DATA EVALUATION RECORD

BAS 510 F'

STUDY TYPE: PRENATAL DEVELOPMENTAL TOXICITY STUDY - RAT [ORAL] OPPTS 870.3700a [§83-3a]; OECD 414.

MRID 45404904

7/23/2002

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task No. 02-06

Primary Reviewer:
Claudia Troxel, Ph.D.

Signature:
Date: JAN 30 2002

Secondary Reviewers:
Carol Forsyth, Ph.D., D.A.B.T.

Signature:
Date: JAN 30 2002

Robert H. Ross, M.S. Group Leader

Signature: Robert H. Ross
Date: JAN 30 2002

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: JAN 30 2002

Disclaimer

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

Oak Ridge National Laboratory is managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

(1)

EPA Reviewer: Alan C. Levy, Ph.D. Signature: Alan C. Levy
Registration Action Branch 2, Health Effects Division (7509C) Date 7-23-2002
EPA Work Assignment Manager: Ghazi Dannan, Ph.D. Signature: _____
Registration Action Branch 3, Health Effects Division (7509C) Date _____

Template version 11/01

DATA EVALUATION RECORD
TXR#: 0050193.

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; OPPTS 870.3700a [§83-3a]; OECD 414.

PC CODE: 128008

DP BARCODE: D278384
SUBMISSION NO.: S 604279

TEST MATERIAL (PURITY): BAS 510 F (94.4% a.i.)

SYNONYMS: none provided

CITATION: Schilling, K. and J. Hellwig (2000) BAS 510 F - Prenatal developmental toxicity study in Wistar rats - oral administration (gavage). Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, FRG. Laboratory Project Identification: 30R0179/97140, September 1, 2000. MRID 45404904. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products Division, RTP, NC 27709.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 45404904), BAS 510 F (94.4% a.i., batch/lot # N37) was administered to 25 female Wistar rats/dose by gavage at dose levels of 0, 100, 300, or 1000 mg/kg bw/day from days 6 through 19 of gestation. Due to the unscheduled death (gavage error) of 3 animals each in the low- and high-dose groups, an additional section with 6 animals (3 each low and high doses) was added to guarantee at least 20 pregnant rats/group. On gestation day 20, dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally. The total numbers of fetuses examined (number of litters) was 319 (22), 246 (18), 333 (22), and 287 (21) for the 0, 100, 300, and 1000 mg/kg bw/day groups, respectively. Approximately one-half of the fetuses were examined viscerally, and the other one-half of the fetuses were examined for skeletal malformations/ variations.

There were no treatment-related effects in survival, clinical signs, body weight, food consumption, or gross necropsy. Deaths of several maternal animals were ascribed to gavage error. **The maternal toxicity NOAEL is ≥ 1000 mg/kg bw/day, and the maternal toxicity LOAEL could not be established.**

2

No treatment-related effects on pregnancy rates, number of corpora lutea, pre- or postimplantation losses, resorptions/dam, fetuses/litter, fetal body weights, or fetal sex ratios were observed in the treated groups compared with the controls.

No treatment-related external, visceral, or skeletal malformations/variations were observed in any groups. Most treated and control litters contained fetuses with minor variations in skeletal ossification. **The developmental toxicity NOAEL is ≥ 1000 mg/kg bw/day, and the developmental toxicity LOAEL could not be established.**

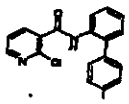
The developmental toxicity study in the rat is classified **Acceptable/Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** BAS 510 F

Description:	Powder, white
Lot/Batch #:	N 37
Purity:	94.4 % a.i.
Compound Stability:	Proven by reanalysis after the in-life phase of the study
CAS #of TGAI:	188425-85-6
Structure:	

2. **Vehicle and/or positive control:** 0.5% Tylose CB 30.000 in doubly distilled water (Lot/Batch # and purity not provided)

