DATA EVALUATION RECORD

BAS 670H

Study Type: Non-guideline; Developmental Toxicity Study in Rabbits

Work Assignment No. 1-01-11 O (MRID 45902213)

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DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; Prenatal Developmental Toxicity Study - Rabbit

PC CODE: 123009 **DP BARCODE:** D292904

TEST MATERIAL (PURITY): BAS 670H (98.8% a.i.)

SYNONYMS: [3-(4,5-Dihydro-isoxazol-3-yl)-4-methanesulfonyl-2-methyl-phenyl]-(5-hydroxy-1-methyl-1H-pyrazol-4-yl)-methanone

CITATIONS: Schneider, S., B.V. Ravenzwaay (2003) BAS 670H - Prenatal developmental toxicity study in New Zealand white rabbits: oral administration (gavage). Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany. Laboratory Project ID: Project No. 40R0124/98150, BASF Registration Document No. 2003/1006259, February 28, 2003. MRID 45902213. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, Research Triangle Park, NC.

EXECUTIVE SUMMARY: In a non-guideline developmental toxicity study (MRID 45902213), one batch of BAS 670H (Lot/Batch # N33, 98.8% a.i.) and 3 chromatographic fractions of BAS 670H (derived from Lot/Batch N17; 95.2% a.i.) in 0.5% (w/v) aqueous carboxymethylcellulose were administered by gavage at a dose volume of 10 mL/kg body weight to 25 female New Zealand White [Crl:KBL (NZW)] rabbits/group on gestation days (GD) 6 through 28. Dose levels of 0, 1.5, or 5.0 mg/kg were used for batch N33; chromatography fractions (CFR) 1 and 2 were given at 1.5 mg/kg, while CFR 3 was administered at 0.5 mg/kg. All does were sacrificed on GD 29; their fetuses were removed by cesarean section and examined.

No effects of treatment were observed on maternal survival, clinical signs, body weights, body weight gains, food consumption, or gross pathology. No differences were noted between Batch N33 and the 3 chromatographic fractions of Batch N17. Serum tyrosine level was not measured.

The maternal LOAEL was not observed. The NOAEL was 5.0 mg/kg/day. The LOAEL/NOAEL could be lower because serum tyrosine level was not evaluated.

There were no treatment-related effects on the numbers of litters, fetuses (live or dead), resorptions (early, late or complete litter), or post-implantation loss.

There were no treatment-related external, visceral, or skeletal malformations and no effects on fetal growth or development.

Increased incidences of supernumerary thoracic vertebrae were observed in the 1.5 mg/kg N17/ CFR 1 and 2, and 1.5 and 5.0 mg/kg N33 fetuses compared to concurrent and historical controls. Increased incidences of supernumerary 13th rib (with cartilage present) were noted in the 1.5 mg/kg N17/ CFR 1 and 2, and 1.5 and 5.0 mg/kg N33 fetuses compared to concurrent and historical controls. The effects observed at 0.5 mg/kg/day for N17/CFR 3 were within the historical control ranges.

The purpose of this study was to ascertain the effects of 3 different chromatographic fractions (CFR) isolated from a previously used batch N17 of BAS 670H on embryonic and fetal development and to compare them with the effects evoked by batch N33 of BAS 670H which has a greater purity compared to N17. However, the results did not achieve the goal because the study design and the dose selection made it difficult to compare the effects among these treatments. No meaningful conclusion can be drawn from the study regarding the comparison of the effects between Batch N33 and the chromatographic fractions of Batch N17 in terms of maternal and prenatal developmental toxicity.

The developmental LOAEL for N33 and N17/CFR 1-2 was 1.5 mg/kg/day based on increased presence of supernumerary thoracic vertebrae and supernumerary 13th rib. The developmental NOAEL was not observed. No effect was observed for N17/CFR 3 at 0.5 mg/kg/day (the only dose tested). There was no evidence of teratogenicity.

