DATA EVALUATION RECORD

BAS 670H

Study Type: §83-3b; Developmental Toxicity Study in Rabbits

Work Assignment No. 1-01-11 P (MRID 46020301)

Prepared for
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U.S. Environmental Protection Agency
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DATA EVALUATION RECORD

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

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

TEST MATERIAL (PURITY): BAS 670H (95.2% 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., and Leibold, E. (2003) BAS 670 H - 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. 40R0124/98121, BASF Registration Document No. 2003/1009191, March 20, 2003. MRID 46020301. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, NC

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46020301), BAS 670H (95.2% a.i.; Lot/Batch # N17) in 0.5% (w/v) aqueous carboxymethylcellulose was administered by gavage at a dose volume of 10 mL/kg body weight to female New Zealand White [Crl:KBL (NZW)] rabbits (30/group) at dose levels of 0, 5, 50, or 450 mg/kg on gestation days (GD) 7 through 28. All does were sacrificed on GD 29; their fetuses were removed by cesarean and examined.

There were no treatment-related effects observed on maternal survival, clinical signs of toxicity, body weights, body weight gains, food consumption, or gross pathology. No effects of treatment were noted on numbers of litters, number of live fetuses per doe, resorptions (early or late), fetal weight, placental weight, sex ratio, or postimplantation loss.

The maternal LOAEL was not observed. The maternal NOAEL is 450 mg/kg/day.

There were no dead fetuses and no treatment-related effects on early, late, or complete litter resorptions. Slight dose-dependent decrease of fetal weight was observed, though no statistical significance was achieved. The following findings were increased over concurrent and historical controls at 5, 50, and 450 mg/kg: (i) fluid-filled abdomen; (ii) pale liver; (iii) dark content of the stomach and intestines; (iv) unilateral ossification or incomplete ossification (with unchanged cartilage) of the centrum of the cervical vertebrae; (v) incomplete ossification of the thoracic centrum (with unchanged cartilage); (vi) supernumerary thoracic vertebrae; (vii) supernumerary 13th rib (with cartilage present); and (viii) incomplete ossification (with cartilage present) of the talus.

Additionally, the following findings were increased over concurrent and historical controls at 50 and 450 mg/kg: (i) increased incidences of infarct of the liver; (ii) unossified cervical centra (with unchanged cartilage); (iii) extra ossification site (with unchanged cartilage) of the sternebrae; (iv) unossified talus (with cartilage present); and (v) short 1st rib with cartilage not present. At 450 mg/kg, the following were increased over concurrent and historical controls: (i) incidences of small thymus; (ii) severely malformed bones of the skull; (iii) increased incidences of absent and misshapen caudal vertebrae; (iv) fused ribs with unchanged cartilage; (v) absent 1st rib; (vi) pale kidney; (vii) incidence of incomplete ossification of the interparietal bone; (viii) unilateral ossification (with dumbbell-shaped cartilage) of the centrum of the cervical vertebrae; (ix) incomplete ossification of the forepaw phalanx; and (x) incomplete ossification (with cartilage present) of the hindpaw phalanx.

The developmental LOAEL is 5 mg/kg/day, based on visceral findings (fluid-filled abdomen, pale liver, and dark content of the stomach and intestines) and alterations in skeletal development (i.e., incomplete ossification of the vertebrae and talus, and supernumerary thoracic vertebrae and 13th rib). The developmental NOAEL was not established.

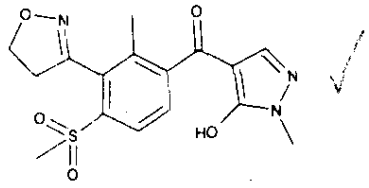
This study is classified **acceptable/guideline (OPPTS 870.3700b)** in conjunction with MRID 45902210 and satisfies the requirements for a developmental study in the rabbit.

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:	Beige crystalline solid
Lot/Batch #:	N17
Purity:	95.2% a.i.
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 0:	21-28 weeks/3249-4741 g
Source:	Elevage Scientifique des Dombes (01400 Chatillon/Chalaronne, France)
Housing:	Individually in stainless steel wire mesh cages
Diet:	Pelleted Kliba maintenance diet type 3418 (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:	5-21 days

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Start: March 13, 2000 End: April 20, 2000

2. **Mating:** After at least 5 days of acclimation, nulliparous nonpregnant females were given an 0.2 mL intramuscular injection of Receptal® (Hoechst AG; a synthetic hormone causing release of luteinizing hormone and follicle stimulating hormone) and were artificially inseminated one hour later with pooled ejaculate samples from male rabbits of the same strain. The day of insemination was designated as gestation (GD) 0.

