EPA Reviewer: Lisa Austin, Ph.D **Signature:**
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 7/30/08
Template version 02/06

TXR#: 0055057

HED Executive Summary Cover for the attached OECD Formatted DATA EVALUATION RECORD

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

PC CODE: 118203 **DP BARCODE:** D349929

TEST MATERIAL (PURITY): BAS 800 H (93.8%)

SYNONYMS: AC 433379; BASF Reg. No. 4054449, saflufenacil

CITATION: BAS 800 H Prenatal Developmental Toxicity Study in Wistar Rats Oral Administration (Gavage). Experimental Toxicology and Ecology, BASF Aktiengesellschaft 67056 Ludwigshafen, FGR. Project Number 30R0414/01178. Study report date: 25-January-2007. MRID 47128115. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, 26 Davis Drive, Research Triangle Park, NC 27709

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 47128115) BAS 800 H (93.8% *a.i.*, batch/lot # COD - 000515) was administered to 25 Crl:WI(Han) female rats/dose in by gavage at dose levels of 0, 5, 20 or 60 mg/kg bw/day from days 6 through 19 of gestation.

There were no treatment-related effects on body weight, body weight gain, food consumption or gross necropsy. Hemoglobin, hematocrit, mean cell volume, and mean cell hemoglobin were slightly decreased (4-5%). Total bilirubin was increased by 11-22% at ≥ 20 mg/kg bw/day and liver porphyrins were increased by 4-13-fold at 5 mg/kg bw/d.

The maternal LOAEL is 60 mg/kg bw/day, based on decrease hemoglobin, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin. The maternal NOAEL is 20 mg/kg bw/day.

There was a decrease (8-16%) in male and female fetal body weights at doses ≥ 20 mg/kg bw/d. There was an increased incidence of the following skeletal malformations observed at 60 mg/kg/day: thick humeri (litter incidence: 3/22), bent radii (2/22), ulnas (2/22) and femurs (2/22) and mal-positioned and bipartite sternebrae (1-2/22), the control litter incidence was 0/22. Skeletal variations consisting of bent scapulae (1-3/22), incomplete ossification in the nasal area (4-9/22) and thoracic centra (3-9/22), bassioccipital holes (4/22), dumbbell ossification of lumbar centrum (6/22) and the finding of wavy ribs (16-17/22) were increased at doses ≥ 20 mg/kg bw/d.

At 60 mg/kg bw/d there was an increase in supraoccipital and bassoccipital holes (4-20/22), incomplete ossification of the hyoid (2/22) and dumbbell ossification of the lumbar centrum (6/22).

The developmental LOAEL is 20 mg/kg bw/day, based on decreased fetal body weights and increase skeletal variations. The developmental NOAEL is 5 mg/kg bw/day.

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, and Data Confidentiality statements were provided.

This Executive Summary was prepared for the United States Environmental Protection Agency, Office of Pesticide Program, Health Effects Division Use.

Much of the text was generated by the submitter(s) in OECD format. However, this document has undergone critical scientific analysis in comparison to the study report and modified as needed.

Reviewer #: 1755, Date: August 14, 2008

Study Type: Prenatal Developmental Study - Rat; OPPTS 870.3700; OECD 414.

Test Material (purity): BAS 800 H (93.8% BAS 800 H)

Synonyms: AC 433379; BASF Reg. No. 4054449

Citation: PMRA 1547124. BAS 800 H Prenatal Developmental Toxicity Study in Wistar Rats Oral Administration (Gavage). Experimental Toxicology and Ecology, BASF Aktiengesellschaft 67056 Ludwigshafen, FGR. Project Number 30R0414/01178. Study report date: 25-January-2007. Applicant Report Number 2007/1008481. DACO 4.5.2.

Sponsor: BASF Corporation, Agricultural Products, 26 Davis Drive, Research Triangle Park, NC 27709

MRID: Unknown

Executive Summary:

In a developmental toxicity study, BAS 800 H (93.8% BAS 800 H) was administered to 25 Crl:WI(Han) female rats/dose by gavage at dose levels of 0, 5, 20 or 60 mg/kg bw/day from days 6 through 19 of gestation.

There were no treatment-related effects on body weight, body weight gain, food consumption or gross necropsy. White blood cell counts were increased and haemoglobin, mean cell volume and mean cell haemoglobin were decreased at 60 mg/kg bw/d. **The maternal LOAEL is 60 mg/kg bw/day, based on decreased haematological parameters. The maternal NOAEL is 20 mg/kg bw/day.**

There was a decrease in male and female foetal body weights at doses ≥ 20 mg/kg bw/d. The skeletal malformation bent scapulae were increased as of 20 mg/kg bw/d and at 60 mg/kg bw/d there was an increase in thick humeri and bent radii, ulnas and femurs and malpositioned and bipartite sternebrae. Skeletal variations consisting of incomplete ossification in the nasal area and thoracic centra and the finding of wavy ribs were increased at doses ≥ 20 mg/kg bw/d. At 60 mg/kg bw/d there was an increase in supraoccipital and bassoccipital holes, incomplete ossification of the hyoid and dumbbell ossification of the lumbar centrum. **The developmental LOAEL is 20 mg/kg bw/day, based on decreased foetal body weights and increase skeletal malformations and variations. The developmental NOAEL is 5 mg/kg bw/day.**

