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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.
Toxicologist, Registration Action Branch 3/HED (7509C)

DATA EVALUATION RECORD

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

EPA ID No.s: EPA MRID No. 45118326
EPA Pesticide Chemical Code 099100
CAS# 175013-18-0
EPA DP Barcode D267732, D269669
EPA Submission No. S583112

Test Material: BAS 500 F

Synonyms: Pyraclostrobin, Reg. No. 304 428

Citation: Schilling V., Hellwig, J., Hildebrand, B. (1999): BAS 500 F - Prenatal Developmental Toxicity Study in Himalayan Rabbits Oral Administration (Gavage); Department of Toxicology of BASF Aktiengesellschaft for BASF Corporation, Agricultural Products; Laboratory Project Identification No.40R0494/96159, BASF Registration Document No. 1999/11512; October 25, 1999 (Unpublished); EPA MRID Number 45118326.

Executive Summary: In a prenatal developmental toxicity study (Teratology) (MRID# 45118326), sexually mature, virgin Chbb:HM (outbred strain) Himalayan rabbits (Supplier: BOEHRINGER INGELHEIM PHARMA KG received either 0, 5, 10, or 20 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 days 7 through 28 p.i., inclusive. A check was made twice daily on working days or once daily (Saturday, Sunday or on public holidays) (days 0 - 29 p.i.). The maternal animals were examined for clinical symptoms with all animals weighed on days 0, 2, 4, 7, 9, 11, 14, 16, 19, 21, 23, 25, 28 and 29 p.i. along with consumption of food determined daily during the entire study

period. On day 29 p.i., the surviving dams were sacrificed and the fetuses were removed from the uterus, the dams were then 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 recorded. The fetused were examined for external, visceral and skeletal anomalies.

No treatment related mortality was noted. Reduced fecal output was seen in 1 mid dose (day 10 p.i.) and 10 high-dose animals (days 10-14 p.i.). Two mid-dose and 4 high-dose animals showed blood in the bedding (between days 16-29 p.i.). No other relevant clinical observations were noted. All treated groups had lower body weight gains during the dosing period (days 7-28) and the overall gestation period (day 0-29) while the mid and high dose groups had lower body weight gains during the post dosing period (days 28-29). The decreased body weight gain, among all treated groups, can mainly be attributed to the earliest post-treatment period, namely gestation days 7-9 (treatment days 0-2). As seen with the body weights and body weight gains, all treated groups had reduced food consumption during the treatment period (days 7-28), and the overall gestation period (days 0-29). Food efficiency was lower in all treated groups during the same periods as food consumption and during the post dosing period (days 28-29). No treatment related pathological observations were noted in the data provided. There was reduced litter size, increased resorptions per dam and increased post implantation loss in the high dose group. **The maternal toxicity NOAEL was less than 5 mg/kg/day and the maternal toxicity LOAEL is less than or equal to 5 mg/kg/day based on body weight gains, reduced food consumption and reduced food efficiency.**

There was reduced litter size, increased resorptions per dam and increased post implantation loss in the high dose group. There was also an increased incidence of the anomaly: lumbar vertebrae absent. **The developmental toxicity NOAEL was 10 mg/dg/day and the developmental toxicity LOAEL was 20 mg/kg/day based on reduced litter size, increased resorptions per litter, increased post implantation loss and increased lumbar verterbrae absent.**

This study is classified as <u>Acceptable-Guideline</u> and satisfies

the guideline requirements (§ 83-3a) for a Prenatal Developmental Toxicity Study (Teratology) in rabbits.

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).

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 Himalayan rabbits
Strain: Chbb:HM (outbred strain)
Source: BOEHRINGER INGELHEIM PHARMA KG (former name: DR. K. THOMAE GmbH), Biberach an der Riss, FRG
Age: 10 weeks
Body Weight: 2601-2711 grams at gestation day 0

B. Study Design

According to the investigators (from pages 17-18 and 25-26 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 Himalayan rabbits from implantation to one day prior to the expected day of parturition (days 7 - 28 p.i.). 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 18-22 weeks on November 24, 1997.