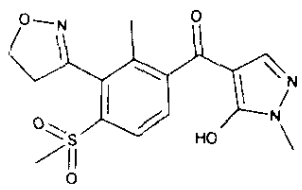
This study is classified as **acceptable/non-guideline.**

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: BAS 670H

Description:	Batch N33; yellow-brown crystalline solid N17/Chromatography fraction (CFR) 1; yellowish crystalline solid N17/CFR 2; yellowish crystalline solid N17/CFR 3; dark brown solid
Lot/Batch #:	N33, N17 (CFRs 1, 2, and 3)
Purity:	N33 = 98.8% a.i., CFRs not specified(chromatographic fractions of N17)
Compound Stability:	Stable suspended in water for up to 7 days (room temperature or refrigerated)
CAS #of TGAI:	210631-68-8
Structure:	

2. Vehicle and/or positive control: 0.5% (w/v) aqueous carboxymethylcellulose

3. Test animals:

Species:	Rabbit	
Strain:	New Zealand White [Crl:KBL (NZW)]	
Age/body weight range on GD 1:	17-19 weeks/2842-3961 g	
Source:	Elevage Scientifique des Dombes, Châtillon/Chalaronne, France	
Housing:	Individually in stainless steel wire mesh cages	
Diet:	Pelleted Kliba maintenance diet type 3418 for rabbits (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	30-70%
	Air changes:	Not provided
	Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	At least 5 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: November 4, 2001 End: December 13, 2001

2. Purpose: The purpose of this study was to ascertain the effects of 3 chromatography fractions (CFR) derived from a batch of BAS 670H used previously (Batch N17) on embryonic and fetal development and to compare them to a batch of BAS 670H of greater purity (Batch N33).

3. Mating: The females were naturally mated with breeder male rabbits of the same strain by the supplier prior to shipment. The day of insemination was designated as gestation day (GD) 0. Rabbits were shipped on GD 0 and arrived at the performing laboratory on GD 1.

4. Animal assignment: After arrival, does were randomly assigned to the treatment groups (stratified by body weight), as indicated in Table 1.

Table 1. Animal assignment[a]

Dose (mg/kg bw/day)	0	0.5	1.5			5.0
Batch/CFR[b]	NA	N17/3	N33	N17/1	N17/2	N33
# of Dams	25	25	25	25	25	25

a Data obtained from page 21 of the study report.
b Chromatography fraction of Batch N17
NA Not applicable

5. Dose selection rationale: It was stated that for comparison purposes, doses of 0.5, 1.5, and 5.0 mg/kg/day were selected to compare the effects of batch N33 to chromatography fractions 1, 2, and 3 of batch N17. No other information regarding dose selection was provided.

6. Dosage preparation and analysis: It was stated that dosing solutions were prepared at the beginning of the study and thereafter at a frequency depending on their stability; however, the precise frequency of preparation was not provided. For each dose group, an appropriate amount of test substance was suspended in (doubly-distilled) aqueous 0.5% (w/v) carboxymethylcellulose using a high-speed homogenizer and stirred during dosing. Homogeneity was confirmed by analyses of three samples (top, middle, and bottom) for dose formulations of batch N33 prepared for use on the first day of treatment; and by analysis of samples of the chromatography fraction dose formulations prepared for use in the last week of treatment. Concentration was confirmed by analysis of samples for all dose formulations prepared at the end of the administration period. For stability analysis, the test substance was suspended at a concentration of 0.1 mg/L in water having different purities (referred to as tap, M4, OECD, and superpure) and stored for 0, 1, or 7 days at room temperature or refrigerated. Stability data in carboxymethylcellulose was not provided.

Results -

Homogeneity (range as % CV): Batch N33 = 0.8-1.0%
Chromatography fractions = 2.7-3.5%

Stability (range as % of nominal value):
0 days at room temperature: 96.2-102.2%
0 days refrigerated: 96.2-102.2%
1 day at room temperature: 98.8-100.4%
1 day refrigerated: 99.8-101.1%
7 days at room temperature: 100.3-103.1%
7 days refrigerated: 102.3-103.8%

Concentration (range as % nominal): Batch N33 = 96.0-100.6%
Chromatography fractions = 87.8-102.0

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

7. **Dosage administration:** All doses were administered once daily by oral gavage, on GDs 6-28, in a volume of 10 mL/kg of body weight. Dosing was adjusted based on the most recent individual body weight determined prior to gavage. Rabbits were dosed in an ascending dose order at approximately the same time each day.