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

Table 1. Animal assignment [a]

Dose (mg/kg bw/day)	0	5	50	450
# Females	30	30	30	30

a Data obtained from page 21 of the study report (MRID 46020301).

4. Dose selection rationale: It was stated that 5 mg/kg was expected to be the NOAEL, 50 mg/kg was chosen as the intermediate dose, and 450 mg/kg was expected to cause some maternal and developmental toxicity. However, no data were presented to support these selections.

5. Dosage preparation and analysis: It was stated that stock dosing solutions were prepared at the beginning of the study and thereafter at intervals which took into account the results of the stability findings. A weighed amount of test substance was suspended in (doubly distilled) aqueous 0.5% (w/v) carboxymethylcellulose. Homogeneity was confirmed by analyses of three samples of the low and high dose formulations taken from the top, middle, and bottom of the mixing container at Week 0. 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. Samples of the dosing mixtures at each dose level were taken at the beginning and towards the end of the study, and concentrations of the test substance were determined.

Results -

Homogeneity (range as % CV): 1.4-1.6%

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): 96.6-110.8%

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.

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

C. OBSERVATIONS

1. **Maternal observations and evaluations:** All does were checked for mortality and morbidity twice daily (daily on weekends and holidays). Does were examined for clinical signs of toxicity at least once per day. Body weights were determined on GD 0, 2, 4, 7, 9, 11, 14, 16, 19, 21, 23, 25, 28, and 29. Food consumption (g/rabbit/day) was measured daily throughout the study period. On GD 29, all surviving does were sacrificed in randomized order and subjected to necropsy, the ovaries and uteri excised, and gravid uterus weights determined. All fetuses were removed by cesarean section. The numbers of corpora lutea, implantations, live fetuses, dead fetuses, and resorptions (early and late) were recorded. Rabbits that died or were sacrificed during the study period were examined using the same procedures as those used for the does killed on schedule except that gravid uteri were not weighed.

2. **Fetal evaluations:** All fetuses were weighed, examined for external abnormalities. Viability and the condition of the placentae, umbilical cords, fetal membranes, and fluids were noted. Individual placental weights were recorded. All fetuses were dissected and visceral alterations were noted; the heart and kidneys were sectioned to examine their internal structures; and the fetuses were sexed. The heads of approximately one-half of the fetuses in each litter and those revealing severe external findings were removed, fixed in Bouin's solution, and processed and evaluated according to Wilson's method. All fetuses were then skinned, fixed in ethyl alcohol, and after 1-5 days, the heads of the remaining fetuses were sectioned with a single cross-sectional incision in the parietal bone area for evaluation of internal brain structures. The skeletons of fixed fetuses were stained for skeletal examination according to a modification of the method of Kimmel and Trammell.

D. DATA ANALYSIS

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

Parameter	Statistical test
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 fetuses, proportions of preimplantation loss, proportions of postimplantation loss, proportions of resorptions, proportion of live fetuses in each litter, litter mean fetal body weight, and litter mean placental weight	Simultaneous comparison of all dose groups with the control group using Dunnett's test (two sided) for the hypothesis of equal means
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
Proportions of fetuses with malformations, variations 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

Only pregnant does were used for calculations of food consumption, body weight, and body weight gain. Only pregnant does sacrificed on GD 29 were used for calculations of gravid uterine weights, net maternal body weight gain, and reproductive data. Significance was denoted at $p \leq 0.05$ or $p \leq 0.01$.

2. Indices: The following indices were calculated:

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

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

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

3. Historical control data: Control data were provided for maternal body weights, cesarean parameters, placenta weights, and external, visceral, and skeletal findings in the fetuses. Data were comprised of 3 studies on 29-80 does and 70-73 litters of the same strain performed from March 2000 through December 2001.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical observations: No treatment-related mortality was observed. Three does in the 450 mg/kg group died/were sacrificed after gavage error, and one doe was sacrificed in moribund condition on GD 27; however, this was considered incidental as no clinical signs of toxicity were observed in this animal prior to GD 27. One control doe was sacrificed after aborting its litter on GD 20; blood was found in the bedding, and considered related to the abortion. Increased incidence and frequency of no defecation (5/26 treated vs 1/29 controls) were observed in the 450 mg/kg does. No other clinical observations were noted.

2. Body weight: No treatment-related effects were observed on body weights or body weight gains (Table 2). Increases ($p \leq 0.05$) in body weight gains were noted but were sporadic and not related to dose; therefore, they were considered incidental. Gravid uterine weights were comparable to controls. Body weight gains for the treatment interval (GD 7-28) and for the overall study (GD 0-29) were comparable to controls whether corrected for gravid uterine weights or not.

