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Archived

BAS 500 F PRENATAL DEVELOPMENTAL TOXICITY(OPPTS 870.3700; OPP 83-3A)

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DATA EVALUATION RECORD

Study Type: Prenatal Developmental Toxicity Study (Teratology)
Species: Rat; Guideline: OPPTS 870.3700; OPP 83-3a

EPA ID No.s: EPA MRID No. 45118325
EPA Pesticide Chemical Code
CAS#
EPA DP Barcode D
EPA Submission No. S

Test Material: BAS 500 F

Synonyms:

Citation: Schilling, K., Hellwig, J., Hildebrand, B. (1999): BAS 500 F - Prenatal Developmental Toxicity Study in Wistar Rats Oral Administration (Gavage); Department of Toxicology of BASF Aktiengesellschaft for BASF Corporation Agricultural Products; Laboratory Project Identification No. 30R0494/96168, BASF Registration Document No. 1999/11511; October 25, 1999 (Unpublished); EPA MRID Number 45118325.

Executive Summary: In a prenatal developmental toxicity study (Teratology) (MRID# 45118325), sexually mature, virgin Chbb:THOM (SPF) Wistar rats (supplier: BOEHRINGER INGELHEIM PHARMA KG received either 0, 10, 25, or 50 mg/kg/day BAS 500 F (Purity: 98.9%; Batch No.: CP028719) in 0.5% Tylose CB 30.000 (in doubly distilled water) by oral gavage from gestation days 6 through 19, inclusive. The animals were examined for clinical symptoms, weighed on days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20 p.c. Also with the exception of day 0, the consumption of food was determined on the same days as was body weight. The animals were sacrificed on day 20 p.c., were necropsied and assessed by gross pathology, the uterus and the ovaries were removed and weighed with the number of corpora lutea, the number and distribution of implantation sites were recorded. The fetuses were weighed and examined for external, visceral and skeletal anomalies.

No deaths, clinical signs of toxicity or gross pathological observations were noted in the maternal animals. The 25 and 50 mg/kg/day dose groups had lower overall body weights at gestation days 19/20 and gained less weight than the control during the dosing period (gestation days 6-19), for the post dosing period

(19-20), for the overall gestation period (0-20) and for the calculated period of gestation days 6-20, also for corrected body weight gains from gestation days 6-20. As seen with the body weights and body weight gains, the 25 and 50 mg/kg/day dose groups had reduced food consumption during the dosing period (gestation days 6-19), for the post dosing period (19-20) and for the overall gestation period (0-20). There was reduced food efficiency in the 50 mg/kg/day dose group during the dosing period (gestation days 6-19) and in the 25 and 50 mg/kg/day dose groups for the post dosing period (19-20), for the overall gestation period (0-20) and for the calculated period of gestation days 6-20. **The maternal toxicity NOAEL was 10 mg/kg/day and the maternal toxicity LOAEL of 25 mg/kg/day based on decreased body weights and body weight gains and reduced food consumption and reduced food efficiency.**

There was an increase in fetal and litter incidence of dilated renal pelvis and increased incidence of cervical ribs with cartilage not present in the high dose group. **The developmental toxicity NOAEL was 25 mg/kg/day and the developmental toxicity LOAEL was 50 mg/kg/day based on increased incidence of dilated renal pelvis and cervical ribs.**

This study is classified as Acceptable-Guideline and satisfies the guideline requirements (OPPTS 870.3700; OPPS 83-3a) for a prenatal developmental toxicity (teratology) study in rats.

Compliance: A signed and dated STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS, GOOD LABORATORY PRACTICES STATEMENT (EPA), FLAGGING CRITERIA Statement (according to the investigators: This study neither meets nor exceeds any of the applicable criteria.), GLP-STATEMENT (OECD) and STATEMENT OF THE QUALITY ASSURANCE UNIT was provided.

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

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A. Materials and Methods

Test Compound: BAS 500 F
Purity: 98.9% (method: HPLC; certificate of analysis, PCP04329)
Description: Crystalline/yellowish
Batch No.: CP028719
other provided information:
the test material was refrigerated.

Vehicle(s): 0.5% Tylose CB 30.000 in doubly distilled water

Test Animal(s): Species: Sexually mature, virgin Wistar rats
Strain: Chbb:THOM (SPF)
Source: BOEHRINGER INGELHEIM PHARMA KG (former name: DR. K. THOMAE GmbH), Biberach an der Riss, FRG
Age: 10 weeks
Body Weight: 216.6-219.4 g on gestation day 0
males of the same strain were used

B. Study Design

According to the investigators (from pages 16-17 and 22-23 of the report):

The purpose of this study was to assess the effects of BAS 500 F, on embryonic and fetal development according to current test guidelines (see also below). BAS 500 F was administered daily as an aqueous suspension to pregnant Wistar rats from implantation to one day prior to the expected day of parturition (days 6 - 19 p.c.). Moreover, information about influences of the test substance on the maternal organism was expected to be obtained.