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

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

I. Materials and Methods

A. Materials:

1 **Test Material:** BAS 800 H (N'-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide)

Description:	Solid / bright-beige
Lot/Batch #:	COD - 000515
Purity:	93.8 % BAS 800 H
Compound Stability:	The stability under the storage conditions present in this study was guaranteed by the Certificate of Analysis. The homogeneity of the test material in the dosing solution was confirmed by analysis.
CAS #:	372137-35-4

2. **Vehicle and/or positive control**: The test substance was given via gavage as an aqueous suspension in 1% Carboxymethylcellulose [CB 30.000].

3 **Test animals:**

Species:	Rat	
Strain:	Crl:WI(Han)	
Age/weight at study initiation:	Age: 10 – 12 weeks Weight: 143.1 – 186.8g	
Source:	Charles River, Germany	
Housing:	DK III stainless steel wire mesh cages (floor area about 800 cm^2)	
Diet:	Kliba maintenance diet mouse/rat "GLP", meal, supplied by Provimi Kliba SA, Kaiseraugst, Switzerland. *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	30-70%
	Air changes:	10/hr
	Photoperiod:	12hrs dark / 12hrs light
Acclimation period:	At least five days prior to application	

B. Study Design

1. **In life dates** - Start: May 11, 2005 End: June 2, 2005

2. **Mating** - Time-mated rats (Crl:WI (Han)) were supplied by Charles River Laboratories. The animals were paired by the breeder and supplied on day 0 post coitum. Confirmation of mating was determined by the presence of vaginal plug or sperm in the vaginal washing and was designated as day [0] of gestation.

3. **Animal Assignment** - Animals were assigned randomly to test groups by random withdrawal from the transport container and random placement of the animals into the test groups as indicated in Table 1.

Table 1. Study design

Dose (mg/kg bw/day)	0	5	20	60
# Females	25	25	25	25

4. **Dose selection rationale** – The investigators stated that 60 mg/kg bw/d was the dose level where some developmental and/or maternal toxicity (but not death or severe suffering) was expected. Five mg/kg bw/d was the expected no adverse effect level and 20 mg/kg bw/d was an intermediate dose level that could provide the opportunity for a gradation of toxic effects.

5. **Dosage preparation and analysis** – For the preparation of the test material-vehicle mixture, an appropriate amount of the test substance was weighed depending on the dose group, in calibrated beakers and subsequently suspended in 1.0% Carboxymethylcellulose CB 30.000 in doubly distilled water using a high-speed homogenizer. A magnetic stirrer was used to keep the suspensions homogeneous during treatment of the animals.

The test material-vehicle mixture was prepared at least every four days. The preparation was stored at room temperature for a maximum of four days.

Prior to the start of the study, stability of the test substance in doubly-distilled water was evaluated for a period of 96 hours (four days) at room temperature. 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 samples, which were taken for the first concentration control analyses at the beginning of the administration period, were also used to verify the homogeneity for the samples at the low and the high concentrations (5 and 60 mg/kg body weight/day). Three samples (one from the top, middle and bottom in each case) were taken for each of these concentrations.

Results - Homogeneity Analysis: For the low concentration preparation (0.05 g/100 mL), the mean concentration was 90.7% of the nominal concentration with a standard deviation of 11.0%. For the high concentration preparation (0.60 g/100 mL), the mean concentration was 95.4% of the nominal concentration with a standard deviation of 4.4%.

Stability Analysis: In doubly distilled water, the material was stable for a period of 96h (0 hr: 99.2% of nominal; 96hr: 99.0% of nominal).

Concentration Analysis: Mean concentrations ranged from 90.7 to 103.2% of the nominal concentrations.

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

6. **Dosage administration** - All doses were administered once daily orally by gavage, on

gestation days 6 through 19, in a volume of 10 mL/kg of body weight/day. Dosing was based on the most recent body weight determination.

C. Observations

1. Maternal Observations and Evaluations - The animals were checked for mortality or clinical signs at least daily. Body weight data were recorded on gestation days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20. With the exception of day 0, food consumption was determined on the same days as body weight. Dams were sacrificed on day 20 of gestation.

Examinations at sacrifice consisted of the following:

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

- Organ weights of spleen and liver, afterwards (and after removal of liver tissue for clinical pathology) fixation in 4% formaldehyde solution for histopathological examination

- Placentas from 5 dams per group and the livers of 10 foetuses/group from different litters (selected for skeletal examinations) were frozen at about –80°C at Clinical Pathology for potential further examinations.

- Weight of the unopened uterus

- Number of corpora lutea

- Number and distribution of implantation sites classified as:
  - live foetuses
  - dead implantations (early resorptions, late resorptions, dead foetuses)

Haematology

The following parameters were determined in blood with EDTA-K3 as anticoagulant using a particle counter.