C. OBSERVATIONS

1. **Maternal observations and evaluations:** All does were checked for mortality and morbidity twice daily (once daily on weekends or holidays) during treatment, and for clinical signs of toxicity at least once per day. Body weights were measured on GD 1, 4, 6, 9, 11, 14, 16, 19, 21, 23, 25, 28, and at sacrifice. Body weight gains were calculated for each of the intervals between body weight measurements. Additionally, body weight gains corrected for gravid uterine weights were calculated for GD 6-29. Food consumption (g/rabbit/day) was measured daily on GD 2-29. On GD 29, surviving does were killed by an intravenous injection of pentobarbital, the uteri excised and weighed, and all fetuses were removed by cesarean section. All rabbits were necropsied and the numbers of corpora lutea, and the number and distribution of live and dead fetuses, resorptions (early and late), and implantation sites were recorded. Does that died or were sacrificed prematurely were examined according to the same procedures as those killed on schedule, except that gravid uterine weights were not determined.

2. **Fetal evaluations:** On removal from the uterus, all fetuses were weighed, given a detailed external examination, and viability and the condition of the placentae, umbilical cords, fetal membranes, and fluids were recorded. Individual placental weights were recorded. Live fetuses were killed by a subcutaneous injection of pentobarbital, dissected for visceral examination, and sexed. The heads of approximately one half of the fetuses per doe (and fetuses having severe external findings of the head) were removed, fixed in Bouin's solution, and assessed by Wilson's method. These heads were subsequently discarded. After skinning, all fetuses were fixed in ethyl alcohol, and a cross sectional cut was made in the heads of the intact fetuses. The brain was examined, then the skeletons were stained according to a modification of the method of Kimmel and Trammel, and a detailed examination of the skeletal bone and cartilage was performed.

D. DATA ANALYSIS

1. **Statistical analyses:** Data were subjected to the following statistical procedures:

Parameter	Statistical test
Body weight, body weight gains (uncorrected and corrected for gravid uterine weight), food consumption, gravid uterus weight, numbers of corpora lutea, implantations, resorptions, and live fetuses, proportions of pre-implantation losses, post-implantation losses, resorptions, and live fetuses, litter mean fetal body weight, and litter mean placental weight	Dunnett's test (two-sided)
Mortality (does), # does pregnant at sacrifice, and number of litters with fetal findings	Fisher's exact test (one-sided)
Proportion of fetuses with malformations, variations, or unclassified observations	Wilcoxon's test (one-sided)

Significance was denoted at $p \leq 0.05$ or $p \leq 0.01$ for each comparison.

2. **Indices:** The following indices were calculated from the cesarean data:

Conception rate (%) = # of pregnant females/# of fertilized animals x 100

Pre-implantation loss (%) = (# of corpora lutea - # of implantations)/# of corpora lutea x 100

Post-implantation loss (%) = (# of implantations - # of live fetuses)/# of implantations x 100

3. **Historical control data:** Historical control data were provided for maternal body weight, cesarean section parameters and external, visceral, and skeletal findings in the fetuses. Data were comprised of 3 studies on 29-77 does and 73 litters of the same strain.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and clinical observations:** Mortality observations are shown in Table 2. No significant treatment-related clinical signs were observed. Two 1.5 mg/kg N33 females and two 5.0 mg/kg N33 females were found dead, and one 0.5 mg/kg N17/CFR 3 female died during gavaging (not due to gavage error). Two 0.5 mg/kg N17/CFR 3 females, one 1.5 mg/kg N33 female, and two 5.0 mg/kg N33 females were sacrificed after abortion. Additionally, one 0.5 mg/kg N17/CFR 3 female was sacrificed in moribund condition following an accidental spinal fracture. No clinical signs were observed in any of these animals prior to death; therefore, these deaths and abortions are considered spontaneous and not treatment-related.