Since BAS 500 F is used as a fungicide and uptake of crop bearing residues by man cannot be ruled out, oral administration of BAS 500 F (by gavage) was selected as the route of choice. Furthermore, oral administration of a test substance has proved to be suitable worldwide in numerous experiments to disclose a toxicological hazard.

The animals were supplied at an age of about 10 weeks on June 23, 1998.

On day 20 p.c., all females were sacrificed in a randomized order and examined macroscopically. The fetuses were removed from the uterus and further investigated with different methods (for details see 3.9.).

Due to technical reasons, the study was carried out in 3 sections. Each dose group was represented in each section. A treatment interval of 1 - 2 days elapsed before the next section. For further details, see Table 3.7.1.

Time schedule

	Acclimatization period	Beginning of study (day 0 p.c.)	Beginning of treatment (day 6 p.c.)	End of treatment (day 19 pc.)	Sacrifice (day 20 p.c.)
1st section	by June 29, 1998	June 30, 1998	July 06, 1998	July 19, 1998	July 20, 1998
2nd section	by June 30, 1998	July 01, 1998	July 07, 1998	July 20, 1998	July 21, 1998
3rd section	by July 01, 1998	July 02, 1998	July 08, 1998	July 21, 1998	July 22, 1998

The study was carried out according or exceeding the requirements of the following test guidelines:
EC Commission Directive 87/302/EEC of Nov. 18, 1987; Part 13: Methods for the determination of toxicity: Teratogenicity study (rodent and non-rodent); Official Journal of the European Communities; No. L 133, pp. 24 - 26 (1988)

OECD Guidelines for Testing of Chemicals; Proposal for updating Guideline 414: Prenatal Developmental Toxicity Study (Draft Document of March 1998)

U.S. EPA, Health Effects Test Guidelines; OPPTS 870.3700: Prenatal Developmental Toxicity Study (Aug. 1998) [When the study protocol was created, U.S. EPA, 40 CRF Part 799; Toxic Substances Control Act Test Guidelines; Final Rule § 799.9370 TSCA Prenatal Developmental Toxicity Study (August. 1997) was available as the latest finalized test guideline. In between the final version of EPA Health Effects Test Guidelines; OPPTS 870.3700 was issued, which does not show any significant differences to the guideline previously referenced.]

Japan/MAFF: Testing Guidelines for Toxicological Studies: Teratogenicity Study, pp. 48 - 49 (1985)

Mating Procedure From page 15 of the report:
After an acclimatization period of at least 5 days, 2-3 untreated female rats were mated with one untreated male animal of the same breed. The male mating partners were kept under conditions (air conditioning, diet, water) comparable to those of the females participating in this study.

Mating took place from about 4.00 p.m. to about 7.30 a.m. on the following day. If sperm were detected microscopically in the vaginal smear in the morning, the animals were considered to be fertilized. This day was designated "day 0" (beginning of the study) and the following day "day 1" post coitum (p.c.).

On day 0 (detection of sperm), the rats were about 11 - 12 weeks old. The mean weight of the rats with positive sperm detection was approx. 218 g.

On day 0, the animals were assigned to the different test groups according to a randomization plan (Nijenhuis and Wilf, 1978).

Animal Husbandry From page 19 of the report:

After randomization the rats were identified uniquely by ear tattoo.

This strain was selected since extensive experience is available on Wistar rats and this strain has been proved to be sensitive to substances with a teratogenic potential.

The rats were housed singly from day 0 - 20 p.c. in type DK III stainless steel wire mesh cages supplied by BECKER & CO., Castrop-Rauxel, FRG (height: 15 cm, length: 37,5 cm, width: 21 cm; floor area about 800 cm^2). Underneath the cages, waste trays were fixed containing absorbent material (type 3/4 dustfree embedding, supplied by SSNIFF, Soest, FRG).

The animals were accommodated in fully air-conditioned rooms 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 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.).

Before the study started, the animal room was completely disinfected using a disinfector ("AUTEX", fully automatic, formalin-ammonia - based terminal disinfection). In general, 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 meal, supplied by PROVIMI KLIBA SA (former name: KLINGENTALMOHLE AG), Kaiseraugst, Switzerland. Food was available to the animals ad libitum throughout the study (from the day of supply to the day of necropsy), as was drinking water of tap water quality from water bottles.