- leukocytes (WBC)
- erythrocytes (RBC)
- haemoglobin (HGB)
- haematocrit (HCT)
- mean corpuscular volume (MCV)
- mean corpuscular haemoglobin (MCH)

- mean corpuscular haemoglobin concentration (MCHC)
- platelets (PLT)
- differential blood count
- reticulocytes

In addition, differential blood smears were prepared and stained according to Wright and then examined by light microscopy. Prothrombin time was determined using the Hepato Quick's test.

Clinical Chemistry

The following parameters were determined:

- alanine aminotransferase (ALT)
- aspartate aminotransferase (AST)
- alkaline phosphatase (ALP)
- γ-glutamyltransferase (GGT)
- Sodium (Na)
- Potassium (K)
- Chloride (Cl)
- inorganic phosphate (INP)
- Calcium (Ca)
- Urea (UREA)
- Creatinine (CREA)
- Glucose (GLUC)
- total bilirubin (TBIL)
- total protein (TPROT)
- Albumin (ALB)
- Globulins (GLOB)
- Triglycerides (TRIG)
- Cholesterol (CHOL)
- Magnesium (Mg)
- Total porphyrin in liver tissue (LTPOR); from 10 randomly selected pregnant animals/group.

2. **Foetal Evaluations** - At necropsy, each foetus was weighed, sexed and examined macroscopically for any external findings. The viability of the foetuses and the condition of the placenta, the umbilical cords, the foetal membranes and fluids was examined. Individual placental weights were recorded. Thereafter, the foetuses were sacrificed by subcutaneous injection of a pentobarbital solution. After these examinations, approximately one half of the foetuses per dam were eviscerated, skinned and placed in ethyl alcohol, the remaining foetuses were placed in Harrison's fluid for fixation and further evaluation.

The foetuses fixed in Harrison's fluid were examined for any visceral findings according to the

method of BARROW and TAYLOR (Barrow and Taylor, 1969). After this examination, these foetuses were discarded.

The skeletons of the foetuses 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 foetuses were examined under a stereomicroscope. After this examination the stained foetal skeletons were retained individually.

The glossary of WISE et al. (Wise et al., 1997) was used as much as possible to describe findings in foetal morphology. Classification of these findings was based on the terms and definitions proposed by CHAHOUD et al. (Chahoud et al., 1999 ; Solecki et al., 2001; Solecki et al., 2003):

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

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

The term **unclassified observation** was used for those foetal findings, which could not be classified as malformations or variations (e.g. focal liver necrosis in foetuses). According to the definitions specified before, the findings obtained in foetuses were classified and listed in the tables accordingly.

D. Data Analysis

1. Statistical analyses -

Parameter	Statistical Test*	References
Food consumption, 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 foetuses, proportions of preimplantation loss, proportions of postimplantation loss, proportions of resorptions, proportion of live foetuses in each litter, litter mean foetal 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	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
Liver and spleen weights	Non-parametric one-way analysis using KRUSKALWALLIS- test (two-sided). If the resulting p-value was equal or less than 0.05, a pair-wise comparison	MILLER, R. G. (1981): Simultaneous Statistical Inference Springer-Verlag New York Inc., 165-167. International Mathematical and Statistical Libraries, Inc., 2500 Park West

Parameter	Statistical Test*	References
	of each dose group with the control group was performed using the WILCOXON-test (two-sided) for the equal medians	Tower One, Houston, Texas 77042-3020, USA, nakl-1 - nakl-3. NIJENHUIS, A. and WILF H.S. (1978): Combinatorial Algorithms, Academic Press, New York, 32-33. HETTMANNSPERGER, T. P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132- 140.
Female mortality, females pregnant at terminal sacrifice, number of litters with foetal findings	Pair wise comparison of each dose group with the control group using FISHER'S EXACT test (one-sided) for the hypothesis of equal proportions	Siegel S. (1956): Non-parametric statistics for behavioural sciences. McGraw-Hill New York
Proportions of foetuses with malformations, variations and/or unclassified observations in each litter	Pair wise comparison of each dose group with the control group using the WILCOXON test (one-sided) for the hypothesis of equal medians	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
Clinical pathology parameters, Except reticulocytes and differential blood count	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided).If the resulting p-value was equal or less than 0.05, a pair-wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians	Siegel S. (1956): Non-parametric statistics for behavioural sciences. McGraw-Hill New York

* for $p < 0.05$; ** for $p < 0.01$

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

Implantation losses were classified as one of the following:

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 foetal tissue in addition to placental tissue visible)
c) Dead foetuses (hypoxemic foetuses which did not breathe spontaneously after the uterus had been opened)

Calculations of conception rate and pre and post-implantation losses were determined according to the following formulas:

$$\text{Conception Rate (\%)} = \frac{\text{Number of pregnant animals}}{\text{Number of fertilized animals}} * 100$$

$$\text{Preimplantation Loss (\%)} = \frac{\text{Number of corpora lutea - Number of implantations}}{\text{Number of corpora lutea}} * 100$$

$$\text{Postimplantation Loss (\%)} = \frac{\text{Number of implantations - Number of live fetuses}}{\text{Number of implantations}} * 100$$

3. **Historical control data**: Historical control data from 2001 – 2004 (same source and strain, see page 361 of study report) were provided to allow comparison with concurrent controls in the analysis of foetal skeletal variations.

