DATA EVALUATION RECORD

BAS 670H

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

Work Assignment No. 1-01-11 Q (MRID 46020302)

Prepared for

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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

CITATION: Schneider, S., E. Leibold (2003) BAS 670H - prenatal developmental toxicity study in Himalayan rabbits: oral administration (gavage). Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany. Laboratory Project Id.: 40R0124/98107, March 20, 2003. MRID 46020302. Unpublished.

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

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46020302), BAS 670H (Batch # N17; 95.2% a.i.) was administered in 0.5% aqueous caroxymethylcellulose orally via gavage, in a dosing volume of 10 mL/kg, to 25 female Himalayan Chbb:HM rabbits/group, at dose levels of 0, 50, 150, or 450 mg/kg/day, on gestation days (GD) 7 through 28. All surviving does were sacrificed on GD 29, and their fetuses were removed by cesarean and examined. There were no treatment-related adverse effects observed from the cesarean section data.

At 450 mg/kg/day, body weight gain decreased ($p \leq 0.01$) by 49% during Days 7-28. Body weight gain from Day 7 until termination (corrected for gravid uterine weight) was decreased by 43% (not statistically significant [NS]). Food consumption was decreased ($p \leq 0.05$) by 22-30% at Days 19-20, 20-21, and 24-28, contributing to a decrease (NS) of 11% on Days 7-28 and 6% on Days 0-29. Three females, which were sacrificed following spontaneous abortion, consumed less than 10 g/day during the last third of the pregnancy. These three females were sacrificed after spontaneous abortions shortly before term (GD 28 and 29), and had markedly decreased body

weights. In addition, five 450 mg/kg/day animals did not defecate for 1 or more days beginning at GD 22.

The maternal LOAEL was 450 mg/kg/day based on decreased body weight gains and food consumption, increased incidences of abortion and lack of defecation. The maternal NOAEL was 150 mg/kg/day.

Increased incidence of short 1st rib (malformation) was observed in all treated groups at ≥50 mg/kg/day (with discontinuous cartilage) and at ≥150 mg/kg/day (with cartilage present). The total number of skeletal and visceral malformations were increased in all treated groups, but these effects were not clearly dose-dependent. The incidence of the following variations were increased compared to concurrent and historical controls: (i) incomplete ossification of cervical centrum, phalanx, and talus; (ii) unossified cervical centrum; (iii) extra ossification site between cervical centers; (vi) supernumerary thoracic vertebra; (vii) misshapen sacral vertebra; and (viii) supernumerary [13th] rib [with or without cartilage present]). An increased incidence of displaced cartilaginous parts of ribs were observed.

Increased incidences relative to the concurrent and historical controls were observed at ≥150 mg/kg/day: dilated aorta (malformation), small cervical arch (malformation), unossified talus (variation), and polyhydramnios (unclassified abnormality). Fetal weights were decreased in all treated groups (decr 8-18%) and was considered to be treatment-related.

Additionally, at 450 mg/kg/day, the following malformations were observed at increased incidences relative to the concurrent and historical controls: enlarged ventricular chamber in the heart, three chambered heart (cor triloculare), small tongue (microglossia), and small thymus. One 450 mg/kg/day female fetus had a distended bladder (visceral unclassified) and dilated ureter (visceral variation), and these findings were not observed in concurrent and historical controls.

The developmental toxicity LOAEL was 50 mg/kg/day based on decreased fetal weight and increased incidence of visceral malformations, and skeletal malformations, variations, and unclassified abnormalities. The developmental toxicity NOAEL was not observed.

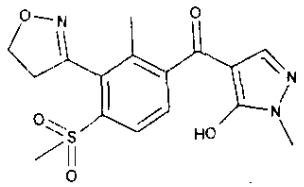
This study is classified **acceptable/guideline** (OPPTS 870.3700b; OECD 414) and satisfies the requirements for a developmental toxicity study in the rabbit.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** BAS 670H

Description:	Beige crystalline solid
Batch #:	N17
Purity:	95.2% a.i.
Compound Stability:	The compound was stable in the vehicle for 4 days at room temperature
CAS #:	210631-68-8
Structure:	

2. **Vehicle and/or positive control:** 0.5% aqueous carboxymethylcellulose

3. **Test animals**

Species:	Rabbit	
Strain:	Himalayan (Chbb:HM)	
Age at mating/ mean weight on GD 0:	26-31 weeks 2144-3070 g	
Source:	Boehringer Ingelheim Pharma KG, Biberach an der Riss., Germany	
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 reported
	Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	≥5 Days	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates** - Start: 10/11/99 End: 11/18/99

2. **Mating:** Nulliparous and nonpregnant rabbits were artificially inseminated using the pooled ejaculate of males from the same strain (source not reported). The day of insemination was designated as gestation day (GD) 0.