Group Arrangement:

From page 20 of the report:

Test group	Dose mg/kg body weight/day	Concentration mg/100 ml1	Volume ml/kg	Number of animals	Animal No.
0	0	0	10[1)]	25	1 - 25
1	10	100	10[2)]	25	26 - 50
2	25	250	10[2)]	25	51 - 75
3	50	500	10[2)]	25	76 - 100

[1)] 0.5% Tylose CB 30.000 in doubly distilled water

[2)] Test substance suspensions in 0.5% Tylose CB 30.000 in doubly distilled water

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Dosing and Dosing Solution Preparations From pages 16 and 20 of the report:

The selection of doses for the present examination was based on the results of a preceding maternal toxicity dose range-finding study in Wistar rats.

The primary aim of this range-finding study was to find a dose which should induce "some overt maternal toxicity such as slight weight loss, but not more than 10 percent maternal deaths" and could be used as the highest dosage for the planned full-scale prenatal toxicity study.

Taking the results of this previous study into consideration, the following doses were chosen for the present full-scale toxicity study in Wistar rats:

10 mg/kg body weight/day:	as the expected no observed adverse effect level
25 mg/kg body weight/day:	as intermediate dose level
50 mg/kg body weight/day:	as the dose level with some overt signs of maternal toxicity and possible substanue-related developmental toxicity

At least every 4th day the test substance suspensions were freshly prepared. For the preparation of the suspensions, an appropriate amount of the test substance was weighed and subsequently suspended in 0.5% Tylose CB 30.000 in doubly distilled water using a high speed sonicator (Ultra Turrax, JANKE & KUNKEL KG,FRG). A magnetic stirrer was used to keep the suspensions homogeneous during treatment of the animals.

Dose Administration: From pages 22 of the report:

The aqueous test substance suspensions were administered to the animals orally (by gavage) once a day from implantation to one day prior to the expected day of parturition (day 6 to day 19 p.c.) always at approx. the same time of day (in the morning). The animals of the control group were treated in the same way with the vehicle (0.5% Tylose CB 30.000 in doubly distilled water). The volume administered each day was 10 ml/kg body weight. The calculation of the volume administered was based on the last individual body weight.

Dosing, Food, Water Analysis From page 21 of the report:

All analyses mentioned under 3.6.1. [of the study report] were carried out at the Ecology and Environmental Analytics of BASF Aktiengesellschaft (Landwirtschaftliche Versuchsstation, Limburgerhof, FRG) or at the Bioanalytical Laboratory, Department of Toxicology of BASF Aktiengesellschaft, Ludwigshafen, FRG.

Analytical verifications of the stability of the test substance in aqueous suspension for a period of at least 96 hours at room temperature were carried out before the study was initiated.

Homogeneity analyses of the test substance preparations in the carrier were determined in comparable concentrations before the beginning of this study.

Samples of the test substance suspensions were sent to the analytical laboratory twice during the study period (at the beginning and towards the end) for verification of the concentrations.

The test substance suspensions were analyzed by HPLC.

More details on the methods used for the analytical investigations of the test substance preparations can be found in Volume III (Supplement: 1. Analyses of the aqueous suspensions of BAS 500 F).

X The investigators determined that the test compound was stable in 0.5% Tylose CB 30.000 in distilled water for at least 96 hours. They also determined that the test suspension were homogeneous for all 3 test groups and that the concentration analyses were within ±5%.

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

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.

Observations

Maternal examinations: From pages 24-25 of the report:

A check was made twice a day on working days or once a day (Saturday, Sunday or on public holidays) (days 0 - 20 p.c.).

The animals were examined for clinical symptoms at least once a day, or more often when clinical signs of toxicity were elicited (days 0 - 20 p.c.).

With the exception of day 0, the consumption of food was determined on the same days as was body weight.

All animals were weighed on days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20 p.c. The body weight change of the animals was calculated from these results.

Furthermore, the corrected body weight gain was calculated after terminal sacrifice (terminal body weight on day 20 p.c. minus weight of the unopened

uterus minus body weight on day 6 p.c.).

On day 20 p.c., the dams were sacrificed in randomized order by cervical dislocation and the fetuses removed from the uterus.

After the dams had been sacrificed, they were necropsied and assessed by gross pathology in randomized order to minimize bias. The uterus and the ovaries were removed and the following data were recorded:

- Weight of the unopened uterus (After the weight of the uterus had been determined, all subsequent evaluations of the dams and the gestational parameters were conducted without knowledge of treatment group in order to minimize bias.)