II. Results

A. Maternal Toxicity:

1. **Mortality and Clinical Observations** – There was no mortality in the dams. There were no treatment-related clinical signs.

2. **Body Weight** - There were decreases in body weight gain on days 3 – 6 and 13 – 15, however, there was a large amount of variability at all doses. Body weight data are summarized in Table 2.

Table 2. Maternal body weight and body weight gain[a]

mg/kg bw/d	0 n = 24	5 n = 23	20 n = 22	60 n = 24
		Females		
Initial BW (pretreatment)	162.3 ± 7.44	162.1 ± 8.32	162.4 ± 9.31	159.9 ± 8.03
BW Day 6 (pretreatment)	188.4 ± 9.90	188.1 ± 10.16	190.8 ± 11.70	186.0 ± 9.54
BW day 15 (treatment)	222.0 ± 13.17	224.4 ± 11.37	226.3 ± 13.89	218.6 ± 15.32
BW day 19 (treatment)	249.1 ± 22.32	255.3 ± 16.81	258.3 ± 16.45	243.5 ± 20.39
Final BW (posttreatment)	260.2 ± 24.96	267.0 ± 19.55	271.1 ± 17.28	254.5 ± 22.84
Gravid uterine weight	40.7 ± 18.01	44.0 ± 15.13	47.6 ± 9.40	38.4 ± 13.62
Corrected final BW	219.5 ± 13.37	223.0 ± 10.15	223.5 ± 17.4	216.2 ± 15.37
BWG Day 0 – 1	11.2 ± 3.69	11.7 ± 3.82	12.6 ± 3.67	12.5 ± 2.66
BWG Days 3 – 6	8.9 ± 2.65	8.3 ± 3.42 (↓6.7)	9.8 ± 1.81 (↑10.1)	7.9 ± 3.75 (↓11.2)
BWG Days 6 - 8	6.6 ± 3.91	7.3 ± 3.33 (↑10.6)	6.3 ± 2.19 (↓4.5)	6.3 ± 3.71 (↓4.5)
BWG Days 8 - 10	5.4 ± 3.37	5.7 ± 2.78 (↑5.6)	6.4 ± 2.72 (↑18.5)	5.6 ± 3.52 (↑3.7)
BWG Days 10 - 13	12.6 ± 4.31	13.9 ± 3.11 (↑10.3)	13.0 ± 3.34 (↑3.2)	12.5 ± 4.65 (↓0.8)
BWG Days 13 – 15	9.0 ± 6.17	9.4 ± 3.83 (↑4.4)	9.7 ± 3.46 (↑7.8)	8.2 ± 5.0 (↓8.9)
BWG Days 19 – 20	11.2 ± 4.37	11.7 ± 4.06 (↑4.5)	12.8 ± 3.54 (↑14.3)	11.0 ± 5.06 (↓1.8)

Overall BWG Days 6 - 19(gestation)	60.7 ± 21.04	67.2 ± 15.0 (↑10.7)	67.4 ± 8.49 (↑11.0)	57.6 ± 17.27 (↓5.1)

[a] Data obtained from pages 72 - 75 in the study report.
* Significantly different from control, p<0.05; ** Significantly different from control, p<0.01
BW body weight, BWG body weight gain

3. **Food Consumption** - There were no treatment-related changes to food consumption. Food consumption data are summarized in Table 3.

Table 3. Food consumption (g/animal/d)[a] (% C)

mg/kg bw/d	0 n = 24	5 n = 23	20 n = 22	60 n = 24
		Female		
FC Days 0 – 1	13.0 ± 1.63	12.8 ± 1.77	13.7 ± 1.96	13.7 ± 1.19
FC Days 3 – 6	17.0 ± 1.14	17.3 ± 1.70	18.2 ± 1.54*	17.0 ± 1.17
FC Days 13 – 15	19.0 ± 1.97	19.4 ± 1.69	19.8 ± 1.82	18.6 ± 2.09
FC Days 19 – 20	17.8 ± 2.27	18.4 ± 2.06	19.7 ± 2.18*	17.4 ± 3.04
Overall FC Days 0 – 20	17.8 ± 2.12	18.3 ± 2.40	18.9 ± 2.33	17.7 ± 1.78

a Data extracted from pages 70 – 71 of the study report
* Statistically different from control, p<0.05; ** Statistically different from control, p<0.01
BW body weight, FC food consumption

4. **Haematological Examinations** – There was an increase in white blood cell counts at 60 mg/kg bw/d, a finding seen throughout the rat database. There were slight changes to the red blood cell parameters (haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin and platelet counts). These changes were slight, however, the blood is a target organ with this compound and the marginal changes were considered to be treatment-related in the presence of statistical significance. Haematocrit changes were not considered treatment-related due to the lack of dose response.