Table 2. Maternal clinical observations(# affected animals)[a]

Observation	Dose (mg/kg bw/day)					
	0	0.5	1.5			5.0
Batch/CFR[b]	NA	N17/3	N33	N17/1	N17/2	N33
Found dead	0	0	2	0	0	2
Died during gavage	0	1	0	0	0	0
Sacrificed after abortion	0	2	1	0	0	2
Sacrificed moribund	0	1	0	0	0	0

a Data obtained from pages 34 and 55-57 of the study report. n=25
b Chromatography fraction of Batch N17
NA Not applicable

2. **Body weight:** Body weight gain data are shown in Table 3. No treatment-related effects were observed on body weights, body weight gains, gravid uterus weights, or body weight gains from GD 6, either uncorrected or corrected for gravid uterus weights. Decreases ($p \leq 0.05$) in body weight gains were observed in the 5.0 mg/kg N33 does on GD 25-28 (↓311%), but this decrease was isolated and not treatment-related.

Table 3. Mean (±SD) maternal body weight gains (g) and gravid uterus weights (g)[a]

Interval	Dose (mg/kg bw/day)					
	0	0.5	1.5			5.0
Batch/CFR[b]	NA (n=24)	N17/3 (n=21-25)	N33 (n=22-25)	N17/1 (n=24)	N17/2 (n=22)	N33 (n=20-24)
Pretreatment: GD 1-6	270.2±99.33	294.7±60.20	243.8±119.09	272.0±89.81	284.3±75.78	303.5±87.65
Treatment GD 6-9	39.1±50.9	56.2±61.4	28.0±74.1	33.1±61.8	6.4±62.5	41.2±108.0
Treatment GD 25-28	18.5±54.8	18.9±81.3	28.3±80.4	3.6±53.6	30.5±52.0	-39.0±85.5*
Treatment: GD 6-28	347.8±167.46	352.8±155.08	386.3±181.93	371.0±151.18	378.5±102.42	335.3±142.02
Overall: GD 1-29	621.8±179.92	616.2±178.71	653.2±206.91	644.2±163.69	669.8±105.35	642.1±190.26
Gravid uterus	481.3±104.56	491.1±82.14	472.1±72.82	495.4±94.76	486.0±85.41	446.1±65.04
Carcass[c]	3557.4±377.34	3532.6±261.66	3540.8±321.09	3604.5±227.75	3481.1±248.03	3598.8±270.22
Net change: GD 6-29[d]	-129.6±224.95	-157.2±134.23	-82.8±180.37	-123.1±189.27	-100.4±145.70	-134.2±125.34

a Data obtained from pages 64-67 of the study report.
b Chromatography fraction of Batch N17
c Carcass = terminal body weight - gravid uterine weight
d Net weight change = carcass - GD 6 body weight
NA Not applicable

3. **Food consumption:** No treatment-related effect was observed on food consumption.

4. **Gross pathology:** No treatment-related macroscopic findings were observed in any group.

5. **Cesarean section data:** Cesarean section data are presented in Table 4. No effects of treatment were noted on numbers of litters, live fetuses, resorptions (early, late, or complete litter), fetal body weight, placental weight, sex ratio, or post-implantation loss.

Table 4. Cesarean section observations[a]