Table 2. Mean (±SD) maternal body weight gain (g)[a]

Interval		Dose in mg/kg bw/day (# of Does)			
		0 (28-29)	5 (30)	50 (27)	450 (26-27)
Pretreatment:	GD 0-7	180.0±118.91	173.5±112.53	208.5±108.10	243.5±133.45
Treatment:	GD 7-11[b]	25.41±41.51	20.00±70.66	46.04±66.50	58.30±60.80
	GD 11-16[b]	118.59±82.06	107.37±121.85	95.89±88.17	90.93±76.64
	GD 16-21[b]	-62.43±102.09	-27.43±107.31	3.30±67.41	-8.85±97.70
	GD 21-25[b]	64.68±80.27	58.53±69.78	49.41±69.89	20.48±80.78
	GD 25-29[b]	35.29±66.76	11.27±78.41	3.37±63.72	-19.65±119.16
Treatment	GD 7-28	159.3±154.27	157.1±244.02	199.6±111.28	154.7±208.21
Overall	GD 0-29	356.5±175.13	343.2±263.56	406.5±155.34	404.5±272.85
Gravid uterus weight (g)		455.4±136.98	431.1±127.60	477.9±75.39	429.1±93.77
Carcass (g)		3867.7±272.4	3896.9±315.6	3875.4±281.4	3882.4±305.4
Net weight gain[c] GD 7-29		-273.2±193.1	-261.4±233.3	-279.9±84.3	-278.2±227.9

a Data obtained from pages 65-68 of the study report (MRID 46020301). Percent difference from controls (calculated by reviewers) is included in parentheses.
b Calculated by reviewers from individual data presented on pages 141-148 of the study report.
c Net body weight gains were calculated by carcass weight minus day 7 body weight.

3. Food consumption: No treatment-related effects were observed on food consumption. Increases ($p \leq 0.05$) were noted at Day 19 in the ≥50 mg/kg groups (↑38-41%) and at Day 20 in the ≥5 mg/kg groups (↑35-36%), but these increases were sporadic and not related to dose.

4. Gross pathology: There were no treatment-related macroscopic findings in any group. Three 450 mg/kg does were found to have bloody fluid in the thoracic cavity and/or congested lungs. These findings correlate with gavage errors and are not related to treatment. The 450 mg/kg doe that was sacrificed in moribund condition was found to have a tympanic distension of the large intestine with watery/bloody feces. This also was not considered treatment-related. All other necropsy observations were unrelated to dose and not corroborated by other findings, and were considered incidental.

5. Cesarean section data: Cesarean section data are presented in Table 3. One control doe aborted on GD 20. Also, one 5 mg/kg doe had only early resorptions with no viable fetuses. These findings were considered to be incidental, as they were not related to dose. Slight dose-dependent decreases of fetal weight were observed (not statistically significant). There were no dead fetuses and no effects of treatment were noted on numbers of litters, number of live fetuses per doe, resorptions (early or late), placental weight, sex ratio, or postimplantation loss.

Table 3. Cesarean section observations[a]

Observation	Dose (mg/kg bw/day)			
	0	5	50	450
# Animals Assigned (Mated)	30	30	30	30
# Animals Pregnant	29	30	27	29
Pregnancy Rate (%)	97	100	90	97
# Nonpregnant[b]	1	0	3	1
Maternal Wastage				
# Died[c]	0	0	0	4
# Died Pregnant[c]	0	0	0	3
# Died Nonpregnant[c]	0	0	0	1
# Aborted	1	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	290	307	282	267
Corpora Lutea/Doe	10.4±2.64	10.2±2.31	10.4±1.58	10.3±2.59
Total # Implantations	265	273	263	239
(Implantations/Doe)	9.5±2.70	9.1±2.67	9.7±2.05	9.2±2.71
Total # Litters	28	29	27	26
Total # Live Fetuses	230	227	240	214
(Live Fetuses/Doe)	8.2±2.62	7.8±2.36	8.9±1.63	8.2±2.20
Total # Dead Fetuses	0	0	0	0
(Dead Fetuses/Doe)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Total # Resorptions	35	46	23	25
Early	15	32	11	20
Late	20	14	12	5
Total Resorptions/Doe	1.3±1.48	1.5±1.66	0.9±1.35	1.0±1.80
Early	0.5±0.69	1.1±1.62	0.4±0.75	0.8±1.63
Late	0.7±1.12	0.5±0.68	0.4±0.93	0.2±0.49
Complete Litter Resorption	0	1	0	0
Mean Fetal Weight (g)/litter	38.3±6.95	38.9±5.58	36.5±4.55	36.5±6.91
Males	37.6±7.36	38.6±5.96	36.9±4.73	35.9±6.81
Females	38.0±5.36	38.5±5.95	36.1±4.48	34.9±7.02
Placenta weight (g)/litter	5.1±0.96	5.4±0.92	5.4±0.77	5.3±1.30
Males	5.1±1.03	5.4±0.99	5.5±0.80	5.3±1.38
Females	5.0±0.71	5.3±1.01	5.3±0.81	5.2±1.34
Sex Ratio (Mean % Male)	50.0	59.0	52.1	50.0
Preimplantation Loss (%)	8.8±16.99	11.7±16.09	7.0±12.73	10.4±14.21
Postimplantation Loss (%)	12.4±13.91	18.1±22.63	7.5±10.54	9.1±14.34