Table 4. Select haematological parameters in the dams [a]

mg/kg bw/d	0 n = 24	5 n = 23	20 n = 22	60 n = 24
		Female		
WBC (giga/L)	4.38 ± 1.21	4.57 ± 0.96 (↑4.33)	4.50 ± 0.96 (↑2.74)	5.14 ± 1.06* (↑17.35)
HGB (mmol/L)	6.9 ± 0.7	6.9 ± 0.5 (0.0)	6.6 ± 0.3 (↓4.35)	6.5 ± 0.6* (↓5.80)
HCT (L/L)	0.320 ± 0.030	0.321 ± 0.024 (↑0.31)	0.303 ± 0.015* (↓5.31)	0.304 ± 0.027* (↓5.00)
MCV (fL)	55.0 ± 1.5	54.6 ± 1.5 (↓0.73)	54.6 ± 1.4 (↓0.73)	52.5 ± 1.5** (↓4.55)
MCH (fmol)	1.19 ± 0.04	1.17 ± 0.04 (↓1.68)	1.19 ± 0.04 (0.0)	1.13 ± 0.04** (↓5.04)
PLT (giga/L)	983 ± 172	984 ± 132 (↑0.10)	960 ± 143 (↓2.34)	945 ± 161 (↓3.87)

a Data extracted from pages 82 – 83 of the study report

95

* Statistically different from control, p<0.05; ** Statistically different from control, p<0.01
Bolded values determined to be treatment-related

5. **Clinical Chemistry** – There was an increase in liver porphyrins at ≥ 5 mg/kg bw/d and a slight increase in total bilirubins at doses ≥ 20 mg/kg bw/d. The absolute change in bilirubin was not considered of sufficient magnitude to indicate an adverse effect and the adverse nature of the change in porphyrins is unknown in the absence of other liver changes.

Table 5. Select clinical chemistry parameters in the dams [a]

mg/kg bw/d	0 n = 24	5 n = 23	20 n = 22	60 n = 24
		Female		
TBIL (µmol/L)	1.58 ± 0.59	1.63 ± 0.79 (↑3.16)	**1.76 ± 0.63 (↑11.39)**	**1.92 ± 0.56 (↑21.52)**
TProt (g/L)	62.59 ± 3.40	63.13 ± 3.52 (↑0.86)	62.81 ± 3.82 (↑0.35)	64.18 ± 3.59 (↑2.54)
LTPor (pmol/g P.)	99.8 ± 23.7	369.7 ± 73.2** (↑270.44)	772.7 ± 178.2** (↑674.25)	1251.0 ± 376.9** (↑1153.51)

a Data extracted from pages 87 - 89 of the study report
* Statistically different from control, p<0.05; ** Statistically different from control, p<0.01
Bolded values determined to be treatment-related

4. **Gross Pathology** - There were no treatment-related changes at gross necropsy and no organ weight changes to the liver or spleen.

5. **Caesarean Section Data** - There was a decrease in foetal body weights as of 20 mg/kg bw/d as summarized in Table 6. While there was an increase in live births per dam at the same doses, it does not entirely account for the reduced foetal weights and is considered treatment-related and adverse.

Table 6. Caesarean section observations [a]

Observation	Dose (mg/kg bw/day)			
	0	5	20	60
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant	24	23	22	24
Pregnancy Rate (%)	96	92	88	96
# Nonpregnant				
Maternal Wastage				
# Died	0	0	0	0
# Died Pregnant	0	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea Corpora Lutea/Dam	231 9.6 ± 176	218 9.5 ± 195	221 10.0 ± 1.46	240 10.0 ± 1.64
Total # Implantations (Implantations/Dam)	185 7.7 ± 3.26	191 8.3 ± 3.07	207 9.4 ± 1.71	203 8.5 ± 2.69

Total # Litters	22	23	22	22
Total # Live Foetuses (Live Foetuses/Dam)	174 7.9 ± 2.67	179 7.8 ± 2.91	196 8.9 ± 1.97	187 8.5 ± 1.74
Total # Dead Foetuses (Dead Foetuses/Dam)	0 0	0 0	0 0	0 0
Total # Resorptions	11	12	11	16
Early	11	11	11	14
Late	0	1	0	2
Resorptions/Dam	0.5 ± 0.66	0.5 ± 0.59	0.5 ± 1.22	0.7 ± 1.05
Early	0.5 ± 0.66	0.5 ± 0.51	0.5 ± 1.22	0.6 ± 1.06
Late	0.0 ± 0.00	0.0 ± 0.21	0.0 ± 0.00	0.1 ± 0.28
Litters with Total Resorptions	2	0	0	2
Mean Foetal Weight (g)	3.7 ± 0.44	3.7 ± 0.21 (0)	**3.4 ± 0.32*** **(↓8.1)**	**3.1 ± 0.25**** **(↓16.2)**
Males	3.8 ± 0.49	3.8 ± 0.19 (0)	**3.5 ± 0.31** **(↓7.9)**	**3.2 ± 0.30**** **(↓15.8)**
Females	3.6 ± 0.44	3.7 ± 0.21 (↑2.8)	**3.4 ± 0.34*** **(↓5.6)**	**3.0 ± 0.30**** **(↓16.7)**
Sex Ratio (% Male)	52.9	49.2	47.4	54.5
Preimplantation Loss (%)	19.6 ± 29.93	16.0 ± 24.11	6.1 ± 11.51	14.1 ± 26.34
Postimplantation Loss (%)	14.0 ± 28.86	5.7 ± 6.40	5.1 ± 12.30	14.8 ± 28.73

