE/ ORGAN WEIGHTS/ GROSS AND HISTOPATHOLOGICAL FINDINGS

- no substance-related adverse effects

F1 and F2 pups

CLINICAL EXAMINATIONS/ SEXUAL MATURATION (F1 PUPS)/ PUP ORGAN WEIGHTS/ GROSS FINDINGS

- no substance-related adverse effects

25 ppm (about 2.7 mg/kg body weight/day)

F0 and F1 parental animals

CLINICAL EXAMINATIONS/ REPRODUCTIVE PERFORMANCE/ORGAN WEIGHTS/GROSS AND HISTOPATHOLOGICAL FINDINGS

- no substance-related adverse effects

F1 and F2 pups

CLINICAL EXAMINATIONS/ SEXUAL MATURATION (F1 PUPS)/PUP ORGAN WEIGHTS/GROSS FINDINGS

- no substance-related adverse effects

CONCLUSION

Under the conditions of this study BAS 500 F had no adverse effects on reproductive performance or fertility of the F0 or F1 parental animals of all substance-treated groups (25, 75 and 300 ppm). Estrous cycle data, mating behavior, conception, gestation, parturition, lactation and weaning as well as sperm parameters, sexual organ weights, gross and histopathological findings of these organs were similar between the substance-treated rats and the corresponding controls. Most of the F0 and F1 parental rats proved to be fertile. The scattered occurrence of individual infertile rats throughout the different dose groups did not suggest any relation to treatment.

Signs of general, systemic toxicity occurred in both parental generations at 300 ppm. Toxicity was characterized by decreased food consumption during the first weeks of the premating period in F0 and F1 parental males as well as in F0 and F1 parental females during premating and gestation (F1 only). The high dose F0 parental females showed impairments in body weight/body weight gain during premating, gestation and lactation, while in the F0 parental males the body weight gain was only transiently reduced during premating weeks 9 - 10. Moreover, impaired body weight data of the F1 parental animals consisted of reduced mean body weights in the males during main phases of treatment period (weeks 4 - 11, week 14) and in the females during the first weeks of premating and after weaning.

Substance-induced signs of developmental toxicity were observed in progeny of the F0 and F1 parents at 300 ppm. The administration led to impairments of pup body weight data and causally related pup organ weight changes occurred in the high dose progeny of the F0 and F1 parental rats (i.e. F1 and F2 litters). Furthermore, a delay in vaginal opening in the selected F1 females was noted as sign of a general delay in physical development. 75 and 25 ppm did not induce any indication of developmental toxicity.

Therefore, under the conditions of this study the NOAEL (no observed adverse effect level) for reproductive performance and fertility is 300 ppm (about 32.6 mg/kg body weight/day) for the F0 and F1 parental rats.

The NOAEL for general, systemic toxicity of the test substance is 75 ppm (about 8.2 mg/kg body weight/day) for the F0 and F1 parental males and females.

The NOAEL for developmental toxicity (growth and development of the offspring) could be fixed at 75 ppm (about 8.2 mg/kg body weight/day) for the F1 and F2 progeny.

Thus, indications for developmental toxicity occurred only at a dose, which was also toxic to the parental animals.

B. Reviewer's conclusions:

No substance related mortality was noted in any of the F0 or F1 parental animals; however, 1 F0 high dose female died on the first day of lactation and assumed to be spontaneous in origin. No clinical signs of toxicity were noted in the F0 or F1 parental animals during the premating periods. For the F0 females during the gestation period, 2 dams, 1 each in the 25 and 75 ppm dose groups, exhibited insufficient nesting behavior and one 75 ppm sperm positive female did not deliver. For the F1 females, 2 sperm positive females, 1 each in the 25 and 75 ppm dose groups, did not deliver. For the F0 females during lactation, 1 high dose female was found dead on day 0 p.p. One control and 3 low dose females did not nurse their pups correctly and the pups were cannibalized, and the only pup of a 75 ppm dose dam died on day 5 p.p. For the F1 females during lactation, one mid dose female did not nurse her pups correctly and therefore all pups were cannibalized by day 7 p.p. and the only pup of a mid dose dam was cannibalized on day 3 p.p. Since no dose response was noted, these observations were not considered to be related to treatment. Further, there was no treatment related effects noted in the body weights, body weight gains, food consumption, reproductive performance (all measured parameters), sexual maturation landmarks, necropsy results, both gross and microscopic including assessment of differential ovarian follicle counts and organ weights of the parental animals and the pups.

Parental (Paternal/Maternal) Systemic Toxicity NOAEL ≥ 300 ppm (29.0-35.0 mg/kg/day for males and 30.4-36.0 mg/kg/day for females)

Parental (Paternal/Maternal) Systemic Toxicity LOAEL > 300 ppm (29.0-35.0 mg/kg/day for males and 30.4-36.0 mg/kg/day for females)

Offspring Systemic/Developmental Toxicity NOAEL ≥ 300 ppm (29.0-35.0 mg/kg/day for males and 30.4-36.0 mg/kg/day for females)

Offspring Systemic/Developmental Toxicity LOAEL > 300 ppm (29.0-35.0 mg/kg/day for males and 30.4-36.0 mg/kg/day for females)

Reproductive Toxicity NOAEL ≥ 300 ppm (29.0-35.0 mg/kg/day for males and 30.4-36.0 mg/kg/day for females)

Reproductive Toxicity LOAEL > 300 ppm (29.0-35.0 mg/kg/day for males and 30.4-36.0 mg/kg/day for females)