3. **Animal assignment:** The does were randomly assigned, stratified by body weight, to dose groups as indicated in Table 1.

Table 1. Animal assignment [a]

Dose (mg/kg bw/day)	0	50 (LDT)	150 (MDT)	450 (HDT)
# Females	25	25	25	25

a Data were obtained from page 21 of MRID 46020302.

4. Dose-selection rationale: A dose-selection rationale was not provided.

5. Dosage preparation and analysis: Dose formulations were prepared by suspending the appropriate amount of test substance in 0.5% aqueous carboxymethylcellulose. The frequency of preparation and the storage conditions were not reported; however, the Sponsor stated that the compound stability was considered. The stability of the test substance for up to 96 hours at room temperature was verified in a 10 mg/kg/day formulation prior to the study. Homogeneity (top, middle, bottom) was determined in the 50 and 450 mg/kg/day formulations at Week 2. Concentration analyses were determined for each dose formulation prepared for Weeks 2 and 5 of the study.

Results -

Homogeneity (range as % CV): 0.9-1.0%

Stability (% of initial concentration): 96.2%

Concentration (range as % of nominal): 93.5-104.9%

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 in 0.5% aqueous carboxymethyl-cellulose orally via gavage on GD 7 through 28 in a dosing volume of 10 mL/kg based on the most recent body weight.

C. OBSERVATIONS

1. Maternal observations and evaluations: All does were checked for clinical signs of toxicity at least once a day and for mortality twice daily (once daily on weekends and holidays). Food consumption (g/animal/day) was measured daily. Body weights were determined on GD 0, 2, 4, 7, 9, 11, 14, 16, 19, 21, 23, 25, 28, and 29. Body weight gain was calculated for these intervals and GD 0-7, 7-28, and 0-29. The corrected body weight gain was determined by subtracting the gravid uterine weight on GD 29 from the body weight gain (GD 7-29). On GD 29, all surviving does were sacrificed and subjected to gross necropsy in randomized order. The gravid uterus was removed from each doe and weighed. All fetuses were removed by cesarean section. The numbers of corpora lutea, implantations, live and dead fetuses, and early, late, and complete litter resorptions were recorded. Does that died intercurrently or were sacrificed after abortion were examined according to the same procedures as those killed on schedule, except that the uterus was not weighed.

2. Fetal evaluations: Each fetus was weighed, sexed, and examined externally. Each fetus was then examined for visceral abnormalities. The heads of approximately one half of the fetuses per doe and the heads of those fetuses with severe external findings (e.g. anolphthalmia, microphthalmia, or hydrocephalus) were removed, fixed in Bouin's solution, and processed and assessed according to Wilson's method. Then all fetuses (approximately half without heads) were fixed in ethanol. Those fetuses with heads were removed from the fixative after approximately 1-5 days, the skull was sectioned across the parietal bone area, and separated to allow examination of the brain. These fetuses were then placed back into the fixative. The skeletons were stained according to a modified method of Kimmel and Trammell.

D. DATA ANALYSIS

1. Statistical analyses: Data were subjected to the following statistical procedures (tested at $p \leq 0.05$ and 0.01):

Parameter	Statistical test
Food consumption Body weight, body weight gain (uncorrected and corrected), and carcass weight Weight of unopened uterus Number of corpora lutea, implantations, resorptions, and live fetuses Proportions of preimplantation loss, postimplantation loss, resorptions, and live fetuses in each litter Litter mean fetal body weight and placental weight	Dunnett's test (two-sided)
Female mortality Females pregnant at terminal sacrifice Number of litters with fetal findings	Fisher's exact test (one-sided)
Proportions of fetuses with malformations, variations, and/or unclassified abnormalities in each litter	Wilcoxon-test (one-sided)

2. Indices: The following indices were calculated:

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

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

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

3. Historical control data: Historical control data were provided for maternal body weight, cesarean section parameters, and fetal external, visceral, and skeletal findings. Data were comprised of 8 studies performed from 1997-2001 on rabbits of the same strain as the current study (190 females mated providing 180 litters with 1188-1189 fetuses examined).