[a] Data obtained from pages 79 – 81 in the study report.
* Significantly different from control, $p<0.05$; ** Significantly different from control, $p<0.01$
Bolded values were determined to be treatment-related

B. Developmental Toxicity

1. External Examination – There was no increase in external malformations or variations.

2. Visceral Examination – There was no increase in visceral malformations or variations.

3. Skeletal Examination – There was a treatment-related increase in the number of skeletal malformations at 60 mg/kg bw/d, with ten individuals and seven litters affected. The malformations consisted of bent scapulae, radii, ulnas and femurs, malpositioned and bipartite sternebrae and thick humeri. At 20 mg/kg bw/d, there was a single incidence of bent scapula. As this occurred in the absence of historical control findings, the malformation was considered treatment-related.

The litters of every dose group, including control, had skeletal variations. However, the proportions of individuals affected were higher in the 60 mg/kg bw/d dose groups, with all individuals affected, a finding above that of historical controls. At doses ≥ 20 mg/kg bw/d, there were increases incomplete ossification of the skull, nasal area and thoracic centrum and wavy ribs. At 60 mg/kg bw/d there were increases in supraoccipital holes, bassioccipital holes, incomplete ossification of the hyoid and thoracic centrum, dumbbell ossification of the lumbar centrum and bipartite ossification of the sternebrae.

Table 4a. External examinations[a]

Observations+	Dose (mg/kg bw/day)			
	0	5	20	60
#Foetuses(litters) examined	174 (22)[b]	179 (23)	196 (22)	187 (22)
Malformations				
#Foetuses(litters) affected	0 (0)	0 (0)	1 (1)	0 (0)
Cleft palate	0 (0)	0 (0)	0 (0)	0 (0)

+Some observations may be grouped together
[a]Data extracted from pg 91 – 94 of the study report.
[b]Foetal (litter) incidence
* Significantly different from control, p<0.05; ** Significantly different from control, p<0.01

TABLE 4b. Visceral examinations[a]

Observations+	Dose (mg/kg bw/day)			
	0	5	20	60
#Foetuses(litters) examined	80 (20)[b]	82 (21)	90 (22)	89 (22)
Variation				
#Foetuses(litters) affected	1 (1)	5 (4)	2 (2)	3 (2)
Short innominate artery	0 (0)	0 (0)	0 (0)	1 (1)

+Some observations may be grouped together
[a]Data extracted from pgs 95 – 100 of the study report.
[b]Foetal (litter) incidence
* Significantly different from control, p<0.05; ** Significantly different from control, p<0.01.

TABLE 4c. Skeletal examinations [a]

Observations+	Dose (mg/kg bw/day)			
	0	5	20	60
#Foetuses(litters) examined	94 (22)[b]	97 (23)	106 (22)	98 (22)
Malformations				
#Foetuses(litters) affected	1 (1)	0 (0)	4 (4) [3.8(18.2)]	10 (7)* [10.2 (31.8)]
HC: Total skeletal malformations	0.0 – 5.1 (0.0 – 25.0)			
Bent scapula: Cartilage present	0 (0)	0 (0)	1 (1)	5 (3)*
HC: Bent scapula	No historical findings			
Malpositioned and bipartite sternebra: Unchanged cartilage	0 (0)	0 (0)	1 (1) [0.9 (4.5)]	2 (2) [2.0 (9.0)]
HC: Malpositioned and bipartite sternebra: Unchanged cartilage	0.0-1.7 (0.0 -8.0)			
Thick humerus: Cartilage present	0 (0)	0 (0)	0 (0)	5 (3)*
HC: Thick humerus	No historical findings			