3. **Test animals:**

Species:	Rats	
Strain:	Wistar (Chbb:Thom(SPF))	
Age/weight at study initiation:	10-11 weeks of age; 187.7 - 235.2 g (individual animals)	
Source:	Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany	
Housing:	singly in stainless steel wire mesh cages (15 cm x 37.5 cm x 21 cm)	
Diet:	Ground Kilba maintenance diet rat/mouse/hamster meal (supplied by Provimi Kliba Sa, Kaiseraugst, Switzerland) *ad libitum*	
Water:	Tap water *ad libitum* (water bottles)	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	30-70%
	Air changes:	information not provided
	Photoperiod:	12hrs dark/12 hrs light
Acclimation period:	At least 5 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: October 27, 1998; End: November 25, 1998

2. Mating: Sexually mature, virgin females were mated with sexually mature males of the same strain as females (not specified if same source; 1-2 females mated with one untreated male). Confirmation of mating was determined by microscopic detection of sperm in a vaginal smear and was designated as day 0 of gestation.

3. Animal Assignment: Animals were assigned randomly to dose groups as indicated in Table 1.

TABLE 1. Animal Assignment

Dose (mg/kg bw/day)	0	100 LDT	300 MDT	1000 HDT
# Females	25	28 [a]	25	28 [a]

[a] Due to the unscheduled death of 3 animals in these test groups, an additional section of animals was added to get at least 20 pregnant rats/group.

4. Dose selection rationale: No information was provided regarding the dose selection process. The authors stated that the low-, middle-, and high-dose concentrations were expected to result in a no-observed-adverse-effect-level, an intermediate dose-level, and a higher level with some overt signs of maternal toxicity and possible developmental toxicity (the limit dose), respectively.

5. Dosage preparation and analysis: Test material-vehicle mixture was prepared at the beginning of the study and thereafter at intervals of 3-4 days by mixing appropriate amounts of test substance with 0.5% Tylose CB 30.000 in doubly distilled water using a high speed sonicator. The storage conditions of the test material-vehicle mixture were not described. Prior to the start of the study (approximately one year before), stability of the test substance in 0.5% Tylose CB 30.000 was evaluated for a period of 4 days at room temperature. Homogeneity (top, middle, and bottom) of the test mixture was evaluated before the beginning of the study (approximately one year before), and concentrations of the test mixture were evaluated before the beginning of the study (approximately one year before) and twice during the study period (beginning and near the end).

Results -

Homogeneity analysis: The mean concentrations of the samples taken from the top, middle, and bottom of the 6.0 and 10.0 g/100 mL test suspensions ranged from 90.0-93.3% of nominal and 95.0-99.0 % of nominal, respectively.

Stability analysis: The mean concentrations of 20 mg/100 mL and 50 mg/100 mL test suspensions at Day 4 as a percentage of nominal were 91% and 96.8%, respectively.

Concentration analysis: The mean concentrations of the 6.0, 8.0, and 10.0 g/100 mL test substance samples taken before study initiation ranged from 96.3 - 105.0% of nominal. The

mean concentrations of the 1.0, 3.0, and 10.0 g/100 mL test substance samples taken near the beginning and end of the study ranged from 90.0 - 97.9% of nominal.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. **Dosage administration:** All doses were administered once daily by (oral) gavage, on gestation days 6 through 19, in a volume of 10 mL/kg of body weight/day. Dosing was based on the last individual body weight.

C. OBSERVATIONS

1. **Maternal observations and evaluations:** Animals were checked for mortality or clinical signs twice/day during the week and once/day on weekends and public holidays. Body weight and food consumption data were recorded on gestation days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19, and 20. Dams were sacrificed on day 20 of gestation and subjected to gross necropsy. The uterus and ovaries were removed. Examinations at sacrifice consisted of weighing the unopened uterus and determining the number and distribution of viable and nonviable fetuses, early and late resorptions, and the number of total implantations and corpora lutea.

2. **Fetal evaluations:** Fetuses were weighed, sexed, and examined macroscopically for any external findings. .Placenta weights were recorded. Approximately one-half of the fetuses were placed in Bouin's fixative for subsequent soft tissue examination by the Barrow and Taylor method. The remaining one-half of the fetuses were eviscerated, skinned, fixed in alcohol, and stained according to a modified method of Kimmel and Trammell for skeletal examination.