a Data obtained from pages 71-74 of the study report (MRID 46020301).
b Calculated by reviewers from data presented in this table
c Calculated by reviewers from individual data presented on pages 157-165 of the study report

B. DEVELOPMENTAL TOXICITY

1. External examination: External malformations are presented in Table 4a. In the 450 mg/kg group, exencephaly was observed in 2 fetuses (0.9% fetuses; 7.7% litters) compared to 0 concurrent controls. This finding was not observed in the historical controls; however, it only occurred in 2 fetuses in 2 litters and was considered to be incidental. Short tail was noted in the 5 (0.4% fetuses; 3.4% litters) and 450 (0.9% fetuses; 7.7% litters) mg/kg groups compared to concurrent controls (0.4% fetuses; 3.6% litters). This finding was over the range of historical controls (0-0.4% fetuses; 0-3.6% litters), but was not dose-dependent and occurred in 2 fetus in 2 litters in the 450 mg/kg group. Therefore, it was considered to be incidental. All other malformations were unrelated to dose. No external variations were noted. Discolored amniotic fluid was observed in the 5 (2.6% fetuses; 3.4% litters), 50 (1.3% fetuses; 7.4% litters), and 450 (13.0% fetuses; 12% litters) mg/kg groups compared to concurrent controls (2.6% fetuses; 3.6% litters) and exceeded historical controls (0-2.8% fetuses; 0-4.2% litters) at 450 mg/kg. Necrobiotic placentae were noted in the 450 mg/kg group (3.3% fetuses; 3.8% litters) compared to 0 concurrent controls or historical controls. However, no morphological abnormalities correlating to these findings were noted; therefore, these findings were not considered adverse. All other unclassified external observations were not related to dose.

2. Visceral examination: Selected visceral abnormalities are presented in Table 4b. Incidences of small thymus, a malformation, were observed at 450 mg/kg (2.3% fetuses; 12.0% litters) compared to concurrent (0.4% fetuses; 3.6% litters) and historical (0-0.6% fetuses; 0-4.8% litters) controls. Small lung and diaphragmatic hernia, both malformations, were observed together in a single 450 mg/kg fetus, and misshapen kidney and fused kidneys, also malformations, were each noted in one 450 mg/kg fetus (0.5% fetuses; 3.8% litters), all compared to 0 concurrent controls. These findings were not reported in the historical controls; however, they were considered incidental. All other visceral malformations, such as absent kidney/ureter, were unrelated to dose. There were no dose-related visceral variations.

Several unclassified visceral findings were observed. Fluid-filled abdomen was noted in the 5 (4.8% fetuses; 28.0% litters), 50 (2.5% fetuses; 15.0% litters), and 450 (5.6% fetuses; 35.0% litters [$p \leq 0.05$]) mg/kg groups compared to concurrent controls (1.3% fetuses; 11.0% litters). Incidences of this finding at ≥5 mg/kg were above the range of historical controls (0.6-1.3% fetuses; 4.2-10.7% litters). Increased incidences of infarct of the liver were observed in the 50 (0.8% fetuses; 7.4% litters) and 450 (1.9% fetuses; 12.0% litters) mg/kg groups compared to concurrent (0.4% fetuses; 3.6% litters) and historical (0-0.4% fetuses; 0-3.6% litters) controls. Pale liver was noted in the 5 (0.4% fetuses; 3.4% litters), 50 (0.4% fetuses; 3.7% litters), and 450 (6.5% fetuses; 15.0% litters) mg/kg groups compared to 0 concurrent or historical controls. Dark content of the stomach was noted in the 5 (7.5% fetuses; 17.0% litters [$p \leq 0.05$]), 50 (4.2% fetuses; 7.4% litters), and 450 (12.0% fetuses; 27.0% litters [$p \leq 0.01$]) mg/kg groups compared to 0 concurrent or historical controls. Pale kidney was observed in the 450 mg/kg group (0.9% fetuses; 3.8% litters) compared to 0 concurrent or historical controls. Dark content of the intestines was observed in the 5 (7.5% fetuses; 17.0% litters), 50 (6.7% fetuses; 7.4% litters) and 450 (13.0% fetuses; 27.0% litters [$p \leq 0.05$]) mg/kg groups compared to concurrent (0.4% fetuses; 3.6% litters) and historical (0-0.4% fetuses; 0-3.6% litters) controls. All other unclassified

visceral findings were unrelated to dose, occurred in a single fetus, and/or fell within the range of historical controls.