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical observations: Three females in the 450 mg/kg/day group were sacrificed after spontaneous abortions shortly before term (GD 28 and 29). These animals also had markedly decreased food consumption and decreased body weights. In addition, one animal at 150 mg/kg did not defecate for 10 days, and five 450 mg/kg/day animals did not defecate for 1 or more days beginning at GD 22 (Table 2). One 50 mg/kg/day female died intercurrently (GD 26), without any clinical signs of toxicity. This death was considered to be incidental. There were no other clinical signs that could be attributed to treatment.

Table 2. Maternal clinical signs of toxicity (# animals affected [mean number days affected]) [a]

	Dose (mg/kg/day)			
Clinical sign	0	50	150	450
No defecation	0	0	1 [10]	5 [3.0]

a Data were obtained from page 57 of MRID 46020302; n = 24-25. Mean numbers were calculated by the reviewers.

2. Body weight and body weight gains: Although initial mean body weights of females ranged from 2600-2613 g, an incidental, dose-dependent increase in body weight gain was observed during the pretreatment period for all treated females (↑521-859%). Despite the increased body weight gain during pretreatment, body weight gain dose-dependently decreased by 17-49% in all treated females during treatment (Days 7-28), and for the overall study (Days 0-29), uncorrected for gravid uterus weight (↓10-20%). The values were highly variable, and a statistically significant effect was only observed in the 450 mg/kg/day females during Days 7-28 (↓49%; $p \leq 0.01$). Body weight gain from Day 7 until termination (corrected for gravid uterine weight) was decreased by 43% (not statistically significant [NS]) in the 450 mg/kg/day females.

Table 3. Mean (±SD) maternal body weight gain (g), gravid uterus weight (g), and corrected weight gain (g) [a]

Interval	Dose in mg/kg/day			
	0	50	150	450
Pre-treatment: GD 0-7	2.90±34.7	18.0±45.4 (↑521)	20.0±57.3 (↑590)	27.8±45.3 (↑859)
Treatment: GD 7-9	10.4±27.6	12.2±31.7	10.6±27.3	11.2±25.8
GD 16-19	9.2±21.1	3.5±40.1	11.2±24.1	-15.8±41.3*
GD 19-21	-5.5±26.4	-10.5±37.2	-0.2±28.2	-16.8±30.0
Treatment: GD 7-28	193±84.3	160±86.6 (↓17)	147±85.5 (↓24)	99.3±132** (↓49)
GD 0-29	218±96.7	197±114 (↓10)	182±111 (↓17)	174±129 (↓20)
Body weight (g) at GD 29	2818±160	2814±195	2783±165	2778±219
Gravid uterus (g)	342±101	296±102 (↓13)	304±85.1 (↓11)	324±83.9 (↓5)
Carcass (g)	2476±147	2518±177	2479±171	2453±203
Net weight change GD 7-29	-126±110	-114±98 (↑9)	-141±86 (↓12)	-181±95 (↓43)

a Data (n=22-25) were obtained from pages 66 and 67 of MRID 46020302. Percent difference from controls, calculated by reviewers, is included in parentheses.
Carcass weight = terminal body weight minus uterine weight.
** Significantly different from the control at p≤0.01

3. **Food consumption:** Treatment-related decreases (p≤0.05) in food consumption were observed at 450 mg/kg/day, during GD 19-20, 20-21, and 24-28 (↓22-30%), contributing to a decrease (NS) of 11% for GD 7-28 and 6% for GD 0-29 (Table 4). Three females in the 450 mg/kg/day group, which were sacrificed following spontaneous abortion, consumed less than 10 g/day during the last third of the pregnancy. There were no other treatment-related differences in food consumption.