On day 29 p.i., all surviving females were sacrificed in 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 4 sections. Each dose group was represented in each section. A treatment interval between 2 - 9 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 7 p.c.)	End of treatment (day 28 pc.)	Sacrifice (day 29 p.c.)
1st section	by Jan. 04, 1998	Jan. 05, 1998	Jan. 12, 1998	Feb. 02, 1998	Feb. 03, 1998
2nd section	by Jan. 06, 1998	Jan. 07, 1998	Jan. 14, 1998	Feb. 04, 1998	Feb. 05, 1998
3rd section	by Jan. 11, 1998	Jan. 12, 1998	Jan. 19, 1998	Feb. 09, 1998	Feb. 10, 1998
4th section	by Jan. 13, 1998	Jan. 14, 1998	Jan. 21, 1998	Feb. 11, 1998	Feb. 12, 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 Aug. 1996)

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 25 of the report:

After an acclimatization period of at least 5 days, the does were fertilized by means of artificial insemination.

This implied that 0.2 ml of a synthetic hormone which releases LH and FSH from the anterior pituitary lobe (Receptal®, trademark of HOECHST AG, Frankfurt/Main) were injected intramuscularly to the female rabbits about 1 hour before insemination. The pooled ejaculate samples used for the artificial insemination were derived from male Himalayan rabbits of the same breed as the females. The male donors were kept under conditions (air conditioning, diet,

water) comparable to those of the females participating in this study. During the acclimatization period the animals were assigned to the different test groups according to a randomization plan (Nijenhuis and Wilf, 1978) and on the basis of their body weights.

The day of insemination was designated as day 0 (beginning of the study) and the following day as day 1 post insemination (p.i.).

On day 0, the does were between 24 and 29 weeks old. Their mean body weight was approx. 2657 g.

Animal Husbandry

From pages 20-21 of the report:

Unique indentification of the rabbits by ear tattoo had already been carried out by the breeder.

This strain was selected since extensive experience is available on Himalayan rabbits and this strain has been proved to be sensitive to substances with a teratogenic potential (Lehmann and Niggeschulze, 1971; Merkle and Zeller, 1980).

During the acclimatization and the study period, the rabbits were housed singly in type 12.2395.C stainless steel wire mesh cages supplied by DRAHT-BREMER GmbH, Marktheidenfeld, FRG (floor area about 3,000 cm). 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 room was completely disinfecied 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 pelleted Kliba maintenance diet type 23-341-4 for rabbits supplied by PROVIMI KLIBA SA (former name: KLINGENTALMOHLE AG), CH-4303 Kaiseraugst, Switzerland, which 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 22 of the report:

Test group	Dose mg/kg bw/day	Concentration mg/100 ml	Volume ml/kg	Number of animals	Tattoo[3] No.
0	0	0	10[1]	25	see page 22 of the study report
1	5	50	10[2]	24[4]	
2	10	100	10[2]	25	
3	20	200	10[2]	25	

[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

[3] Due to technical reasons, the animals were numbered consecutively, beginning with the first animal of test group 0 (computer No. 1) and ending with the last animal of test group 3 (computer No. 99). These computer Nos. are printed in the Tables of Volume 11 and can also be found in the relevant record sheets (raw data).

[4] It was the original intention to place 25 animals in each test group (i.e 100 rabbits in total). One of the rabbits, however, broke its leg shortly before the study started and thus had to be sacrificed; therefore only 24 rabbits were available in one group.

Dosing and Dosing Solution Preparations

From pages 17 and 23 of the report:

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

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 developmental toxicity study.

Taking the results of this previous study into consideration the following doses were chosen for the present full-scale prenatal toxicity study in Himalayan rabbits:

5 mg/kg body weight/day:	as the expected no adverse effect level
10 mg/kg body weight/day:	as intermediate dose level
20 mg/kg body weight/day:	as the dose level with some overt signs of maternal toxicity and possible substance-related

of developmental toxicity

Each day the aqueous test substance suspensions were freshly prepared before the test substance was administered. 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 the treatment of the animals.