D. DATA ANALYSIS:

1. **Statistical analyses:** Multiple comparisons with the control group using Dunnett's test were used to evaluate food consumption; body weight, body weight gain, corrected body weight gain, and carcass weights; weight of unopened uterus; number of corpora lutea, implantations, resorptions, and live fetuses; proportions of preimplantation loss, postimplantation loss, resorptions, and of live fetuses in each litter; litter mean fetal body weight; and litter mean placental weight. The reviewer does not think that the use of Dunnett's test without first performing an analysis of variance is appropriate. Female mortality, females pregnant at terminal sacrifice, and the number of litters with fetal findings were analyzed using a pairwise comparison of each dose group with the control using Fisher's Exact test (one-sided). Proportions of fetuses with malformations, variations, and/or unclassified observations in each litter were analyzed using pairwise comparison of each dose group with the control group using the Wilcoxon-test (one-sided).

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study:

conception rate (%): $\frac{\text{number of pregnant animals}}{\text{number of fertilized animals}} \times 100$

preimplantation loss (%): $\frac{\text{number of corpora lutea - number of implantations}}{\text{number of corpora lutea}} \times 100$

postimplantation loss (%): $\frac{\text{number of implantations - number of live fetuses}}{\text{number of implantations}} \times 100$

3. **Historical control data:** Two sets of historical control data were provided to allow comparison with concurrent controls. The first was called "old" and consisted of 10 studies dated March 1995 - October 1997. The other set was called "recent" because of the updated classification used for fetal findings and consisted of 2 studies dated March 1997 - June 1998.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** Three low-dose and 3 high-dose dams were found dead or were sacrificed in moribund condition on GDs 7-9. The deaths were the result of gavage error. No other mortalities were noted. No treatment-related signs of toxicity were observed.

2. **Body weight:** Body weight gain data are summarized in Table 2. No statistically significant differences were observed in mean absolute body weight or body weight gain of treated animals as compared to controls.

TABLE 2. Mean (±SD) Maternal Body Weight Gain (g) [a]

Interval	Dose in mg/kg bw/day			
	0	100	300	1000
Pretreatment: Days 0-6	28.3 ± 6.8	29.2 ± 5.1	32.2 ± 4.6	30.7 ± 5.4
Treatment: Days 6 -19	106.6 ± 13.9	92.9 ± 31.2	107.0 ± 14.7	102.9 ± 14.5
Total Study Days 0-20	150.1 ± 22.2	135.5 ± 36.2	156.4 ± 18.9	149.1 ± 18.9
Corrected BW Gain	38.1 ± 9.7	36.2 ± 10.8	39.5 ± 8.3	39.6 ± 7.8

[a]Data obtained from Table 1A; pages 53-56 in the study report.

3. **Food consumption:** No treatment-related differences were observed in food consumption of treated animals compared with the controls.

4. **Gross pathology:** Gross pathological examination of the 3 low-dose and 3 high-dose dams that died or were sacrificed in moribund condition revealed findings consistent with gavage error including congested lungs and bloody fluid in the thoracic cavity. No pathological changes related to the test substance were observed in any animal at scheduled sacrifice.

5. Cesarean section data: Data are summarized in Table 3. No statistically significant, substance-related changes were observed. A reduced number of litters (18) was noted in the 100 mg/kg/day group. Of the 28 mated animals in this group, 3 died early, 5 were not pregnant, and 2 had complete litter resorptions. Although not statistically significant, the low-dose group additionally had an elevated postimplantation loss and decreased mean gravid uterine weight.