Table 4. Mean (±SD) maternal food consumption (g/animal/day) [a]

Interval	Dose (mg/kg/day)			
	0	50	150	450
Pretreatment GD 0-7	92.6±5.4	94.1±5.6	95.8±5.6	99.9±6.0
Treatment GD 7-8	77.8±15.1	82.9±17.5	88.0±26.8	81.0±27.3
Treatment GD 19-20	77.7±25.3	76.4±26.1	76.2±28.3	55.1±33.6*
Treatment GD 24-25	75.6±21.3	73.5±18.4	69.8±19.4	58.6±30.7* (↓22)
Treatment GD 25-26	82.9±17.3	80.4±14.2	72.6±21.6	58.2±32.5** (↓30)
Treatment GD 7-28	75.0±7.9	79.8±8.0	79.3±7.4	66.5±13.4 (↓11)
Overall study: GD 0-29	79.5±10.5	83.6±9.6	83.2±9.9	74.9±18.6 (↓6)

a Data (n=22-25) were obtained from pages 60 and 61 of MRID 46020302. Percent difference from the controls, calculated by reviewers, is included in parentheses.
* Significantly different from the control at p≤0.05
** Significantly different from the control at p≤0.01

4. Gross pathology: There were no treatment-related macroscopic findings in the maternal organs/tissues.

5. Cesarean section data: Fetal weights were decreased in all treated groups (↓8-18%, Table 5). The number of resorptions/doe was increased in all treated animals (0.9-1.3) compared to concurrent controls (0.5) and historical controls (0.3-1.2), and the increase was significant ($p \leq 0.05$) at 150 mg/kg/day (1.3). There was an increased resorptions/doe at 50 (0.9), 150 (1.3) and 450 (1.0) mg/kg/day vs 0.5 in the concurrent controls and 0.3-1.2 in the historical controls. This increase corresponded to an increase total number of resorptions and increases in both early and late resorptions. The effect on resorptions was not clearly dose-dependent, and were within (or slightly exceeded) the range of historical controls. Furthermore, post-implantation loss was comparable to concurrent controls. Consequently, the effect on resorptions was not considered to be adverse and was probably unrelated to treatment. There were no effects of treatment on the numbers of litters, live fetuses, resorptions (early, late, or complete litter), placental weight, sex ratio, or post-implantation loss.

Table 5. Cesarean section observations in the main study [a]

Observation	Dose (mg/kg bw/day)			
	0	50	150	450
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant	24	24	25	25
Pregnancy Rate (%)	96	96	100	100
# Nonpregnant	1	1	0	0
Maternal Wastage				
# Died	0	1	0	3
# Died Pregnant	0	1	0	0
# Died Nonpregnant	1	0	0	0
# Aborted	0	0	0	3
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	198	188	210	197
Corpora Lutea/Doe	8.3±1.87	8.2±1.75	8.4±1.66	9.0±1.79
Total # Implantations	172	162	186	181
(Implantations/Doe)	7.2±2.22	7.0±2.27	7.4±1.87	8.2±1.54
Total # Litters	24	22	25	22
Total # Live Fetuses	161	142	154	159
(Live Fetuses/Doe)	6.7±2.39	6.5±2.09	6.2±2.01	7.2±2.09
Total # Dead Fetuses	0	0	0	0
(Dead Fetuses/Doe)	0	0	0	0
Total # Resorptions	11	20	32	22
Early	9	14	23	12
Late	2	6	9	10
Resorptions/Doe	0.5±0.72	0.9±1.06	1.3±0.98* (↑160)	1.0±1.31
Early	0.4±0.71	0.6±0.99	0.9±0.81	0.5±0.86
Late	0.1±0.28	0.3±0.64	0.4±0.64	0.5±0.96
Complete Litter Resorption	0	1	0	0
Fetal Weight/litter (g) -				
Combined sexes	37.8±3.37	33.8±3.87** (↓11)	34.6±4.45* (↓8)	30.9±3.39** (↓18)
Male	37.9±3.90	33.1±4.06** (↓13)	34.5±4.43* (↓9)	31.1±2.80** (↓18)
Female	37.3±3.33	34.1±4.16* (↓9)	34.5±4.92 (↓8)	30.9±4.32** (↓17)
Placental weight (g)	4.5±0.6	4.4±0.6	4.5±0.6	4.2±0.5
Sex Ratio (% male)	54.0	51.4	45.5	45.3
Preimplantation Loss (%)	14.4±14.7	13.9±20.1	11.4±14.2	7.4±11.0
Postimplantation Loss (%)	8.0±13.3	14.1±22.3	18.3±15.9	12.6±16.9

a Data were obtained from pages 70-73 of MRID 46020302.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