Observation	Dose (mg/kg bw/day)					
	0	0.5	1.5			5.0
Batch/CFR[b]	NA	N17/3	N33	N17/1	N17/2	N33
# Animals Assigned (Mated)	25	25	25	25	25	25
# Animals Pregnant	24	25	25	24	22	24
Pregnancy Rate (%)	96	100	100	96	88	96
# Nonpregnant	1	0	0	1	3	1
Maternal Wastage						
# Died	0	4	3	0	0	4
# Died Pregnant	0	2	2	0	0	2
# Died Nonpregnant	0	0	0	0	0	0
# Aborted	0	2	1	0	0	2
# Premature Delivery	0	0	0	0	0	0
Total # Corpora Lutea Corpora Lutea/Doe	250 10.4±2.02	226 10.8±1.89	218 9.9±1.95	260 10.8±2.44	230 10.5±2.06	207 10.4±1.87
Total # Implantations (Implantations/Doe)	232 9.7±2.39	216 10.3±1.79	204 9.3±2.07	242 10.1±2.52	220 10.0±1.85	191 9.6±1.76
Total # Litters	24	21	22	24	22	20
Total # Live Fetuses (Live Fetuses/Doe)	211 8.8±2.54	196 9.3±1.49	198 9.0±2.00	227 9.5±2.60	197 9.0±2.06	179 8.9±1.76
Total # Dead Fetuses (Dead Fetuses/Doe)	1 0.0±0.2	0 0.0±0.0	0 0.0±0.0	0 0.0±0.0	0 0.0±0.0	0 0.0±0.0
Total # Resorptions	20	20	6	15	23	12
Early	12	14	5	1	18	5
Late	8	6	1	14	5	7
Total Resorptions/Doe	0.8±1.43	1.0±1.12	0.3±0.46	0.6±1.86	1.0±1.00	0.6±0.99
Early	0.5±1.10	0.7±0.97	0.2±0.43	0.0±0.20	0.8±1.01	0.3±0.55
Late	0.3±0.76	0.3±0.72	0.0±0.21	0.6±1.86	0.2±0.43	0.3±0.81
Complete Litter Resorption	0	0	0	0	0	0
Mean Fetal Weight/litter (g)	38.7±5.59	36.8±4.10	37.5±4.11	36.4±3.87	38.4±3.82	36.3±4.98
Males	38.6±5.58	37.2±4.81	37.9±4.39	36.5±4.48	38.9±4.32	36.8±6.25
Females	38.6±6.19	36.7±4.04	37.2±4.16	36.8±4.38	37.5±3.86	36.1±4.89
Placental Weight/litter (g)	5.1±0.80	4.9±0.58	5.1±0.85	4.9±0.57	5.1±0.80	4.9±0.75
Males	5.1±0.87	4.9±0.62	5.1±0.89	4.9±0.65	5.1±0.88	5.0±0.83
Females	5.2±0.77	5.1±0.61	5.1±0.86	5.0±0.58	5.0±0.86	4.9±0.86
Sex Ratio (Mean % Male)	50.7	46.4	48.0	56.4	55.8	52.0
Pre-implantation Loss (%)	7.8±14.68	4.2±6.70	6.4±10.16	6.8±10.81	3.9±7.02	7.3±8.78
Post-implantation Loss (%)	8.4±14.53	8.5±9.93	2.7±4.62	5.2±13.63	10.7±11.38	6.0±9.92

a Data obtained from pages 34, 70-73 and 180-185 of the study report.
b Chromatography fraction of Batch N17
NA Not applicable

B. DEVELOPMENTAL TOXICITY

1. **External examination:** External abnormalities are presented in Table 5a. All observed external malformations and variations were not dose-dependent and were not considered treatment-related.

2. **Visceral examination:** Selected visceral abnormalities are presented in Table 5b. Increased incidence of microphthalmia, a malformation, was observed in the 5.0 mg/kg N33 fetuses (0.6% fetuses; 5.0% litters) compared to concurrent (0) and historical (0-0.4% fetuses; 0-3.6% litters) controls . There were no other dose-related visceral malformations.