Dose Administration: From page 25 of the report:

The test substance was administered to the animals orally (by gavage) in an ascending dose once a day from implantation to one day prior to the expected day of parturition (days 7 - 28 p.i.) always at approximately 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 pages 23-24 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 performed in a previous study at identical concentrations.

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).

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 ±12%.
The food used in the study was assayed for chemical and 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 27-28 of the report:

A check was made twice daily on working days or once daily (Saturday, Sunday or on public holidays) (days 0 - 29 p.i.).

The animals were examined for clinical symptoms at least once a day, or more often when clinical signs of toxicity were elicited (days 0 - 29 p.i.).

The consumption of food was determined daily during the entire study period.

All animals were weighed on days 0, 2, 4, 7, 9, 11, 14, 16, 19, 21, 23, 25, 28 and 29 p 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 29 p.i. minus weight of the unopened uterus minus body weight on day 7 p.i.).

On day 29 p.i., the surviving dams were sacrificed in randomized order by intravenous injection of a pentobarbital (Nembutal@, Sanofi ceva, FRG; dose: 1 ml/kg body weight + 1 ml) and the fetuses were 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 30-31 of the report:

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

At necropsy each fetus was weighed and examined macroscopically for any external findings. 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.

Thereafter, the fetuses were sacrificed by the subcutaneous injection of a pentobarbital (Nembutal®, Sanofi ceva, FRG; dose: about 0.2 ml/fetus).

After the fetuses had been sacrificed, the abdomen and thorax were opened in order to be able to examine the organs in situ before they were removed. The heart and the kidneys were sectioned in order to assess the internal structure.

The sex of the fetuses was determined by internal examination of the gonads.

After these examinations, the heads of approximately of one half of the fetuses per dam (and the heads of those fetuses, which revealed severe findings (e.g. anophthalmia, microphthalmia or hydrocephalus) already during the external examination) were severed from the trunk. These heads were fixed in BOUIN's solution and processed and assessed according to WILSON's method subsequently (Wilson and Warkany, 1965). About 10 transverse sections were prepared per head. After the examination the heads treated in this way were discarded.

After skinning, all fetuses (partly without heads) were fixed in ethyl alcohol. After fixation for approx. 1 - 5 days, the fetuses were removed from the fixative for a short while and a cross section of the heads from all intact fetuses was made in the parietal bone area using a scalpel. The two halves of the heads were carefully bent to allow a thorough examination of the brain. Subsequently, the fetuses were placed back into the fixative for further fixation.

After fixation in ethyl alcohol the skeletons (partly without heads (see

3.9.2.)) were stained according to a modified method of DAWSON (Dawson, 1926). The stained skeletons were placed on an illuminated plate and examined, evaluated and assessed. After the examination the stained 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 CHAHOUD 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 morphongenesis that has otherwise followed a normal pattern of development.

Moreover, the term **"Unclassified observation"** was used for those fetal findings, which could not be classified as malformations or variations (e.g. focal liver necrosis in fetuses).

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 29 and 32-33 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, proportions 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 Hettmansperger, T.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); the are not exactly precise values, because the size of the intervals taken for calculation differs. Fo the "mean of means" values no statistical analysis was performed.

References (from pages 53-54 of the report):

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

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Reproductive Toxicology 13, 77 - 82 (1999)

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LEHMANN, H. and NIGGESCHULZE, A.
The teratologic effects of thalidomide in Himalayan rabbits.
Toxicol. Appl. Pharmacol. 18, 208 - 219 (1971)

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Untersuchungen von Acetamiden und Formamiden auf embryotoxische und teratogene.
Wirkung bei Kaninchen. Arzneim. Forsch. 30, 1557 - 1562 (1980)

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Combinatorial Algorithms, Academic Press, New York, San Francisco, London, 62 - 64 (1978)

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Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. 247, 367 - 368 (1964)

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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)

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 §83-3b.