3. **Skeletal examination:** Selected skeletal malformations are presented in Table 4c. Severely malformed bones of the skull were observed in the 450 mg/kg group (0.9% fetuses; 7.7% litters) compared to 0 concurrent or historical controls. Increased incidences of absent and misshapen caudal vertebrae were noted in the 450 (0.9% fetuses; 7.7% litters) mg/kg group compared to concurrent (0.4% fetuses; 3.6% fetuses) and historical (0-0.4% fetuses; 0-3.6% litters [absent], 0-0.5% fetuses; 0-4.2% litters [misshapen]) controls. Fused ribs with unchanged cartilage were observed at 450 mg/kg (0.5% fetuses; 3.8% litters); short 1st rib with cartilage not present was noted at 50 (0.4% fetuses; 3.7% litters) and 450 (0.5% fetuses; 3.8% litters) mg/kg; and absent 1st rib was observed in the 450 mg/kg group (0.5% fetuses; 3.8% litters), all compared to 0 concurrent or historical controls. All other skeletal malformations were unrelated to dose.

Selected skeletal variations are presented in Table 4d. The following findings were observed: (i) increased ($p \leq 0.01$) incidence of incomplete ossification of the interparietal bone of the skull at 450 mg/kg (9.3% fetuses; 46.0% litters) compared to concurrent (0.9% fetuses; 7.1% litters) and historical (0.9-4.6% fetuses; 7.1-29.2% litters) controls; in the centrum of the cervical vertebrae, (ii) unilateral ossification (with unchanged cartilage) in the 5 (6.2% fetuses; 28.0% litters), 50 (10.0% fetuses; 56.0% litters, $p \leq 0.01$), and 450 (12.0% fetuses; 50.0% litters, $p \leq 0.01$) mg/kg groups compared to concurrent (1.3% fetuses; 11.0% litters) and historical (0.5-1.3% fetuses; 4.2-10.7% litters) controls; (iii) unilateral ossification (with dumbbell-shaped cartilage) in the 450 mg/kg group (0.9% fetuses; 7.7% litters) compared to 0 concurrent or historical controls; (iv) incomplete ossification (with unchanged cartilage) in the 5 (33.0% fetuses; 79.0% litters), 50 (40.0% fetuses; 96.0% litters, $p \leq 0.01$), and 450 (32.0% fetuses; 96.0% litters) mg/kg groups compared to concurrent (13.0% fetuses; 64.0% litters) and historical (2.1-12.6% fetuses; 12.5-64.3% litters) controls; (v) unossified cervical centra (with unchanged cartilage) at 50 (1.3% fetuses; 11.0% litters) and 450 (3.3% fetuses; 15.0% litters) mg/kg compared to concurrent (0.4% fetuses; 3.6% litters) and historical (0-0.6% fetuses; 0-4.8% litters) controls; (vi) incomplete ossification of the thoracic centrum (with unchanged cartilage) at 5 (9.3% fetuses; 48.0% litters), 50 (7.1% fetuses; 44.0% litters), and 450 (16.0% fetuses; 54.0% litters) mg/kg compared to concurrent (4.8% fetuses; 36.0% litters) and historical (0-4.8% fetuses; 0-35.7% litters) controls; (vii) supernumerary thoracic vertebrae at 5 (76.0% fetuses; 100% litters), 50 (90.0% fetuses; 100% litters), and 450 (94.0% fetuses; 100% litters) mg/kg compared to concurrent (30.0% fetuses; 93.0% litters) and historical (21.4-30.4% fetuses; 57.1-92.9% litters) controls; (viii) extra ossification site (with unchanged cartilage) of the sternebrae at 50 (1.3% fetuses; 11.0% litters) and 450 (1.9% fetuses; 12.0% litters) mg/kg compared to concurrent (0.4% fetuses; 3.6% litters) and historical (0-0.5% fetuses; 0-4.2% litters) controls; (ix) supernumerary 13th rib (with cartilage present) at 5 (88.0% fetuses; 100% litters), 50 (95.0% fetuses; 100% litters), and 450 (94.0% fetuses; 100% litters) mg/kg compared to concurrent (57.0% fetuses; 96.0% litters) and historical (62.3-81.7% fetuses; 85.7-96.4% litters) controls; (x) incomplete ossification of the forepaw phalanx at 450 mg/kg (0.5% fetuses; 3.8% litters) compared to 0 concurrent or historical controls; (xi) incomplete ossification (with cartilage present) of the talus at 5 (7.9% fetuses; 24.0% litters), 50 (7.9% fetuses; 44.0% litters, $p \leq 0.05$), and 450 (18.0% fetuses; 54.0% litters, $p \leq 0.01$) mg/kg compared to concurrent (4.8% fetuses; 18.0% litters) and historical (0-4.8%