TABLE 3. Cesarean Section Observations [a]

Observation	Dose (mg/kg bw/day)			
	0	100	300	1000
# Animals Assigned (Mated)	25	28	25	28
# Animals Pregnant	22	23	22	24
Pregnancy Rate (%)	88	82	88	86
# Nonpregnant (At Terminal Sacrifice)	3	5	3	4
Maternal Wastage				
# Died	0	3	0	3
# Died Pregnant	0	3	0	3
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	369	308	375	333
Corpora Lutea/Dam	16.8 ± 1.8	15.4 ± 2.39	17.0 ± 1.7	15.9 ± 2.1
Total # Implantations	354	278	352	306
(Implantations/Dam)	16.1 ± 2.5	13.9 ± 4.2	16.0 ± 3.2	14.6 ± 3.5
Total # Litters	22	18	22	21
Total # Live Fetuses	319	246	333	287
(Live Fetuses/Dam)	14.5 ± 2.6	13.7 ± 2.2	15.1 ± 3.2	13.7 ± 3.5
Total # Dead Fetuses	0	0	0	0
(Dead Fetuses/Dam)	0	0	0	0
Total # Resorptions	35	32	19	19
Early	34	27	19	17
Late	1	5	0	2
Resorptions/Dam	1.6 ± 1.4	1.6 ± 1.7	0.9 ± 0.8	0.9 ± 0.9
Early	1.5 ± 1.3	1.4 ± 1.6	0.9 ± 0.8	0.8 ± 0.9
Late	0.0 ± 0.2	0.3 ± 0.7	0.0 ± 0.0	0.1 ± 0.3
Litters with Total Resorptions	0	2	0	0
Mean Fetal Weight (g)	3.9 ± 0.2	3.8 ± 0.3	3.7 ± 0.2	3.9 ± 0.3
Males	4.0 ± 0.2	3.9 ± 0.4	3.8 ± 0.2	4.0 ± 0.3
Females	3.8 ± 0.3	3.7 ± 0.3	3.6 ± 0.2	3.8 ± 0.3
Sex Ratio (% Male)	55.5	52.0	50.5	49.8
Preimplantation Loss (%)	4.1 ± 11.5	9.6 ± 21.6	6.5 ± 16.9	8.6 ± 16.5
Postimplantation Loss (%)	9.7 ± 8.7	17.8 ± 29.0	5.3 ± 4.8	6.2 ± 6.0
Mean Gravid Uterine Weight (g)	83.8 ± 14.2	70.1 ± 26.8	84.8 ± 16.7	78.8 ± 17.4
Mean Placental Weight (g)	0.45 ± 0.06	0.45 ± 0.08	0.44 ± 0.05	0.44 ± 0.04

[a] Data obtained from Tables IA and IB, pages 56 and 58-61 in the study report.

B. DEVELOPMENTAL TOXICITY

1. External examination: External fetal examination data are presented in Table 4. There were no statistically significant differences between treated and control litters in the incidence rate

of external malformations/variations. The only malformation observed was anophthalmia in two fetuses from one low-dose litter. Additionally, a fused placenta was noted in 2 fetuses from 2 control litters and 2 fetuses from 1 low-dose litter.

2. **Visceral examination:** Visceral fetal examination data are presented in Table 4. No statistically significant differences were observed between the treated groups and the control group in the incidence rates of soft tissue malformations/variations. Malformations included a retroesophageal aortic arch in 1 fetus each from the control and low-dose groups, and an enlarged ventricular chamber in 1 control fetus. Variations that occurred in low or comparable incidences in all groups included dilated cerebral ventricle, dilated renal pelvis, and dilated ureter.

3. **Skeletal examination:** Skeletal fetal examination data are presented in Table 4. Skeletal malformations were limited to malpositioned and bipartite sternebra. The most common skeletal variations were incomplete, bipartite, or dumbbell ossification; unossification; and a short 13th rib. The high-dose group had a statistically increased litter incidence of incomplete ossification of thoracic centrum. Other skeletal variations generally occurred in low or comparable incidences in all groups.