Absent lung lobe (left inferior medialis), a variation, was observed in the N17 CFR 1, 2, and 3 (1.8-2.6% fetuses; 17.0-19.0% litters) and 1.5 and 5.0 mg/kg N33 (2.0-2.2% fetuses; 14.0-15.0% litters) fetuses compared to concurrent (1.4% fetuses; 13.0% litters) and historical (1.3-1.7% fetuses; 9.5-14.3% litters) controls. A clear cut dose-dependency was not observed and the increase in incidence over controls was minor; therefore, this finding was not considered treatment-related. Cystic dilatation of the brain, a variation, was observed in one 1.5 mg/kg N17 CFR 2 (0.5% fetuses; 4.5% litters) and one 5.0 mg/kg N33 (0.6% fetuses; 5.0% litters) fetus compared to 0 concurrent or historical controls. Enlarged ventricular chamber of the heart, a variation, was observed in one 5.0 mg/kg N33 fetus compared to 0 concurrent or historical controls. Both of these findings were considered incidental. Dilated renal pelvis, a variation, was noted in the 1.5 and 5.0 mg/kg N33 fetuses (0.5-0.6 fetuses; 4.5-5.0% litters) compared to 0 concurrent controls; however, this finding fell within the range of historical controls (0-1.3% fetuses: 0-10.7% litters). All other visceral findings were unrelated to dose.

3. **Skeletal examination:** Selected skeletal abnormalities are presented in Table 5c. There were no treatment-related skeletal malformations. However, increased incidence of supernumerary thoracic vertebrae, a variation, was observed in the 1.5 mg/kg N17 CFR 1 and 2 (47.0-50.0% fetuses; 77.0-91.0% litters), and 1.5 and 5.0 mg/kg N33 (56.0-77.0% fetuses; 89.0-95.0% litters) fetuses compared to concurrent (23.0% fetuses; 63.0% litters) and historical (21.4-30.4% fetuses; 57.1-92.9% litters) controls. Increased incidence of supernumerary thoracic vertebrae was observed in the 0.5 mg/kg N17 CFR 3 (25.0% fetuses; 71.0% litters) but within historical (21.4-30.4% fetuses; 57.1-92.9% litters) controls ranges. Supernumerary 13th rib (with cartilage present), a variation, was noted in the 1.5 mg/kg N17 CFR 1 and 2 (68.0-78.0% fetuses; 100% litters), and 1.5 and 5.0 mg/kg N33 (87.0% fetuses; 100% litters) fetuses compared to concurrent (50.0% fetuses; 83.0% litters) and historical (62.3-81.7% fetuses; 85.7-96.4% litters) controls. Supernumerary 13th rib (with cartilage present) was noted in the 0.5 mg/kg N17 CFR 3 (54.0% fetuses; 95.0% litters) but within the historical (62.3-81.7% fetuses; 85.7-96.4% litters) controls range. These variations were considered treatment related.

Incomplete ossification of the cervical centrum (with unchanged cartilage), a variation, was observed in the 1.5 mg/kg N17 CFR 1 and 2 (8.3-9.5% fetuses; 35.0-36.0% litters) and 1.5 and 5.0 mg/kg N33 (4.1-5.1% fetuses; 18.0-26.0% litters) fetuses compared to concurrent (2.1% fetuses; 13.0% litters) controls; however, this finding fell within the range of historical controls (2.1-12.6% fetuses; 12.5-64.3% litters). All other skeletal variations were unrelated to dose.

Table 5a. Selected external abnormalities [% fetuses affected (% litters affected)][a]

Observations	Dose (mg/kg bw/day)						
	0	0.5	1.5			5.0	Historical controls[b]
Batch/CFR[c]	NA	N17/3	N33	N17/1	N17/2	N33	
# Fetuses (# litters) examined	212 (24)	196 (21)	198 (22)	227 (24)	197 (22)	179 (20)	601 (73)
Malformations							
Ectrodactyly	0 (0)	0 (0)	0 (0)	0.9 (8.3)	0 (0)	0 (0)	Not observed
Brachydactyly	0 (0)	0 (0)	0 (0)	2.2 (13.0)	0 (0)	0 (0)	Not observed
Total malformations	0.9 (8.3)	0.5 (4.8)	1.0 (9.1)	3.1 (17.0)	1.5 (14.0)	0 (0)	0.6-1.3 (4.8-8.3)
Variations							
Paw hyperflexion	0 (0)	0 (0)	0 (0)	0.9 (8.3)	0 (0)	0 (0)	0-0.6 (0-4.8)
Total variations	0 (0)	0 (0)	0 (0)	0.9 (8.3)	0 (0)	0 (0)	0-0.6 (0-4.8)

a Data obtained from pages 75-80 in the study report.
b Historical control data obtained from page 388-390 in the study report.
c Chromatography fraction of Batch N17
NA Not applicable