fetuses; 0-17.9% litters) controls; (xii) unossified talus (with cartilage present) at 50 (1.3% fetuses; 11.0% litters) and 450 (5.6% fetuses; 23.0% litters, $p \leq 0.01$) mg/kg compared to concurrent (0) and historical (0-0.5% fetuses; 0-4.2% litters) controls; and (xiii) incomplete ossification (with cartilage present) of the hindpaw phalanx at 450 mg/kg (0.9% fetuses; 7.7% litters) compared to 0 concurrent or historical controls. All other skeletal variations were unrelated to dose, occurred in a single fetus, and/or fell within the range of historical controls. There were no treatment-related unclassified skeletal findings.

Table 4a. External findings [% fetuses affected (% litters affected)][a]

Observation	Dose (mg/kg bw/day)				
	0	5	50	450	Historical controls[b]
Malformations					
# Fetuses (# litters) examined	230 (28)	227 (29)	240 (27)	214 (26)	599 (73)
Exencephaly	0 (0)	0 (0)	0 (0)	0.9 (7.7)	Not observed
Short tail	0.4 (3.6)	0.4 (3.4)	0 (0)	0.9 (7.7)	0-0.4 (0-3.6)
Spina bifida	0.4 (3.6)	0 (0)	0.4 (3.7)	0 (0)	0-0.5 (0-4.2)
Malrotated limb	0.9 (3.6)	0 (0)	0 (0)	0 (0)	0-0.9 (0-3.6)
Unclassified observations					
Discolored amniotic fluid	2.6 (3.6)	2.6 (3.4)	1.3 (7.4)	13.0 (12.0)	0-2.8 (0-4.2)
Necrobiotic placentae	0 (0)	0 (0)	0 (0)	3.3 (3.8)	Not observed

a Data obtained from pages 42, 76-77, and 79-80 in the study report (MRID 46020301).
b Historical control data obtained from pages 370-372 in the study report.

Table 4b. Selected visceral findings [% fetuses affected (% litters affected)][a]

Observation	Dose (mg/kg bw/day)				
	0	5	50	450	Historical controls[b]
Malformations					
#Fetuses (# litters) examined	230 (28)	227 (29)	240 (27)	214 (26)	599 (73)
Small thymus[c]	0.4 (3.6)	0.4 (3.4)	0 (0)	2.3 (12.0)	0-0.6 (0-4.8)
Small lung[c]	0 (0)	0 (0)	0 (0)	0.5 (3.8)	Not observed
Diaphragmatic hernia[c]	0 (0)	0 (0)	0 (0)	0.5 (3.8)	Not observed
Misshapen kidney	0 (0)	0 (0)	0 (0)	0.5 (3.8)	Not observed
Fused kidneys	0 (0)	0 (0)	0 (0)	0.5 (3.8)	Not observed
Variations					
Absent lung lobe (lobus inferior medialis)	1.7 (14.0)	2.6 (17.0)	0.8 (7.4)	1.9 (12.0)	1.3-1.7 (9.5-14.3)
Malpositioned carotid branch	0.9 (7.1)	2.2 (17.0)	0.4 (3.7)	0 (0)	0-5.0 (7.1-28.6)
Brain - cystic dilatation	0 (0)	0.4 (3.4)	0 (0)	0 (0)	Not observed
Unclassified observations					
Fluid-filled abdomen	1.3 (11.0)	4.8 (28.0)	2.5 (15.0)	5.6 (35.0*)	0.6-1.3 (4.2-10.7)
Infarct of liver	0.4 (3.6)	0.4 (3.4)	0.8 (7.4)	1.9 (12.0)	0-0.4 (0-3.6)
Pale liver	0 (0)	0.4 (3.4)	0.4 (3.7)	6.5 (15.0)	Not observed
Stomach - dark content	0 (0)	7.5 (17.0*)	4.2 (7.4)	12.0 (27.0**)	Not observed
Pale kidney	0 (0)	0 (0)	0 (0)	0.9 (3.8)	Not observed
Intestines - dark content	0.4 (3.6)	7.5 (17.0)	6.7 (7.4)	13.0 (27.0*)	0-0.4 (0-3.6)

a Data obtained from pages 43 and 82-91 in the study report (MRID 46020301).
b Historical control data obtained from pages 373-375 in the study report.
c Small thymus, small lung, and diaphragmatic hernia were observed in a single 450 mg/kg fetus.
* Significantly different from controls, $p \leq 0.05$
** Significantly different from controls, $p \leq 0.01$