Bent radius: Cartilage present	0 (0)	0 (0)	0 (0)	4 (2)
HC: Bent radius	No historical findings			
Bent ulna: Cartilage present	0 (0)	0 (0)	0 (0)	3 (2)
HC: Bent ulna	No historical findings			
Bent femur: Cartilage present	0 (0)	0 (0)	0 (0)	3 (2)
HC: Bent femur	No historical findings			
Misshapen lumbar vertebrae	0 (0)	0 (0)	1 (1)	0 (0)
Notched scapula: Cartilage present	0 (0)	0 (0)	1 (1)	0 (0)
Cleft sternum: Split cartilage	0 (0)	0 (0)	1 (1)	0 (0)
Variations				
#Foetuses(litters) affected	90 (22)	94 (23)	105 (22) [99.0 (100.0)]	98 (22)* [100.0 (100)]
HC: Total skeletal variations	88.0 – 99.2 (100.0 – 100.0)			
Supraoccipital hole(s)	26 (13)	30 (15)	33 (18) [31.4 (81.8)]	49 (20)** [50.0 (90.9)]
HC: Supraoccipital hole(s)	8.0 – 59.3 (33.3 – 100.0)			
Incomplete ossification of skull: Unchanged cartilage	3 (3)	5 (2)	5 (4) [4.8 (18.2)]	7 (5) [7.1 (22.7)]
HC: Incomplete ossification of skull	0 – 10.8 (0.0 – 30.4)			
Bassioccipital hole(s)	1 (1)	0 (0)	1 (1)	5 (4) [5.1 (18.2)]
HC: Bassioccipital hole(s)	0 – 4.0 (0.0 – 12.5)			
Incomplete ossification of hyoid: Cartilage present	0 (0)	0 (0)	0 (0)	2 (2) [2.0 (9.1)]
HC: Incomplete ossification of the hyoid	0 – 3.6 (0 – 8.3)			
Incomplete ossification of nasal: Unchanged cartilage	0 (0)	0 (0)	4 (4)*	15 (9)**
HC: Incomplete ossification of nasal	No historical findings			
Incomplete ossification of thoracic centrum: Unchanged cartilage	2 (2)	1 (1)	5 (3) [4.7 (13.6)]	12 (9)** [12.2 (40.9)]
HC: Incomplete ossification of the thoracic centrum	0.0 – 1.0 (0.0 – 4.6)			
Dumbbell ossification of lumbar centrum: Unchanged cartilage	3 (3)	4 (4)	1 (1)	6 (6) [6.1 (27.3)]
HC: Dumbbell ossification of lumbar centrum	0.0 – 2.0 (0.0 – 8.7)			
Incomplete ossification of sternebra: Unchanged cartilage	56 (21)	64 (21)	73 (20) [68.9 (90.9)]	76 (21)* [77.6 (95.5)]
HC: Incomplete ossification of sternebra	38.5 – 74.5 (70 – 100.0)			

Misshapen sternebra: Unchanged cartilage	39 (20)	37 (20)	40 (19)	64 (20)* [65.3 (90.9)]
HC: Misshapen sternebra	7.7 – 50.0 (37.5 – 96)			
Bipartite ossification of sternebra: Unchanged cartilage	0 (0)	0 (0)	0 (0)	2 (2) [2.0 (9.1)]
HC: Bipartite ossification of sternebra	0 – 4.9 (0.0 – 13.0)			
Wavy rib	17 (9)	11 (7)	34 (16)* [33.3 (72.7)]	51 (17)** [52.0 (77.3)]
HC: Wavy rib	1.0 – 9.3 (4.8 – 26.1)			
Incomplete ossification of pubis: Cartilage present	0 (0)	0 (0)	1 (1)	2 (1)
Incomplete ossification of ischium: Cartilage present	0 (0)	0 (0)	1 (1)	1 (1)

[a]Some observations may be grouped together
[b]Data extracted from pgs 100 – 123 of the study report; Historical control (HC) data from pages 372 – 377 of the study report
[b]Foetal (litter) incidence
* Significantly different from control, $p<0.05$; ** Significantly different from control, $p<0.01$.
[ ] percent of foetuses and litters with finding in that treatment group
Bolded values were determined to be treatment-related

III. DISCUSSION

A. Investigators' conclusions:

"**BAS 800 H** 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.]).

There were no test substance-related effects on the dams concerning food consumption, body weight, body weight change, uterine, spleen, and liver weights, corrected body weight change or clinical and necropsy observations up to and including a dose of 60 mg/kg body weight/day.

Haematological examinations in dams revealed statistically significantly decreased values for haemoglobin, haematocrit, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) at the 60 mg/kg body weight/day dose level, and statistically significantly decreased haematocrit and decreased haemoglobin at the 20 mg/kg body weight/day dose level. These changes represent a mild microcytic anaemia, which correlates with the mode of action of the test compound. Although foetal haematological measurements were not performed, it is assumed that the foetuses also experienced anaemic responses.

In addition to the haematological evidence for microcytic anaemia in the dams at 20 and 60 mg/kg body weight/day, a dose-related, significant increase in liver porphyrins was noted for dams of all treatment groups, being approximately 4-fold, 8-fold and 13-fold greater than control at the low-, mid- and high-dose levels, respectively. The test compound, which is a peroxidizing herbicide, acts as an inhibitor of protoporphyrinogen IX oxidase, which results in interference with porphyrin biosynthesis at the penultimate stage of haeme synthesis. The disruption of

porphyrin biosynthesis results in decreased formation of protoporphyrin which results in reduced haemoglobin production. Prolonged interference of haeme biosynthesis manifests itself toxicologically as a microcytic anaemia.

Normally, porphyrin levels can vary by several fold in tissues and serum, depending on the normal biological variation in the balance between red blood cell synthesis and recycling. Although a 13-fold increase in liver porphyrins in the high-dose dams in this experiment was not associated with liver toxicity, previous subacute and subchronic experiments with BAS 800 H have demonstrated liver organ weight and pathological changes that reflect metabolic stress on the liver caused by large increases (approximately 8-10 fold) in liver porphyrin concentrations. In this study the adverse changes associated with an 8-fold increase in liver porphyrin concentrations, as it was found in the mid-dose dams, were limited to anaemia, as evidenced by decreased haemoglobin and haematocrit.