C. Results

Maternal Toxicity:

Mortality

One control animal died by accident (was not pregnant) and 1 mid-dose animal, by accident (was pregnant, gavage error).

Clinical Observations

The investigators supplied group mean and individual animal data. Two mid-dose animals were excluded from the calculations due to not being pregnant. Reduced fecal output was seen in 1 mid dose (day 10 p.i.) and 10 high-dose animals (days 10-14 p.i.). Also 2 mid-dose and 4 high-dose animals showed blood in the bedding (between days 16-29 p.i.). No other relevant clinical observations were noted.

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	7	28	29
Dose:				
0 mg/kg/day	2711±195.6	2744±217.3	2935±242.6	2961±238.5
5 mg/kg/day	2601±204.2	2647±216.1	2778±217.9	2807±222.8
10mg/kg/day	2679±221.1	2726±227.9	2828±229.4	2851±231.0
20mg/kg/day	2633±198.0	2685±217.1	2729**±224.8	2748**±230.5
Historical control	2625	2611	2847	2881

[a] = data from Table IA 006 and 007, pages 63-64 of the report; ** = $p < 0.01$

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

Gestation Day	0-7	7-28	28-29	7-9	C7-29[1]
Dose (mg/kg/day):					
0	32.4±49.1	191.5±87.4	25.4±20.2	-3.8±24.63	-135.7±62.9
5	46.0±41.5	131.7±96.8	28.5±18.3	-43.8**±33.34	-142.4±85.8
10	47.2±37.9	116.0*±82.8	22.4±25.8	-85.5**±61.59	-132.9±84.5
20	52.3±44.2	44.1**±116.6	18.6±20.7	-146.3**±51.49	-146.8±84.2

[a] = data from Tables IA 008-011, pages 65-68 of the report; [1] = corrected body weight gain (minus uterine weight); * = $p < 0.05$; ** = $p < 0.01$

All treated groups had lower body weight gains during the dosing period (days 7-28) and the overall gestation period (day 0-29) while the mid and high dose groups had lower body weight gains during the post dosing period (days 28-29). The decreased body weight gain, among all treated groups, can mainly be attributed to the earliest post-treatment period, namely gestation days 7-9 (treatment days 0-2).

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]

Gestation Days	0-7	7-28	28-29	0-29
Dose:				
0 mg/kg/day	117.1±5.0	89.8±6.3	83.5±23.9	96.2±13.4
5 mg/kg/day	113.6±4.9	78.2±11.0	89.3±18.7	87.1±18.1
10 mg/kg/day	118.3±2.7	77.5±17.6	84.3±23.8	87.6±23.1
20 mg/kg/day	117.6±3.7	67.6±26.1	85.4±29.2	80.3±30.9

[a] = data from Tables IA 002-005, pages 59-62 of the report.

Table IV: Food Efficiency Data (%)*

Dose (mg/kg/day):	Gestation Days 0-7	7-28	28-29	0-29
0	3.5	10.2	15.2	8.6
5	5.1	8.0	16.0	7.9
10	5.0	7.1	13.3	7.1
20	5.6	6.2	10.9	4.8

* = calculated by the reviewer.

As seen with the body weights and body weight gains, all treated groups had reduced food consumption during the treatment period (days 7-28), and during the overall gestation period (days 0-29). Food efficiency was lower in all treated groups during the same periods as food consumption and during the post dosing period (days 28-29).

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[4]