Table 4c. Selected skeletal malformations [% fetuses affected (% litters affected)][a]

Observation	Dose (mg/kg bw/day)				
	0	5	50	450	Historical controls[b]
#Fetuses (# litters) examined	230 (28)	227 (29)	240 (27)	214 (26)	583 (73)
Skull					
severely malformed bones	0 (0)	0 (0)	0 (0)	0.9 (7.7)	Not observed
Vertebrae					
caudal, absent[c]	0.4 (3.6)	0.4 (3.4)	0 (0)	0.9 (7.7)	0-0.4 (0-3.6)
caudal, misshapen[c]	0.4 (3.6)	0.4 (3.4)	0 (0)	0.9 (7.7)	0-0.5 (0-4.2)
Ribs					
fused (unchanged cartilage)	0 (0)	0 (0)	0 (0)	0.5 (3.8)	Not observed
short 1st (cartilage not present)	0 (0)	0 (0)	0.4 (3.7)	0.5 (3.8)	Not observed
absent 1st	0 (0)	0 (0)	0 (0)	0.5 (3.8)	Not observed

a Data obtained from pages 46 and 93-98 in the study report (MRID 46020301).
b Historical control data obtained from page 376 in the study report.
c These findings occurred together in the fetuses but were listed separately, and are therefore tabulated separately.

Table 4d. Selected skeletal variations [% fetuses affected (% litters affected)][a]

Observation	Dose (mg/kg bw/day)				Historical controls
	0	5	50	450	
#Fetuses (# litters) examined	230 (28)	227 (29)	240 (27)	214 (26)	583 (73)
Skull interparietal bone					
incomplete ossification	0.9 (7.1)	4.4 (28.0*)	2.5 (22.0)	9.3 (46.0**)	0.9-4.6 (7.1-29.2)
holes	0 (0)	0 (0)	0.4 (3.7)	0.5 (3.8)	Not observed
Vertebrae					
cervical centrum					
unilateral ossification (unchanged cartilage)	1.3 (11.0)	6.2 (28.0)	10.0 (56.0**)	12.0 (50**)	0.5-1.3 (4.2-10.7)
unilateral ossification (dumbbell-shaped cartilage)	0 (0)	0 (0)	0 (0)	0.9 (7.7)	Not observed
incomplete ossification (unchanged cartilage)	13.0 (64.0)	33.0 (79.0)	40.0 (96.0**)	32.0 (96.0**)	2.1-12.6 (12.5-64.3)
unossified (unchanged cartilage)	0.4 (3.6)	0.4 (3.4)	1.3 (11.0)	3.3 (15.0)	0-0.6 (0-4.8)
thoracic centrum					
incomplete ossification (unchanged cartilage)	4.8 (36.0)	9.3 (48.0)	7.1 (44.0)	16.0 (54.0)	0-4.8 (0-35.7)
supernumerary thoracic	30.0 (93.0)	76.0 (100)	90.0 (100)	94.0 (100)	21.4-30.4 (57.1-92.9)
Sternebrae					
extra ossification site (unchanged cartilage)	0.4 (3.6)	0(0)	1.3 (11.0)	1.9 (12.0)	0-0.5 (0-4.2)
Ribs					
supernumerary 13th (cartilage present)	57.0 (96.0)	88.0 (100)	95.0 (100)	94.0 (100)	62.3-81.7 (85.7-96.4)
supernumerary 13th (cartilage not present)	25.0 (79.0)	7.5 (34.0)	5.4 (37.0)	4.2 (23.0)	Not observed
extra ossification site (cartilage not present)	5.2 (25.0)	4.4 (21.0)	0.8 (7.4)	0.9 (7.7)	0-5.2 (0-25.0)
Forepaw phalanx					
incomplete ossification	0 (0)	0 (0)	0 (0)	0.5 (3.8)	Not observed
Talus					
incomplete ossification (cartilage present)	4.8 (18.0)	7.9 (24.0)	7.9 (44.0*)	18.0 (54.0**)	0-4.8 (0-17.9)
unossified (cartilage present)	0 (0)	0 (0)	1.3 (11.0)	5.6 (23.0**)	0-0.5 (0-4.2)
Hindpaw phalanx					
incomplete ossification (cartilage present)	0 (0)	0 (0)	0 (0)	0.9 (7.7)	Not observed

a Data obtained from pages 48 and 99-113 in the study report (MRID 46020301).
b Historical control data obtained from pages 377-379 in the study report.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The NOAEL for maternal toxicity is 450 mg/kg, the highest dose level. The test substance caused delay of skeletal ossification, embryotoxic, and fetotoxic effects at all dose levels tested. A NOAEL for developmental toxicity was not

established.