B. DEVELOPMENTAL TOXICITY

1. External examination: Selected external findings are presented in Table 6a. Microglossia (small tongue) was observed (% fetuses affected [% litters affected]) at 450 mg/kg/day (0.6 [4.5]) compared to concurrent controls and historical controls (0 [0], each). Polyhydramnios (excess amniotic fluid) was observed at ≥150 mg/kg/day (0.6-1.3 [4.0-4.5]) compared to concurrent and historical controls (0 [0], each); however, this effect did not show dose-response relationship. Malrotated limb, paw hyperflexion, and necrobiotic placentae were increased in incidence at 450 mg/kg/day relative to the concurrent controls; however, the incidences were approximately within the historical control ranges. No other external malformation, variation, or unclassified was regarded as treatment-related, because the effect was unrelated to dose.

2. Visceral examination: Selected visceral findings are presented in Table 6b. The following visceral malformations were observed (% fetuses affected [% litters affected]) compared to concurrent and historical controls (0 [0], each) as treatment-related effects: dilated aorta at ≥150 mg/kg (0.6-1.3 [4.0-9.1]); and enlarged ventricular chamber in the heart and cor triloculare (3 chambered heart), and small thymus at 450 mg/kg/day (0.6 [4.5]). The total number of soft tissue malformations was increased ($p \leq 0.05$) in all treated animals (6.5-11 [36-59]) compared to concurrent controls (1.2 [8.3]) and historical controls (0.6-4.8 [4.2-27.3]); however, this effect may be unrelated to dose. One 450 mg/kg/day female fetus had a distended bladder and dilated ureter, and these findings were not observed in historical controls. The incidence of other visceral abnormalities (including absent kidney and ureter) were similar to the concurrent controls, within the range of historical controls, or were clearly unrelated to dose.

3. Skeletal examination: Treatment-related effects on the skeletal system were observed (Table 6c). Short 1st rib was observed (% fetuses affected [% litters affected]) compared to concurrent and historical controls (0 [0], each) in all treated groups (2.5-3.9 [18-20]; $p \leq 0.05$) with discontinuous cartilage, and with cartilage present at ≥150 mg/kg/day (0.6-1.3 [4.5-8.0]). A small cervical arch was also observed at ≥150 mg/kg/day (0.6-1.3 [4.5-8.0]). The total number of skeletal malformations were increased ($p \leq 0.05$ at 150 mg/kg/day only) in all treated groups (4.4-11 [23-40]) compared to concurrent controls (1.9 [13]) and historical controls (1.1-3.6 [8.3-25.0]). Except where noted otherwise, the incidence of the following skeletal variations were increased ($p \leq 0.05$) in all treated groups compared to concurrent and historical controls: (i) incomplete ossification of cervical centrum (37-45 [86-91]); (ii) unossified cervical centrum (2.1-7.8 [14-27]; NS at 50 mg/kg/day); (iii) extra ossification site between cervical centers (11-12 [36-56]); (iv) supernumerary thoracic vertebra (46-58 [84-91]; (v) misshapen sacral vertebra (1.9-5.6 [12-32]; NS at 150 mg/kg/day); (vi) supernumerary (13th) rib with carilage present (58-62 [92-95]) or absent (16-27 [64-73]); (vii) incomplete ossification of phalanx (0.7-2.5 [4.5-14]; NS at any dose); (viii) unossified talus at ≥150 mg/kg/day (1.3-4.4 [4-18]; NS at 150 mg/kg/day); and (ix) incomplete ossification of talus (2.8-9.7 [18-28]). Only the incomplete ossification of the cervical centrum and phalanx, and unossified talus were clearly dose-related findings. Similarly, the total number of skeletal variations were increased (NS) in all treated groups (92 [100], each) compared to the concurrent (71 [96]) and historical (17.4-78.1 [64.3-100.0]) controls. The only unclassified finding that may have been treatment-related is the cartilaginous parts of ribs displaced in all treated groups (1.3-7.1 [9.1-24]; $p \leq 0.05$ at 150 mg/kg/day only) compared to the concurrent (0 [0]) and historical (0-1.2 [0-8.3]) controls. No other skeletal finding was