TABLE 4. External, Visceral, and Skeletal Examinations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	100	300	1000
External Examinations				
#Fetuses(litters) examined	319 (22)	246 (18)	333 (22)	287 (21)
#Fetuses(litters) affected with malformations	0 (0)	2 (1)	0 (0)	0 (0)
#Fetuses(litters) affected with variations	2 (2)	2 (1)	0 (0)	0 (0)
Anophthalmia	0 (0) [c]	2 (1)	0 (0)	0 (0)
Visceral Examinations				
#Fetuses(litters) examined	155 (22)	118 (18)	159 (22)	139 (21)
#Fetuses(litters) affected with malformations	2 (2)	1 (1)	0 (0)	0 (0)
#Fetuses(litters) affected with variations	18 (12)	21 (11)	22 (12)	28 (15)
Retroesophageal aortic arch	1 (1)	1 (1)	0 (0)	0 (0)
Heart: enlarged ventricular chamber	1 (1)	0 (0)	0 (0)	0 (0)
Skeletal Examinations				
#Fetuses(litters) examined	164 (22)	128 (18)	174 (22)	148 (21)
#Fetuses(litters) affected with malformations	2 (2)	1 (1)	1 (1)	0 (0)
#Fetuses(litters) affected with variations	145 (22)	111 (18)	160 (22)	132 (21)
Malpositioned and bipartite sternebra	2 (2)	1 (1)	1 (1)	0 (0)
Incomplete ossification of thoracic centrum [d]	5 (3)	4 (3)	3 (2)	14 (10)*

[a] Data obtained from Table 1B, pages 62-92 in the study report.
[b] Except where otherwise noted, only the incidences of individual malformations are reported
[c] Fetal (litter) incidence
[d] This finding is a variation
* Statistically different ($p < 0.05$) from the control.

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that oral administration of BAS 510 F to pregnant dams over GD 6-19 did not result in any overt maternal or developmental toxicity. The NOAEL was therefore 1000 mg/kg bw/day for both maternal and developmental toxicity, and a LOAEL could not be established.

B. **REVIEWER COMMENTS:**

1. **Maternal toxicity:** Treatment with BAS 510 F did not result in measurable maternal toxicity at doses tested up to 1000 mg/kg bw/day. The six premature deaths were caused by gavage error, and no treatment-related differences in clinical signs, body weights, body weight gains, food consumption, or gross pathological findings were noted in any of the treatment groups compared with the controls.

   Therefore, the maternal toxicity NOAEL is ≥ 1000 mg/kg/day, and the maternal toxicity LOAEL could not be established.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** Treatment of pregnant rats with BAS 510 F did not result in increased embryonic or fetal death. Although a reduced number of litters (18) was noted in the 100 mg/kg/day group, it did not appear to be an effect of treatment: of the 28 mated animals, 3 died early from gavage trauma, 5 were not pregnant, and 2 had complete litter resorptions. This group additionally had an increased postimplantation loss and decreased mean gravid uterine weight, but these decreases were the consequence of the 2 dams with complete litter resorptions.

   b. **Altered growth:** Although the high-dose group had a statistically increased litter incidence of incomplete ossification of the thoracic centrum, no other evidence of delayed fetal growth (such as decreased fetal weights or other significant effects on ossification) were observed. Therefore, this fetal variation is not ascribed to treatment. It was concluded that treatment did not result in any alterations of fetal growth.

   c. **Developmental variations:** Treatment with BAS 510 F did not result in increases of developmental variations. Variations noted occurred equally in all groups or at low incidences. As discussed above, the statistically increased incidence of incomplete ossification of the thoracic centrum in the high-dose group was not ascribed to treatment.

   d. **Malformations:** No major fetal malformations could be attributed to maternal treatment with BAS 510 F.

   Therefore, the developmental toxicity NOAEL is ≥ 1000 mg/kg/day, and the developmental toxicity LOAEL could not be established.

C. **STUDY DEFICIENCIES:** Study deficiencies include: inappropriate statistical analyses, method of storage of dosing solutions not described, the dose selection rationale was not

provided, and the low-dose group had a reduced number of litters. These deficiencies do not compromise the study, however, since no maternal or developmental effects were observed at the highest dose tested, the limit dose. Having the missing information and data available would not have changed any of the conclusions of the study.

DATA FOR ENTRY INTO ISIS

Developmental Study - rats (870.3700a)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
128008	45404904	developmental	rats	GD 6-19	oral	gavage	100-1000	0, 100, 300, 1000	≥1000	not established	none identified	Maternal
128008	45404904	developmental	rats	GD 6-19	oral	gavage	100-1000	0, 100, 300, 1000	≥1000	not established	none identified	Developmental