- Number of corpora lutea

- Number and distribution of implantation sites classified as:

· live fetuses

· dead implantations:

a) early resorptions (only decidual or placental tissues visible or according to SALEWSKI (Salewski, 1964) from uteri from apparently non-pregnant animals and the empty uterus horn in the case of single-horn pregnancy)

b) late resorptions (embryonic or fetal tissue in addition to placental tissue visible)

c) dead fetuses (hypoxemic fetuses which did not breathe spontaneously after the uterus had been opened)

Fetal examinations: From pages 27-28 of the report:

All fetal analyses were conducted without knowledge of treatment group in order to minimize bias.

At necropsy each fetus was weighed, sexed and examined macroscopically for any external findings. The sex was determined by observing the distance between the anus and the base of the genital tubercle and was later confirmed in all fetuses fixed in BOUIN'S solution by internal examination. If there were discrepancies between the "external" and the "internal" sex of a fetus, the fetus was finally sexed according to the appearance of its gonads.

Furthermore, the viability of the fetuses and the condition of the placentae, the umbilical cords, the fetal membranes and fluids were examined. Individual placental weights were recorded.

After these examinations, approximately one half of the fetuses per dam was

placed in ethyl alcohol and the other half was placed in BOUIN's solution for fixation and further evaluation.

The fetuses fixed in BOUIN's solution were examined for any visceral findings according to the method of BARROW and TAYLOR (Barrow and Taylor, 1969). After this examination these fetuses were discarded.
The skeletons of the fetuses fixed in ethyl alcohol were stained according to a modified method of KIMMEL and TRAMMELL (Kimmel, C.A. and Trammell C., 1981). Thereafter, the skeletons of these fetuses were examined under a stereomicroscope. After this examination the stained fetal skeletons were retained individually.

There are differing opinions on classification and assessment of fetal findings (e g. Beltrame and Giavini, 1990, Chahoud et al., 1999). Moreover, according to WISE et al. (Wise et al., 1997) "nomenclature used to describe observations of fetal morphology often varies considerably among laboratories, investigators, and textbooks in the fields of teratology and developmental toxicity'.

In the present study the glossary of WISE et al. (Wise et al., 1997) was used as much as possible to describe findings in fetal morphology. Classification of these findings was based on the terms and definitions proposed by CHAHOUID et al. (Chahoud et al., 1999):

- **Malformation**

A permanent structural change that is likely to adversely affect the survival or health.

- **Variation**

A change that occurs also in fetuses of control animals and is unlikely to adversely affect the survival or health. This includes delays in growth or morphon.enesis that has otherwise followed a normal pattern of development.

Moreover, the terms **"unclassified observation"** or **"unclassified cartilage observation"** were used for those fetal findings, which could not be classified as malformations or variations (e.g. focal liver necrosis in fetuses, isolated cartilage findings without any impact on the respective bony structure).

According to the definitions specified before, the findings obtained in fetuses were classified and listed in the tables accordingly.

Historical control data were provided to allow comparison with concurrent controls.

Statistical analysis From pages 26 and of the study report:

Furthermore, calculations of conception rate and pre-and postimplantation losses were carried out:

- The conception rate (in %) was calculated according to the following formula:

$$\frac{\text{number of pregnant animals}}{\text{number of fertilized animals}} \times 100$$

The preimplantation loss (in %) was calculated according to the following formula:

$$\frac{\text{number of corpora lutea - number of implantations}}{\text{number of corpora lutea}} \times 100$$

The postimplantation loss (in %) was calculated from the following formula:

$$\frac{\text{number of implantations - number of live fetuses}}{\text{number of implantations}} \times 100$$

both pre and post implantation loss was calculated on the basis of each individual pregnant animal with scheduled sacrifice

Statistical analyses were performed according to following tables:

Parameter	Statistical test	Markers in the tables	References
Food consumption[1], body weight, body weight change, corrected body weight gain (net maternal body weight change), carcass weight, weight of unopened uterus, number of corpora lutea, number of implantations, number of resorptions, number of live fetuses, proportions of preimplantation loss, portions of postimplantation loss, proportions of resorptions, proportions of live fetuses in each litter, litter mean fetal body weight, litter mean placental weight	Simultaneous comparison of all dose groups with the control group using the DUNNETT-test (two-sided) for the hypothesis of equal means	* for p ≤ 0.05 ** for p ≤ 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
Female mortality, females pregnant at terminal sacrifice, number of litters with fetal findings	Pairwise comparison of each dose group with the control group using FISHER'S EXACT test (one-sided) for the hypothesis of equal proportions	* for p ≤ 0.05 ** for p ≤ 0.01	Siegel S. (1956): Non-parametric statistics for behavioral sciences. McGraw-Hill New York
Proportions of fetuses with malformations, variations, retardations and/or unclassified observations in each litter	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians	* for p ≤ 0.05 ** for p ≤ 0.01	Nijenhuis, A.; Wilf H.S. (1978): Combinatorial Algorithms. Academic Press New York, 32-33 HettmanspergerT.P. (1984); Statistical Inference based on Ranks. John Wiley & Sons New York, 132-142

1 = 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 different time intervals (pretreatment, treatment and posttreatment period); they are not exactly precise values, because the size of the intervals taken for calculation differs. For the "mean of means" values no statistical analysis was performed.