considered to be treatment-related because incidences were similar to the concurrent controls, within the range of historical controls, or were clearly unrelated to dose.

Table 6a. Selected external findings (% fetuses affected [% litters affected]) [a]

Observations	Dose (mg/kg/day)				Historical Control Range
	0	50	150	450	
# Fetuses (litters) examined	161 (24)	142 (22)	153 (25)	159 (22)	1189 (180)
Malformations					
Microglossia	0 (0)	0 (0)	0 (0)	0.6 (4.5)	0 (0)
Malrotated limb	0 (0)	0 (0)	0 (0)	0.6 (4.5)	0-1.1 (0-4.2)
Total external malformations	1.2 (8.3)	2.1 (9.1)	2.0 (8.0)	0.6 (4.5)	0-1.1 (0-4.2)
Variations					
Paw hyperflexion	1.9 (8.3)	1.4 (9.1)	0 (0)	3.1 (18)	0-3.7 (0-17.4)
Total external variations	1.9 (8.3)	1.4 (9.1)	0 (0)	3.1 (18)	0.6-3.7 (4.0-21.4)
Unclassified					
Polyhydramnios	0 (0)	0 (0)	1.3 (4.0)	0.6 (4.5)	0 (0)
Placentae necrobiotic	0 (0)	0 (0)	0 (0)	0.6 (4.5)	0-0.7 (0-4.0)
Total external unclassified	0 (0)	0 (0)	1.3 (4.0)	1.3 (9.1)	0-0.7 (0-4.0)

a Data were obtained from pages 76-80 and 286-288 of MRID 46020302.

Table 6b. Selected visceral findings (% fetuses affected [% litters affected]) [a]

Observations	Dose (mg/kg/day)				Historical Controls
	0	50	150	450	
# Fetuses (litters) examined	161 (24)	142 (22)	154 (25)	159 (22)	1188 (180)
Malformations					
Dilated aorta	0 (0)	0 (0)	0.6 (4.0)	1.3 (9.1)	0 (0)
Heart, enlarged ventricular chamber	0 (0)	0 (0)	0 (0)	0.6 (4.5)	0 (0)
Cor triloculare	0 (0)	0 (0)	0 (0)	0.6 (4.5)	0 (0)
Small thymus	0 (0)	0 (0)	0 (0)	0.6 (4.5)	0 (0)
Microphthalmia	0 (0)	0 (0)	0.6 (4.0)	0.6 (4.5)	0-0.5 (0-4.2)
Hydrocephaly	0 (0)	0 (0)	0 (0)	0.6 (4.5)	0-0.6 (0-4.2)
Retroesophageal aortic arch	0 (0)	0 (0)	0 (0)	0.6 (4.5)	0-0.7 (0-4.3)
Total visceral malformations	1.2 (8.3)	11 (59)**	6.5 (36)*	7.5 (36)*	0.6-4.8 (4.2-27.3)
Variations					
Malpositioned carotid branch	16 (58)	7 (27)	5.2 (28)	3.8 (9.1)	0-18.3 (0-70.8)
Dilated renal pelvis	0 (0)	0 (0)	0 (0)	0.6 (4.5)	0-0.7 (0-4.5)
Dilated ureter	0 (0)	0 (0)	0 (0)	0.6 (4.5)	0 (0)
Total visceral variations	16 (58)	11 (45)	7.1 (40)	4.4 (14)	5.2-28.8 (26.1-86.4)
Unclassified					
Fluid-filled abdomen	5.0 (17)	4.9 (32)	1.3 (8.0)	3.8 (18)	0-2.7 (0-9.1)
Blood coagulum around urinary bladder	1.2 (4.2)	1.4 (9.1)	3.2 (16)	0 (0)	0-0.7 (0-4.5)
Distended urinary bladder	0 (0)	0 (0)	0 (0)	0.6 (4.5)	0 (0)
Total visceral unclassified	6.2 (21)	7.0 (41)	5.2 (28)	4.4 (23)	0-8.5 (0-27.3)

a Data were obtained from pages 82-93 and 289-292 of MRID 46020302.