Table 5b. Selected visceral abnormalities [% fetuses affected (% litters affected)][a]

Observations	Dose (mg/kg bw/day)						
	0	0.5	1.5			5.0	Historical controls[b]
Batch/CFR[c]	NA	N17/3	N33	N17/1	N17/2	N33	
# Fetuses (# litters) examined	212 (24)	196 (21)	198 (22)	227 (24)	197 (22)	179 (20)	601 (73)
Malformations							
Microphthalmia	0 (0)	0 (0)	0 (0)	0.4 (4.2)	0 (0)	0.6 (5.0)	0-0.4 (0-3.6)
Total malformations	2.4 (17.0)	0 (0)	1.5 (14.0)	1.8 (13.0)	0.5 (4.5)	0.6 (5.0)	1.3-2.4 (9.5-16.7)
Variations							
Absent lung lobe (left Inferior Medialis)	1.4 (13.0)	2.6 (19.0)	2.0 (14.0)	1.8 (17.0)	2.5 (18.0)	2.2 (15.0)	1.3-1.7 (9.5-14.3)
Brain: cystic dilatation	0 (0)	0 (0)	0 (0)	0 (0)	0.5 (4.5)	0.6 (5.0)	Not observed
Heart: enlarged ventricular chamber	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.6 (5.0)	Not observed
Dilated renal pelvis	0 (0)	0 (0)	0.5 (4.5)	0 (0)	0 (0)	0.6 (5.0)	0-1.3 (0-10.7)
Total variations	5.2 (38.0)	3.1 (24.0)	3.5 (23.0)	2.6 (25.0)	4.1 (27.0)	4.5 (35.0)	3.9-6.3 (25.0-38.1)
Unclassified							
Total unclassified	4.2 (21.0)	9.2 (43.0)	6.1 (36.0)	6.2 (29.0)	5.6 (36.0)	3.4 (25.0)	1.7-6.3 (14.3-42.9)

a Data obtained from pages 82-92 in the study report.
b Historical control data obtained from page 391-393 in the study report.
c Chromatography fraction of Batch N17
NA Not applicable

Table 5c. Skeletal abnormalities [% fetuses affected (% litters affected)][a]

Observations	Dose (mg/kg bw/day)						
	0	0.5	1.5			5.0	Historical controls[b]
Batch/CFR[c]	NA	N17/3	N33	N17/1	N17/2	N33	
#Fetuses (# litters) examined	194 (24)	163 (21)	171 (22)	210 (23)	181 (22)	156 (19)	583 (73)
Malformations							
Malpositioned and bipartite sternebra	0 (0)	0.6 (4.8)	0 (0)	0.5 (4.3)	0.6 (4.5)	0 (0)	0-0.6 (0-4.8)
Small paw phalanx	0 (0)	0 (0)	0 (0)	2.4 (13.0)	0 (0)	0 (0)	Not observed
Absent paw phalanx	0 (0)	0 (0)	0 (0)	1.0 (8.7)	0 (0)	0 (0)	Not observed
Total malformations	5.2 (33.0)	0.6 (4.8)	1.2 (9.1)	3.8 (17.0)	2.2 (14.0)	1.9 (11.0)	2.2-5.2 (14.3-33.3)
Variations							
Supernumerary thoracic vertebrae	23.0 (63.0)	25.0 (71.0)	56.0 (95.0)**	50.0 (91.0)*	47.0 (77.0)	77.0 (89.0)*	21.4-30.4 (57.1-92.9)
Supernumerary rib (13th)	50.0 (83.0)	54.0 (95.0)	87.0 (100)	78.0 (100)	68.0 (100)	87.0 (100)	62.3-81.7 (85.7-96.4)
Incomplete ossification of cervical centrum	2.1 (13.0)	1.8 (9.5)	4.1 (18.0)	9.5 (35.0)	8.3 (36.0)	5.1 (26.0)	2.1-12.6 (12.5-64.3)
Incomplete ossification of sternebrae	39.0 (95.0)	56.0 (90.0)	48.0 (91.0)	53.0 (96.0)	40.0 (100)	37.0 (95.0)	30.4-41.5 (75.0-95.8)
Total variations	94.0 (100)	97.0 (100)	99.0 (100)	99.0 (100)	94.0 (100)	98.0 (100)	88.1-95.7 (100)