References (from page 46 of the report):

BARROW, M.V. and TAYLOR, W.J.
A rapid method for detecting malformations in rat fetuses.
J. Morph. 127, 291 - 306 (1969)

BELTRAME, D., GIAVINI, E.
Morphological abnormalities in experimental Teratology: Need for a standardization of current terminology.
Cong. Anom. 30 (3), 187 - 195 (1990)

CHAHOUD, I., BUSCHMANN, J., CLARK, R., DRUGA, A., FALKE, H., FAQI; A., HANSEN, E., HEINRICH - HIRSCH, B., HELLWIG, J., LINGK, W., PARKINSON, M., PAUMGARTTEN, F., PFEIL, R., PLATZEK, T., SCIALLI, A., SEED, J., STAHLMANN, R., ULBRICH, B., WU, X., YASUDA, M., YOUNES, M. and SOLECKI, R.
Classification terms in developmental toxicology: Need for harmonisation
Reproductive Toxicology 13, 77 - 82 (1999)

KIMMEL, C.A. and TRAMMELL, C.
A rapid procedure for routine double staining of cartilage and bone in fetal and adult animals.
Stain Technology 56, 271 - 273 (1981)

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

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

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

WISE, D., BECK, S., BELTRAME, D., BEYER, B., CHAHOUD, I., CLARK, R.L., CLARK, R., DRUGA, A., FEUSTON, M., GUITTIN, P., HENWOOD, S., KIMMEL, C., LINDSTROM, P., PALMER, A., PETRERE, J., SOLOMON, H., YASUDA, M. and YORK, R.
Terminology of developmental abnormalities in common laboratory mammals (Version 1)
Teratology 55, 249 - 292 (1997)

WOO, D.C. and HOAR, R.M.
"Apparent hydronephrosis" as a normal aspect of renal development in late gestation of rats: The effect of methyl salicylate
Teratology 6, 191 - 196 (1972)

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 870.3700; OPP §83-3a.

C. Results

Maternal Toxicity:

Mortality

No deaths were reported in this study.

Clinical Observations

No treatment related clinical signs were reported in this study (individual animal observations were provided).

Body Weight

The investigators supplied group mean and individual animal data. The following tables present selected body weights and body weight gains:

Table I: Body Weights (grams)[a]

Gestation Day	0	6	19	20
Dose:				
0 mg/kg/day	218.4±11.0	249.1±13.6	352.8±20.1	369.1±22.8
10 mg/kg/day	219.4±12.0	248.9±11.8	356.0±20.5	372.7±22.3
25 mg/kg/day	216.6±11.2	245.2±12.2	341.2±22.1	354.5±25.1
50 mg/kg/day	217.9±10.1	249.8±13.3	337.4±27.1	350.1*±30.8
Historical control	239.1	266.7		383.5

[a] = data from Table IA 004, page 52 of the report; * = $p < 0.05$

Table II: Body Weight Gains (grams)[a]

Dose (mg/kg/day):	Gestation Day 0-6	6-19	19-20	6-20[1]	0-20	C6-20[2]
0	30.7±5.5	103.7±9.7	16.3±3.8	120.0	150.7±14.3	40.7±9.3
10	29.5±6.6	107.1±11.3	16.7±3.7	123.8	153.3±14.6	40.9±10.6
25	28.7±3.7	96.0±13.8	13.3±4.7	109.3	137.9±19.3	31.9*±7.4
50	31.9±6.9	87.6**±22.3	12.7*±6.1	100.3	132.2**±28.6	22.3**±11.9

[a] = data from Tables IA 005, 006 and 007, pages 53-55 of the report; [1] = calculated by reviewer from mean data on Table II above; [2] = corrected body weight gain (minus uterine weight); * = $p < 0.05$; ** = $p < 0.01$

The 25 and 50 mg/kg/day dose groups had lower overall body weights at gestation days 19/20 and gained less weight than the control during the dosing period (gestation days 6-19), for the post dosing period (19-20), for the overall gestation period (0-20) and for the calculated period of gestation days 6-20, also for corrected body weight gains from gestation days 6-20.