Table 6c. Selected skeletal findings (% fetuses affected [% litters affected]) [a]

Observations	Dose (mg/kg/day)				Historical Controls
	0	50	150	450	
# Fetuses (litters) examined	161 (24)	142 (22)	154 (25)	159 (22)	1189 (180)
Malformations					
Short 1st rib, discontinuous cartilage	0 (0)	2.8 (18)*	3.9 (20)*	2.5 (18)*	0 (0)
cartilage present	0 (0)	0 (0)	1.3 (8.0)	0.6 (4.5)	0 (0)
Cervical arch, small	0 (0)	0 (0)	1.3 (8.0)	0.6 (4.5)	0 (0)
Lumbar hemivertebra	0 (0)	0 (0)	0 (0)	0.6 (4.5)	0-0.7 (0-4.2)
Total skeletal malformations	1.9 (13)	5.6 (27)	11 (40)*	4.4 (23)	1.1-3.6 (8.3-25.0)
Variations					
Hyoid, incomplete ossification	9.3 (50)	15 (50)	21 (72)	19 (77)	0-24.6 (0-76.0)
Cervical Centrum, incomplete ossification	5.0 (21)	37 (86)**	40 (88)**	45 (91)**	0-12.3 (0-45.8)
unossified	0 (0)	2.1 (14)	7.8 (24)*	3.8 (27)**	0-1.2 (0-8.3)
Extra ossification site between cervical centers	0 (0)	11 (36)**	12 (56)**	11 (55)**	0-0.7 (0-4.5)
Supernumerary thoracic vertebra	0 (0)	48 (91)**	58 (84)**	46 (86)**	0-0.7 (0-4.5)
Misshapen sacral vertebra	0 (0)	5.6 (32)**	1.9 (12)	4.4 (27)**	0-0.7 (0-4.5)
Supernumerary (13th) rib, cartilage present	1.9 (8.3)	62 (95)**	62 (92)**	58 (95)**	0-14.0 (0-50.0) [b]
cartilage absent	3.1 (17)	27 (73)**	16 (64)**	21 (73)**	0-14.0 (0-50.0) [b]
Phalanx, incomplete ossification with cartilage present	0 (0)	0.7 (4.5)	1.9 (8.0)	2.5 (14)	0 (0)
Talus, unossified	0 (0)	0 (0)	1.3 (4.0)	4.4 (18)*	0 (0)
incomplete ossification	0 (0)	2.8 (18)*	9.7 (28)**	5.0 (27)**	0-2.2 (0-8.7)
Total skeletal variations	71 (96)	92 (100)	92 (100)	92 (100)	17.4-78.1 (64.3-100.0)
Unclassified					
Cartilaginous parts of ribs displaced	0 (0)	1.4 (9.1)	7.1 (24)*	1.3 (9.1)	0-1.2 (0-8.3)
Total skeletal unclassified	42 (92)	12 (55)	23 (72)	18 (64)	0-38.4 (0-86.4)

a Data were obtained from pages 96-114 and 293-297 of MRID 46020302.
b The historical control data did not specify if cartilage was present or absent.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: It was concluded that the maternal LOAEL was 450 mg/kg/day, based on body weight gain, food consumption, and clinical signs. The maternal NOAEL was 150 mg/kg/day. The developmental LOAEL was 50 mg/kg/day, based on a delay or interference with the ossification of the axial skeleton and other embryo-/fetotoxic effects in all dose groups. The developmental NOAEL was not observed.