Dose (mg/kg/day):	0	5	10	20	HC[1]
#Animals Assigned	25	24	25	25	
#Animals Mated/Inseminated	25	24	25	25	122
#Animals Pregnant	24	24	23	25	120
Pregnancy Rate (%)	96	100	92	100	96
Maternal Wastage					
#Died/Sacrificed	1	0	1	0	0
#Died/pregnant	0	0	1	0	
#Non pregnant	1	0	2	0	
#Aborted	0	0	0	0	0
#Premature Delivery	0	0	0	0	0
Total litters examined	24	24	22	25	
Total Corpora Lutea	193	189	171	193	1053
Corpora Lutea/dam	8.0±1.6	7.9±2.2	7.8±1.5	7.7±1.6	8.8±1.5
Total Implantations	177	159	152	174	856
Implantations/Dam	7.4±1.4	6.6±2.3	6.9±1.7	7.0±1.7	7.1±2.2
Total Live Fetuses	166	145	123	107	778
Live Fetuses/Dam	6.9±1.5	6.0±2.3	6.2±1.8	4.9**±2.6	6.5±2.3
Total Resorptions	11	14	29	67	120
Early	10	13	26	65	
Late	1	1	3	2	
Resorptions/Dam	0.5±0.7	0.6±0.7	1.3±2.3	2.7**±2.8	0.6±1.0
Total Dead Fetuses	0	0	0	0	1
Mean Fetal Weight (gm)	37.0±2.6	37.0±3.3	35.2±3.7	35.1±4.2	39.3
Preimplantation Loss(%)[2]	8.3	15.9	11.1	9.8	18.7
Postimplantation Loss(%)[2]	6.2	8.8	19.1	38.5	9.1
Sex Ratio (% Male)	50.0	53.1	52.8	52.3	51

[1] = HC = Historical Control; [2] = calculated by reviewer from mean data
[4] = data from Tables IA 014-016 and IB 001, pages 71-74 of the report.

There was reduced litter size, increased resorptions per dam and increased post implantation loss in the high dose group. The post implantation loss was increased in the mid dose group; however, there was no biologically relevant decrease in litter size.

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):	0	5	10	20
Observations				
#pups/litters examined	166/24	145/24	123/20	107/22
Acephaly	0/0	0/0	1/1	0/0
Fetus w/multiple malformations	0/0	1/1	0/0	0/0
Ectrodactyly	0/0	0/0	1/1	0/0
Brachydactyly	0/0	0/0	1/1	0/0
Short tail	1/1	0/0	0/0	0/0
Thread-like tail	0/0	0/0	1/1	0/0
Paw hyperflexion	1/1	1/1	2/2	0/0
Malrotated limb	0/0	0/0	2/2	0/0

[a] = data from Table IB-003, 004 and 006, pages 76, 77 and 79 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*

Dose (mg/kg/day):	0	5	10	20
Observations				
#pups/litters examined	166/24	145/24	123/20	107/22
Hydrocephaly	1/1	0/0	0/0	0/0
Dilated cerebral ventricle	0/0	0/0	1/1	0/0
Overriding aorta	0/0	0/0	2/2	0/0
Narrowed pulmonary trunk	0/0	0/0	2/2	0/0
Transposition of great vessels	0/0	0/0	1/1	0/0
Malpositioed aortic arch	0/0	0/0	1/1	0/0
Dilated aortic arch	0/0	0/0	1/1	0/0
Septal defect	0/0	1/1	3/3	0/0
Malpositioned carotid branch	19/14	4/4	10/7	7/7
Diaphragmatic hernia	0/0	0/0	1/1	0/0
Absent gallbladder	0/0	1/1	1/1	1/1
Fused kidney	0/0	0/0	1/1	0/0
Dilated renal pelvis	0/0	0/0	1/1	0/0
Hydroureter	0/0	0/0	1/1	0/0
Fused adrenal	0/0	0/0	1/1	0/0
Malpositioned ovary	0/0	0/0	1/1	0/0
Infarct of liver	1/1	0/0	1/1	0/0
Blood coagulum around urinary bladder	0/0	1/1	0/0	0/0

* = data from Table IB- 008 to 016, pages 81-89 of the report.