Food Consumption

The investigators supplied group mean and individual animal data. The following table presents selected food consumption data in grams/animal and food efficiency data:

Table III: Food Consumption (grams)[a]

Dose (mg/kg/day):	Gestation Days 0-6	6-19	19-20	0-20
0	21.9±2.3	26.3±1.9	26.2±3.0	25.0±2.8
10	21.4±2.4	26.1±1.9	26.1±2.6	24.7±2.9
25	21.3±2.4	24.5±2.3	24.8±3.3	23.6±2.6
50	22.0±2.2	23.3±3.1	23.3*±4.4	22.9±2.6

[a] = data from Tables IA 002 and 003, pages 50-51 of the report.

Table IV: Food Efficiency Data (%)[a]

Dose (mg/kg/day):	Gestation Days 0-6	6-19	19-20	6-20	0-20
0	20.0	28.2	31.1	30.4	28.7
10	19.7	29.3	32.0	31.6	30.0
25	19.3	28.0	26.8	29.7	27.8
50	20.3	26.9	27.2	28.7	27.5

[a] = calculated by the reviewer.

As seen with the body weights and body weight gains, the 25 and 50 mg/kg/day dose groups had reduced food consumption during the dosing period (gestation days 6-19), for the post dosing period (19-20) and for the overall gestation period (0-20). There was reduced food efficiency in the 50 mg/kg/day dose group during the dosing period (gestation days 6-19) and in the 25 and 50 mg/kg/day dose groups for the post dosing period (19-20), for the overall gestation period (0-20) and for the calculated period of gestation days 6-20.

Gross Pathological Observations

No treatment related effects were noted in the data provided.

Cesarean Section Observations

The following table presents the cesarean section observations:

Table V: Cesarean Section Observations

Dose (mg/kg/day):	0	10	25	50	HC[1]
#Animals Assigned	25	25	25	25	
#Animals Mated/Insem.	25	25	25	25	400
#Animals Pregnant	22	20	21	25	381
Pregnancy Rate (%)	88	80	84	100	95
Maternal Wastage					
#Died/Sacrificed	0	0	0	0	1
#Died/pregnant	0	0	0	0	
#Non pregnant	3	5	4	0	
#Aborted	0	0	0	0	0
#Premature Delivery	0	0	0	0	0
Total litters examined	22	20	21	25	
Total Corpora Lutea	348	323	338	394	6059
Corpora Lutea/dam	15.8±1.5	16.1±1.8	16.1±1.3	15.8±2.6	15.9±2.2
Total Implantations	327	315	313	363	5580
Implantations/Dam	14.9±2.4	15.8±2.8	14.9±3.4	14.5±3.7	14.7±2.9
Total Live Fetuses	306	291	283	343	5145
Live Fetuses/Dam	13.9±2.2	14.6±2.7	13.5±3.2	13.7±3.8	13.5±3.1
Total Resorptions	21	24	30	20	380
Early	21	24	26	20	
Late	0	0	4	0	
Resorptions/Dam	1.0±1.0	1.2±1.1	1.4±1.2	0.8±1.0	1.1±1.3
Total Dead Fetuses	0	0	0	0	1
Mean Fetal Weight (gm)	3.7±0.2	3.7±0.2	3.7±0.3	3.7±0.2	3.8
Preimplantation Loss(%)[2]	6.0	2.5	7.4	7.9	7.9
Postimplantation Loss(%)[2]	6.4	7.6	9.6	5.5	7.8
Sex Ratio (% Male)	53.3	47.1	47.7	52.5	51

[1] = HC = Historical Control; [2] = calculated by reviewer from mean data
[a] = data from Tables IA 009-011 and IB 001, pages 57-60 of the report.

No treatment related effects were noted in the above data.

2. Developmental Toxicity

The investigators provided group mean and individual animal data as well as affected fetuses per litter.

a. External Examinations

The following table presents the external examination data:

Table VI: External Examinations[a]

Dose (mg/kg/day): Observations	0	10	25	50
#pups/litters examined	306/22	291/20	283/21	343/25
Anasarca	0/0	0/0	0/0	1/1
Macroglossia	0/0	0/0	0/0	1/1
Absent eye bulge	1/1	0/0	0/0	0/0
Thread like tail	0/0	1/1	0/0	0/0
Local edema	0/0	0/0	0/0	1/1
Malrotated limb	0/0	0/0	0/0	1/1
Polyhyramnios	0/0	0/0	0/0	1/1
Fused placenta	0/0	0/0	2/2	0/0

[a] = data from Table IB 003 to 006, pages 62-65 of the report.