B. REVIEWER COMMENTS

1. **Maternal toxicity:** Despite an incidentally increased (↑521-859%) body weight gain during pretreatment, body weight gain dose-dependently decreased by 17-49% in all treated females during treatment (Days 7-28), and Days 0-29, uncorrected for gravid uterus weight (↓10-20%). Body weight gain decreased ($p \leq 0.01$) by 49% at 450 mg/kg/day during Days 7-28. After correcting body weight gain for gravid uterine weight, an effect of treatment was only apparent at 450 mg/kg/day. Body weight gain from Day 7 until termination (corrected for gravid uterine weight) was decreased by 43% (not statistically significant [NS]) at 450 mg/kg/day.

At 450 mg/kg/day, decreased ($p \leq 0.05$) food consumption was observed at Days 19-20, 20-21, and 24-28 (↓22-30%), contributing to a decrease (NS) of 11% on Days 7-28 and 6% on Days 0-29. Three does, which were sacrificed following spontaneous abortion, consumed less than 10 g/day during the last third of the pregnancy. These three does were sacrificed after spontaneous abortions shortly before term (GD 28 and 29), and had markedly decreased body weights. In addition, five 450 mg/kg/day animals did not defecate for 1 or more days beginning at GD 22.

The maternal LOAEL was 450 mg/kg/day based on decreased body weight gain and food consumption, increased incidences of abortion and lack of defecation. The maternal NOAEL was 150 mg/kg/day.

2. **Developmental toxicity**

a. Deaths/Resorptions: There were no effects of treatment on resorptions (early, late or complete litter) and there were no dead fetuses.

b. Altered Growth: The incidence of the following skeletal variations were increased in all treated groups compared to concurrent and historical controls: (i) incomplete ossification of cervical centrum, phalanx, and talus; (ii) unossified cervical centrum; (iii) unossified talus at ≥150 mg/kg/day only; and (iv) extra ossification site between cervical centers. The incomplete ossification of the cervical centrum and phalanx, and unossified talus were clearly dose-related findings. Fetal weights were decreased ($p \leq 0.05$, except in 150 mg/kg/day females) in all treated groups (↓8-18%; 30.9-34.6 g), but were within the range of historical controls (23.9-48.5 g). However, due to the treatment-related effect on growth, the effect on fetal weights was also considered to be treatment-related.

c. Developmental Variations: The incidence of supernumerary thoracic vertebra, misshapen sacral vertebra, and supernumerary (13^{th}) rib (with or without cartilage present) were increased in all treated groups. One 450 mg/kg/day female fetus had a distended bladder and dilated ureter, and these findings were not observed in historical controls.

d. Malformations: Increased incidences of the following visceral malformations were observed and were considered treatment-related: dilated aorta at ≥150 mg/kg; and small thymus, enlarged ventricular chamber in the heart, and cor triloculare at 450 mg/kg/day.

The following increased incidences over concurrent and historical controls were observed but were not clearly dose-dependent: (i) short 1st rib at ≥50 mg/kg/day (with discontinuous cartilage) and at ≥150 mg/kg/day (with cartilage present); (ii) a small cervical arch and polyhydramnios at ≥150 mg/kg/day; and (iii) microglossia at 450 mg/kg/day. Also, the total number of skeletal and soft tissue malformations were increased in all treated groups compared to concurrent and historical controls, but these effects were not clearly dose-dependent.

e. Unclassified abnormalities: An increased incidence of displaced cartilaginous parts of ribs were observed in all treated groups compared to the concurrent and historical controls.

The developmental toxicity LOAEL was 50 mg/kg/day based on decreased fetal weight and increased incidence of visceral malformations, and skeletal malformations, variations, and unclassified abnormalities. The developmental toxicity NOAEL was not observed.

This study is classified **acceptable/guideline** (OPPTS 870.3700b; OECD 414) and satisfies the requirements for a developmental study in the rabbit.

C. **STUDY DEFICIENCIES:** No study deficiency was noted.

DATA FOR ENTRY INTO ISIS

Developmental Study - rabbit (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	Endpoint(s)	Comments
123009	46020302	developmental	rabbit	GD 7-28	oral	gavage	50-450	0, 50, 150, 450	150	450	clinical signs, decr. BWG, decr. FC	maternal
123009	46020302	developmental	rabbit	GD 7-28	oral	gavage	50-450	0, 50, 150, 450	not observed	50	incr. visceral malformations; and skeletal malformations, variations, and unclassified abnormalities	developmental

Deleted