No treatment related effects were noted in the above soft tissue observation data.

c. Skeletal Examinations

The following table presents the skeletal examination data:

Table VIII: Skeletal Examinations[a]

Dose (mg/kg/day):	0	5	10	20
Observations				
#pups/litters examined	166/24	145/24	123/20	107/22
Cervical centrum				
hemicentric	0/0	1/1	1/1	1/1
unossified	2/2	0/0	0/0	0/0
Thoracic vertebra				
absent	0/0	0/0	0/0	1/1
misshapen	0/0	0/0	1/1	0/0
supernumerary	1/1	1/1	2/1	0/0
Thoracic centrum				
incomplete ossification	1/1	0/0	0/0	1/1
dumbbell ossification	0/0	0/0	0/0	1/1
Thoracic arch				
incomplete ossification	0/0	0/0	1/1	0/0
Lumbar vertebra				
absent	1/1	1/1	1/1	4/4
misshapen	1/1	1/1	0/0	2/2
Lumbar arch				
incomplete ossification	3/3	3/2	2/2	3/3
Sacral vertebra				
misshapen	1/1	0/0	0/0	0/0
Sacral arch				
incomplete ossification	1/1	1/1	2/2	2/2
Sternebra				
unossified	40/19	25/15	37/16	23/10
incomplete ossification	41/21	37/18	22/11	18/12
fused	6/6	1/1	2/2	7/7
misshapen	11/8	6/5	4/4	11/7
extra ossification site	0/0	1/1	0/0	2/2
bipartite ossification	0/0	1/1	0/0	0/0
Misshappen scapula	2/2	1/1	1/1	0/0
Bony plate	1/1	0/0	1/1	1/1
Incomplete oss. of hyoid	22/14	20/14	13/8	16/12

Continued

Table VIII: Skeletal Examinations continued

Dose (mg/kg/day):	0	5	10	20
Observations				
#pups/litters examined	166/24	145/24	123/20	107/22
Splitting of skull bone(s)	3/3	3/3	0/0	1/1
Parietal hole(s)	3/3	3/3	4/4	2/2
Extra oss. site (between nasal & frontal)	4/4	1/1	0/0	0/0
Enlarged fontanel	0/0	0/0	1/1	0/0
Incomplete oss. of cervical centrum	6/5	5/4	10/7	6/5
Rib				
supernumerary (13th)	8/7	13/9	13/8	9/6
short (12th)	0/0	0/0	1/1	0/0
cervical	0/0	1/1	1/1	1/1
absent (12th)	0/0	0/0	0/0	1/1
incomplete ossification	0/0	0/0	1/1	0/0
Talus				
incomplete ossification	1/1	1/1	2/2	3/3
unossified	0/0	0/0	0/0	1/1
Unossified pubis	0/0	0/0	0/0	1/1

* = data from Table IB- 017 to 031, pages 90-104 of the report

The incidence of absent lumbar vertebrae fell outside of the historical control range of values provided by the sponsor (from page 257 of the study report), i.e., mean fetal incidence of 0.3%, range of 0.0% to 0.9%, mean litter incidence of 1.7%, range of values 0.0% to 5.9%.

D. Discussion/Conclusions

i. Investigators Summary:

From pages 51-52 of the report:

BAS 500 F was administered to pregnant Himalayan rabbits daily by stomach tube from implantation to one day prior to the expected day of parturition (days 7 - 28 p.i.).

20 mg BAS 500 F/kg body weight/day revealed overt signs of maternal toxicity. 4 high dose females showed blood in bedding on several days of the treatment period (3 of these animals had no live fetuses but only early resorptions). The defecation of a considerable number of high dose rabbits was reduced between days 10 - 14 p.i., which was a consequence of the statistically significant reductions in the does' food consumption between days 7 - 14 p.i.. The mean body weight of the high dose rabbits was statistically significantly lower than that of the concurrent controls between days 9 - 29 p.i. (7% below the corresponding control value on day 29 p.i.) and body weight gain was statistically significantly impaired (77% below the mean weight gain of the concurrent control group) if calculated for the entire treatment period (days 7 - 28 p.i.). The impairments in food intake and body weight gain (actually a body weight loss) at initiation of dosing are considered to reflect direct, substance-induced maternal toxicity on the high dose females. The increased resorption rate at 20 mg/kg body weight/day (see below) accounts for the distinctly reduced mean gravid uterus weights and the impairments in body weight gain towards the termination of the study. Similar, but less pronounced substance-induced signs of maternal toxicity occurred in the mid dose (10 mg/kg body weight/day) group in the form of reduced defecation (1 doe), blood in bedding (2 does), reduced food consumption on days 7 - 12 p.i., statistically significantly impaired body weight gain (39% below the mean weight gain of the concurrent control group) if calculated for the entire treatment period (days 7 - 28 p.i.) and slightly reduced mean gravid uterus weight as a consequence of a marginally increased resorption rate.