No treatment related effects were noted in the external observation data.

b. Visceral Examinations

The following table presents the soft tissue examination data:

Table VII: Visceral Examinations[a]

Dose (mg/kg/day): Observations	0	10	25	50
#pups/litters examined	148/22	140/20	136/21	165/24
Anophthalmia	1/1	0/0	0/0	0/0
Heart: enlarged ventricular chamber	1/1	0/0	0/0	1/1
Dilated cerebral ventricle	0/0	0/0	1/1	0/0
Dilated renal pelvis	8/6	16/10	20/9	31/15*
Dilated ureter	0/0	4/4*	1/1	5/3

* = $p < 0.05$

[a] = data from Table IB 008 to 011, pages 67-70 of the report.

There was an increase the fetal and litter incidence of dilated renal pelvis (Historical Control not provided for this obsservation) in the high dose group.

c. Skeletal Examinations

The following table presents the skeletal examination data:

Table VIII: Skeletal Examinations[a]

Dose (mg/kg/day): Observations	0	10	25	50
#pups/litters examined	158/22	151/20	147/21	178/25
Fused cervical arches[uc]	0/0	0/0	0/0	1/1
Absent lumbar vertebra[cmp]	1/1	0/0	0/0	1/1
Misshapen lumbar vertebra				
unchanged cartilage	1/1	0/0	0/0	0/0
dumbbell-shaped cartilage of centrum	1/1	0/0	0/0	0/0
changed cartilage	0/0	0/0	0/0	1/1
Malpositioned & bipartite sternebra[uc]	1/1	1/1	0/0	2/1
Incomplete ossification of hyoid[uc]	0/0	1/1	2/2	1/1
Supraoccipial hole(s)	64/19	63/20	65/17	77/24
Incomplete ossification:				
parietal[uc]	1/1	0/0	3/1	1/1
supraoccipial[uc]	0/0	1/1	1/1	2/2
interparietal[uc]	5/4	3/3	6/4	1/1
skull[uc]	0/0	2/1	0/0	1/1
thoracic centrum[uc]	3/3	3/3	10/6	5/5
Extra ossification site (between parietal & interparietal)[uc]	0/0	1/1	0/0	1/1
Dumbbell ossification of thoracic centrum				
unchanged cartilage	13/8	10/8	12/8	14/9
dumbbell-shaped cartilage of centrum	18/11	11/7	13/11	26/17
Hemicentric thoracic centrum[d-scofc]	0/0	0/0	0/0	1/1
Hemicentric thoracic centrum[scofc]	0/0	0/0	0/0	1/1
Bipartite ossification of thoracic centrum[d-scofc]	1/1	0/0	1/1	2/2
Unossified thoracic centrum[uc]	0/0	0/0	0/0	1/1
Dumbbell ossification of lumbar centrum[d-scofc]	0/0	0/0	0/0	1/1
Supranumerary lumbar vertebra[d-scofc]	1/1	0/0	0/0	0/0
Incomplete ossification of sacral arch[cp]	6/4	11/4	6/3	7/3
Incomplete ossification of sacral arch[cmp]	1/1	1/1	0/0	1/1

continued

Table VIII continued: Skeletal Examinations[a]

Dose (mg/kg/day):	0	10	25	50
Observations				
#pups/litters examined	158/22	151/20	147/21	178/25
Unossified sternebra				
unchanged cartilage	22/9	16/11	11/8	21/12
split cartilage of centrum	0/0	0/0	1/1	0/0
Incomplete ossification sternebra				
unchanged cartilage	29/15	39/14	43/16	61/22
dumbbell-shaped cartilage of centrum	1/1	0/0	2/1	1/1
Hemicentric sternebra[uc]	16/10	25/11	21/12	17/12
Misshapen sternebra[uc]	79/21	78/20	62/20	84/23
Misshapen sternebra[scofc]	1/1	0/0	0/0	0/0
Bipartite ossification sternebra[uc]	4/2	2/2	1/1	3/3
Extra sternabral ossification site[uc]	0/0	3/3	1/1	0/0
Short Rib (13th)[cmp]	17/8	36/12	13/7	29/11
Cervical rib[cp]	0/0	1/1	0/0	1/1
Cervical rib[cmp]	1/1	2/2	2/2	9/8*
Supernumerary rib (14th)[cmp]	3/3	4/2	0/0	4/3
Absent rib (13th)[cp]	1/1	0/0	0/0	0/0
Incomplete ossification of metacarpal[uc]	0/0	0/0	0/0	2/2
Unossified metacarpal[uc]	0/0	0/0	0/0	1/1
Incomplete ossification of metatarsal[uc]	0/0	0/0	0/0	1/1
Dumbbell-shaped cartilage of thoracic centrum	2/1	2/2	2/2	3/3
Fused thoracic centrum cartilage	0/0	0/0	0/0	1/1
Dumbbell-shaped cartilage of lumbar centrum	0/0	0/0	0/0	1/1
Fused sacral arch cartilage	8/5	4/2	10/4	3/2
Fused caudal centrum cartilage	1/1	0/0	0/0	0/0
Bipartite processus xiphoideus	35/16	32/15	45/17	36/19
Fused rib cartilage	4/3	2/2	1/1	5/4
Branched rib cartilage	2/2	0/0	1/1	2/2
Bipartite rib cartilage	1/1	0/0	0/0	0/0