B. REVIEWER COMMENTS

1. **Maternal toxicity:** There were no effects of treatment on maternal survival, clinical signs of toxicity, body weights, body weight gains, food consumption, or gross pathology. No effects of treatment were noted on numbers of litters, number of live fetuses per doe, resorptions (early or late), fetal weight, placental weight, sex ratio, or postimplantation loss.

The maternal LOAEL was not observed. The maternal NOAEL is 450 mg/kg/day.

2. **Developmental toxicity:**
   a. Deaths/Resorptions: There were no dead fetuses and no treatment-related effects on early, late, or complete litter resorptions.
   b. Altered Growth: Slight dose-dependent decrease of fetal weight was observed, though no statistical significance was achieved. The following findings were increased over concurrent and historical controls: (i) incidence of incomplete ossification of the interparietal bone at 450 mg/kg; (ii) unilateral ossification (with unchanged cartilage) of the centrum of the cervical vertebrae in the 5, 50, and 450 mg/kg groups; (iii) unilateral ossification (with dumbbell-shaped cartilage) of the centrum of the cervical vertebrae in the 450 mg/kg group; (iv) incomplete ossification (with unchanged cartilage) of the centrum of the cervical vertebrae in the 5, 50, and 450 mg/kg groups; (v) unossified cervical centra (with unchanged cartilage) at 50 and 450 mg/kg; (vi) incomplete ossification of the thoracic centrum (with unchanged cartilage) at 5, 50, and 450 mg/kg; (vii) supernumerary thoracic vertebrae at 5, 50, and 450 mg/kg; (viii) extra ossification site (with unchanged cartilage) of the sternebrae at 50 and 450 mg/kg; (ix) supernumerary 13th rib (with cartilage present) at 5, 50, and 450 mg/kg; (x) incomplete ossification of the forepaw phalanx at 450 mg/kg; (xi) incomplete ossification (with cartilage present) of the talus at 5, 50, and 450 mg/kg; (xii) unossified talus (with cartilage present) at 50 and 450 mg/kg; and (xiii) incomplete ossification (with cartilage present) of the hindpaw phalanx at 450 mg/kg.
   c. Developmental Variations: The following findings were increased over concurrent and historical controls: (i) fluid-filled abdomen at 5, 50, and 450 mg/kg; (ii) increased incidences of infarct of the liver at 50 and 450 mg/kg; (iii) pale liver at 5, 50, and 450 mg/kg; (iv) dark content of the stomach at 5, 50, and 450 mg/kg; (v) pale kidney at 450 mg/kg; and (vi) dark content of the intestines at 5, 50, and 450 mg/kg.
   d. Malformations: Incidences of small thymus, severely malformed bones of the skull, and increased incidences of absent and misshapen caudal vertebrae were noted in the 450 mg/kg group compared to concurrent and historical controls. Fused ribs with unchanged cartilage were observed at 450 mg/kg; short 1st rib with cartilage not present was noted at 50 and 450 mg/kg; and absent 1st rib was observed in the 450 mg/kg group compared to concurrent and historical controls. There were no treatment-related external malformations.

The developmental LOAEL is 5 mg/kg/day, based on visceral findings (fluid-filled abdomen, pale liver, and dark content of the stomach and intestines) and alterations in skeletal development (i.e., incomplete ossification of the vertebrae and talus, and

supernumerary thoracic vertebrae and 13th rib). The developmental NOAEL was not established. There was no evidence of teratogenicity.

This study is classified **acceptable/guideline (OPPTS 870.3700b)** in conjunction with MRID 45902210 and satisfies the requirements for a developmental study in the rabbit.

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

- Stability of the test compound was tested in water and not in carboxymethylcellulose vehicle.
- No maternal LOAEL was observed; however, in the definitive study (MRID 45902210), a maternal LOAEL was observed at 450 mg/kg/day. Therefore, this study is acceptable in conjunction with the definitive study.

DATA FOR ENTRY INTO ISIS

Developmental Study - rabbits (870.3700b)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
123009	46020301	developmental	rabbit	GD 7-28	oral	gavage	5-450	0, 5, 50, 450	450	Not observed		Maternal
123009	46020301	developmental	rabbit	GD 7-28	oral	gavage	5-450	0, 5, 50, 450	Not established	5	Fetal viscera, skeleton	Developmental

Deleted