No signs of substance-induced maternal toxicity occurred at the low dose level (5 mg/kg body weight/day).

The oral administration of BAS 500 F affected the gestational parameters in a dose related manner at 10 and 20 mg/kg body weight/day, but was devoid of any influence at 5 mg/kg body weight/day.

The resorption rate was statistically significantly increased at 20 mg/kg body weight/day due to the fact that 3 high dose dams resorbed

all of their implants and several other females of this group showed a clustered increase in the number of resorptions. The postimplantation loss value was moderately increased to 38.6% (concurrent control group: 6.2%; historical control range: 5.2% - 20.1 %) and the mean number of live fetuses per rabbit was decreased in comparison to the concurrent control group (4.9 versus 6.9) and the historical control range (6.1 - 7.1).

The dams of test group 2 (10 mg/kg body weight/day) showed a slight increase in the overall resorption rate with two dams with total embryolethality and marginally elevated postimplantation loss value (17.8% versus 6.2% in the concurrent control).

There occurred no further signs of substance-induced developmental toxicity - in addition to the increased embryolethality at 10 and 20 mg/kg - in any of the dose groups (5, 10 or 20 mg/kg body weight/day). Mean placental and fetal body weights were unaffected and there were no indications for substance-related influences on fetal morphology. Several malformations were observed in fetuses of all test groups including the controls, but these were finally considered to be spontaneous in nature.

This is also true for different fetal external, soft tissue and skeletal variations, which appeared at incidences, which were consistent with the historical background data for the rabbit strain used in the present study.

Based on these results, the no observed adverse effect level (NOAEL) for both, maternal and developmental toxicity is 5 mg/kg body weight/day.

ii. Reviewers Conclusions:

a. Maternal Toxicity:

Two animals, 1 control and 1 mid-dose died by accident. Reduced fecal output was seen in 1 mid dose (day 10 p.i.) and 10 high-dose animals (days 10-14 p.i.). Also 2 mid-dose and 4 high-dose animals showed blood in the bedding (between days 16-29 p.i.). No other relevant clinical observations were noted. All treated groups had lower body weight gains during the dosing period (days 7-28), (days 7-9) and the overall gestation period (day 0-29) while the mid and high dose groups had lower body weight gains during the post dosing period (days 28-29). As seen with the body weights and body weight gains, all treated groups had reduced food consumption during the treatment period (days 7-28), and the overall gestation

period (days 0-29). Food efficiency was lower in all treated groups during the same periods and the post dosing period (days 28-29). No treatment related pathological observations were noted in the data provided. There was reduced litter size, increased resorptions per dam and increased post implantation loss in the high dose group. The post implantation loss was increased in the mid dose group; however, there was no significant decrease in litter size.

b. Developmental Toxicity:

i. Deaths/Resorptions:

There was reduced litter size, increased resorptions per dam and increased post implantation loss in the high dose group. The post implantation loss was increased in the mid dose group; however, there was no biologically relevant decrease in litter size.

ii. Altered Growth:

No treatment related effects were noted.

iii. Developmental Anomalies:

The incidence of absent lumbar vertebrae was increased at 20 mg/kg/day.

iv. Malformations:

No treatment related effects were noted.

c. Conclusions:

Maternal Toxicity NOAEL < 5 mg/kg/day
Maternal Toxicity LOAEL ≤ 5 mg/kg/day
Developmental Toxicity NOAEL = 10 mg/kg/day
Developmental Toxicity LOAEL = 20 mg/kg/day

d. Study Deficiencies:

No major deficiencies were noted.

e. Classification: Acceptable-Guideline