[uc] = unchanged cartilage; [cmp] = cartilage not present; d-scofc = dumbbell-shaped cartilage of centrum; [scofc] = split cartilage of centrum; [cp] = cartilage present; * = $p < 0.05$

[a] = data from Table IB 013 to 032, pages 72-91 of the report

There was an increased fetal and litter incidence of cervical ribs with cartilage not present (Historical Control for the closest type of observatic was 3/779 fetuses in 3/118 litters) in the high dose group.

D. Discussion/Conclusions

i. Investigators Summary:

From page 45 of the report:

BAS 500 F was administered to pregnant Wistar rats daily by stomach tube from implantation to one day prior to the expected day of parturition (days 6 - 19 post coitum [P.C.]).

50 mg BAS 500 F/kg body weight/day revealed overt signs of maternal toxicity. The high dose dams' food consumption was statistically significantly reduced on several days of the treatment period; if calculated for days 6 - 19 p.c. it was about 11% below the concurrent control value. The mean body weight of the high dose rats was statistically significantly lower than that of the concurrent controls on day 20 p.c. (about 5% below the corresponding control value) and body weight gain was statistically significantly impaired (16% below the mean weight gain of the concurrent control group) if calculated for the entire treatment period (days 6 - 19 p.c.). Moreover, carcass weight and corrected body weight gain were statistically significantly lower at 50 mg/kg body weight/day (carcass weigth: about 6%, corrected body weight gain: about 45% below the concurrent control value), which demonstrate treatment-related, direct signs of maternal toxicity in line with the decrements in food uptake and/or body weight gain. Similar, but less pronounced substance-induced signs of maternal toxicity occurred in the mid dose (25 mg/kg body weight/day) group in the form of reduced food consumption (about 7% below controls if calculated for days 6 - 19 p.i.) and clearly reduced corrected body weight gain (about 22% below the concurrent cont-ml value).

No signs of substance-induced maternal toxicity occurred at the low dose level (10 mg/kg body weight/day).

The oral administration of BAS 500 F to the dams at all 3 dose levels (10, 25 and 50 mg/kg body weight/day) had no influence on the gestational parameters and induced no signs of developmental toxicity; especially, no indications for substanceinduced teratogenicity were seen up to and including the highest dose level. Several malformations and variations were observed scattered throughout the dose levels, which showed either no dose dependency or occurred at incidences consistent with the historical background data for the rat strain used in the present study.

Based on these results, the no observed adverse effect level (NOAEL) for maternal toxicity is 10 mg/kg body weight/day, while it is 50 mg/kg body weight/day for developmental toxicity.

ii. Reviewers Conclusions:

a. Maternal Toxicity:

No deaths, clinical signs of toxicity or gross pathological observations were noted in this study. The 25 and 50 mg/kg/day dose groups had lower overall body weights at gestation days 19/20

and gained less weight than the control during the dosing period (gestation days 6-19), for the post dosing period (19-20), for the overall gestation period (0-20) and for the calculated period of gestation days 6-20, also for corrected body weight gains from gestation days 6-20. As seen with the body weights and body weight gains, the 25 and 50 mg/kg/day dose groups had reduced food consumption during the dosing period (gestation days 6-19), for the post dosing period (19-20) and for the overall gestation period (0-20). There was reduced food efficiency in the 50 mg/kg/day dose group during the dosing period (gestation days 6-19) and in the 25 and 50 mg/kg/day dose groups for the post dosing period (19-20), for the overall gestation period (0-20) and for the calculated period of gestation days 6-20.

b. **Developmental Toxicity:**

i. **Deaths/Resorptions:**

No treatment related effects were noted.

ii. **Altered Growth:**

No treatment related effects were noted.

iii. **Developmental Anomalies:**

There was an increase the fetal and litter incidence of dilated renal pelvis and increased incidence of cervical ribs with cartilage not present in the high dose group.

iv. **Malformations:**

No treatment related effects were noted.

c. **Conclusions:**

Maternal Toxicity NOEL = 10 mg/kg/day
Maternal Toxicity LOEL = 25 mg/kg/day
Developmental Toxicity NOEL = 25 mg/kg/day
Developmental Toxicity LOEL = 50 mg/kg/day

d. **Study Deficiencies:**

No major deficiencies were noted.

e. **Classification: Acceptable-Guideline**

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