a Data obtained from pages 94-111 in the study report.
b Historical control data obtained from pages 394-398 in the study report.
c Chromatography fraction of Batch N17
* Significantly different from controls, $p \leq 0.05$
** Significantly different from controls, $p \leq 0.01$
NA Not applicable

III. DISCUSSION and CONCLUSIONS

A. <u>INVESTIGATORS' CONCLUSIONS</u>: No significant difference was noted between Batch N33 and the chromatographic fractions of Batch N17 in terms of maternal and prenatal developmental toxicity. Neither batch/fraction caused maternal toxicity or malformations at doses of 0.5, 1.5, and 5.0 mg/kg body weight. All batch/fractions at all administered doses slightly influenced the ossification of the thoracic/lumbar vertebral column and/or the sternebrae. No teratogenic potential was noted for Batch N33 or each individual chromatographic fraction of Batch N17.

B. REVIEWER COMMENTS

1. **Maternal toxicity:** No effects of treatment were observed on maternal survival, clinical signs, body weights, body weight gains, food consumption, or gross pathology. No differences were noted between Batch N33 and the 3 chromatographic fractions of Batch N17. Serum tyrosine level was not measured.

The maternal LOAEL was not observed. The NOAELwas 5.0 mg/kg/day. The LOAEL/NOAEL could be lower because serum tyrosine level was not measured.

2. **Developmental toxicity:**
   a. Deaths/Resorptions: There were no treatment-related effects on the numbers of litters, fetuses (live or dead), resorptions (early, late or complete litter), or post-implantation loss.
   b. Altered Growth: Minor alterations in skeletal ossification were observed without statistical significance or dose-dependency. Fetal body weights of the treated groups were comparable to controls.
   c. Developmental Variations: Increased incidence of supernumerary thoracic vertebrae was observed in the 1.5 mg/kg N17 CFR 1 and 2, and 1.5 and 5.0 mg/kg N33 fetuses compared to concurrent and historical controls. Increased incidence of supernumerary 13th rib (with cartilage present) was noted in the 1.5 mg/kg N17 CFR 1 and 2, and 1.5 and 5.0 mg/kg N33 fetuses compared to concurrent and historical controls. The effects observed at 0.5 mg/kg/day for N17/CFR 3 were within the historical control ranges.
   d. Malformations: There were no treatment-related external, visceral, or skeletal malformations.

   The purpose of this study was to ascertain the effects of 3 different chromatographic fractions (CFR) isolated from a previously used batch N17 of BAS 670H on embryonic and fetal development and to compare them with the effects evoked by batch N33 of BAS 670H which has a greater purity compared to N17. The results did not achieve the goal because the study design and the dose selection made it difficult to compare the effects among these treatments. No meaningful conclusion can be drawn from the study regarding the comparison of the effects between Batch N33 and the chromatographic fractions of Batch N17 in terms of maternal and prenatal developmental toxicity.

   The developmental LOAEL for N33 and N17/CFR 1-2 was 1.5 mg/kg/day based on increased presence of supernumerary thoracic vertebrae and supernumerary 13th rib. The developmental NOAEL was not observed. No effect was observed for N17/CFR 3 at 0.5 mg/kg/day (the only dose tested). There was no evidence of teratogenicity.

This study is classified as **acceptable/non-guideline.**

C. **STUDY DEFICIENCIES:** The following deficiencies were noted but do not alter the conclusions of this DER:

- The chromatographic fractions were not characterized for content or purity.
- A dose selection rationale was not provided.

- Stability data for the test compound in vehicle was not provided