Indications of an adverse effect, such as changes in haemoglobin concentrations, red blood cell synthesis, and liver toxicity were not noted in this experiment, and have not been noted in other experiments, when smaller (e.g., 5-6 fold) changes in liver porphyrin levels occurred. As such, although statistically significant changes in liver porphyrin levels were observed in low-dose animals, without other pathological or functional changes, the single biochemical change is not considered adverse.

Adverse foetal findings in this study were primarily limited to bent limb bones in the mid-dose (single occurrence) and high-dose groups and to an increase in delayed ossification in the high-dose group. The hypothesized mechanism of action for bent limb bones is that the tested compound acts as an inhibitor of protoporphyrinogen IX oxidase and interferes with haeme biosynthesis in both dams and foetuses. Inhibition of porphyrin production results in maternal and foetal anaemia, followed secondarily by foetal hypoxia. The foetal hypoxia results in decreased ossification in the primary (central) ossification site of the developing bone structure. Thus, **BAS 800 H shows no direct and specific effect on the respective morphological structures, and there was no evidence of an adverse developmental effect in the absence of maternal toxicity.**

Slight differences to control were recorded for mid- and high-dose placental and foetal body weights. Similar to the skeletal effects, they are presumably secondary to microcytic anaemia caused by the test compound in dams and foetuses. The decreased mean foetal weight compared to control in the mid-dose group was also likely influenced by the larger litter size in this group compared to the controls. The slightly increased incidence in skeletal variations in the mid- and high-dose foetuses is believed to be secondary to maternal toxicity and primarily related to delayed ossification. No test substance-induced effects on foetal morphology occurred at the low dose level (5 mg/kg body weight/day).

A similar mechanism for developmental toxicity in the rat has been demonstrated by the herbicide Flumioxazin, which produced wavy ribs and bent long bones. In the case of

101

Flumioxazin, protoporphyrin IX was shown to accumulate in rat embryos correlating with hypoxia, but that was a species-specific effect, not seen in rabbit embryos (KAWAMURA S). Protoporphyrin IX accumulation corresponded with the findings of developmental effects in rats, but not in rabbits, even at very high doses (KAWAMURA S). Similar to the Protox inhibitors Lactofen, Sulfentrazone and Flumioxazin, although bent bones were observed in the rat developmental studies, no foetal effects were observed in pregnant rabbits exposed to an order of magnitude greater concentrations of BAS 800 H. The species-specific selectivity for developmental effects is believed to be due to known species differences in the binding of Protox inhibitors to protoporphyrinogen IX oxidase, with rat protoporphyrinogen IX oxidase being approximately 8 times more sensitive to Protox inhibition than the rabbit enzyme (US EPA, BOUIS P, KAWAMURA S).

In conclusion, the **no observed adverse effect level (NOAEL) for maternal** and **prenatal developmental toxicity is 5 mg/kg body weight/day** based on statistically significant haematological changes in the dams at 20 and 60 mg/kg/day as well as decreased foetal growth and bone malformations at 20 and 60 mg/kg/day. **There were no adverse foetal findings evident at a dose not producing maternal toxicity"**

B. <u>Reviewer's discussion</u>:

1. <u>Maternal toxicity</u>: The reviewer does not agree with the investigators' assessment as to the adversity of the haematological changes in the dams. While statistically significant, the haematological changes were not great enough to be considered biologically significant in the dams.

There were no changes to body weight, body weight gain, food consumption, clinical signs or gross necropsy. There were adverse, treatment-related increases in WBCs and decreases in haemoglobin, MCV and MCH. **The maternal LOAEL is 60 mg/kg bw/day, based on haematological changes. The maternal NOAEL is 20 mg/kg bw/day.**

2. <u>Developmental toxicity</u>: Changes to foetal development occurred at the same doses as the dams, but to much greater severity.

The developmental LOAEL is 20 mg/kg bw/day, based on increased decreased foetal body weights in males and females and increased skeletal malformations and variations. The developmental NOAEL is 5 mg/kg bw/day.

a. Deaths/Resorptions: There were no changes to death and resorptions.

b. Altered Growth: At doses ≥ 20 mg/kg bw/d there is a decrease in male and female foetal body weights.

c. Developmental Variations: Incomplete ossification of the nasal area and thoracic centra is increased at doses ≥ 20 mg/kg bw/d, potentially as a result of the decreased foetal body weights. There is also an increase in the number of wavy ribs at the same dose. At 60 mg/kg bw/d, there is an increase in supraoccipital and bassoccipital holes, incomplete ossification of the hyoid and dumbbell ossification of the lumbar centrum.

d. Malformations: There was an increase in skeletal malformations at 20 mg/kg bw/d, with bent scapulae. At 60 mg/kg bw/d there was also an increase in thick humeri, bent radii, ulnas and femurs and malpositioned and bipartite sternebrae.

C. Study deficiencies: There were no deficiencies that would affect the outcome of the study.