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DATA EVALUATION RECORD

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

P.C. CODE: 118601

DP BARCODE: D280761
SUBMISSION CODE: S579862

TEST MATERIAL (PURITY): DPX-W4189-165 [Chlorsulfuron] (98.2% a.i.)

SYNONYMS: Benzenesulfonamide, 2-chloro-N-[[(4-methoxy-6 methyl-1,3,5-triazin-2-yl)amino]carbonyl]; Benzoic acid, 2[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino] sulfonylmethyl]-methylester; 2-chloro-N-[(4-methoxy-4-methyl-1,3,5-triazin-2-yl)amino-carbonyl] benzenesulfonamide; INF-5384-38, INF-5384-52, DPX-W4189

CITATION: Alvarez, L. (1991). Teratogenicity Study of DPX-W4189-165 (Chlorsulfuron) in Rabbits. Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Delaware. Medical Research No. 8965-001; Haskell Laboratory Project ID 306-90. August 12, 1991. MRID 41983101. Unpublished.

SPONSOR: Agricultural Products, E. I. du Pont de Nemours and Company

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 41983101), chlorsulfuron (98.2% a.i.; Lot# 12-51, Drum 14/Batch # 12-51-88) was administered to 20 artificially-inseminated female Hra: (NZW)SPF rabbits/dose once daily *via* gavage at dose levels of 0, 25, 75, 200, and 400 mg/kg/day [original study] and at 400 and 1000 mg/kg/day [supplemental study] from day 7 to 19 of gestation.

Maternal toxicity was evident at the 1000 mg/kg/day dose level, as evidenced by the death of 8 of the 20 does and 6 abortions. One doe in the 200 mg/kg/day dose group and one doe in one of the 400 mg/kg/day groups also aborted. Additionally, there was a negative body-weight gain during the initial 3 days of dosing at 200 mg/kg/day and 400 mg/kg/day in the original study and a substantial decrease in body-weight gain in the supplemental study at 400 and 1000 mg/kg/day. Adjusted maternal body-weight gain was substantially lower than control at the 200 [original study], 400 [original and supplemental studies], and 1000 mg/kg/day [supplemental study] dose levels [days 0-29: 78%, 54%, 43%, and 43% of control, respectively; days 7-29: 24% of control, -24 grams, -25 grams, -67 grams, respectively].

There were no treatment-related effects on pregnancy rate, numbers of corpora lutea/doe, implantations/doe, live fetuses/doe, resorptions/doe, or the sex ratio. In the supplementary study, there was an apparent treatment-related increase in the incidence of enlarged gallbladders [0, 2, 4 at 0, 400, and 1000 mg/kg/day, respectively] and misshapened gallbladders [0, 0, 2 at 0, 400, and 1000 mg/kg/day, respectively] .

The maternal toxicity LOAEL is 200 mg/kg/day, based on decreased body-weight gain. The maternal toxicity NOAEL is 75 mg/kg/day.

Developmental toxicity was observed at the 400 mg/kg/day dose level, as evidenced by the slight increase in the incidence of visceral malformations [absent gallbladder, doubled aorta, ventricular septal defect] compared to the control. Additionally, the female fetuses at the 400 mg/kg/day dose level displayed a slightly lower body weight [90% of control] compared to the control, and the mean litter weight at this dose level was slightly decreased [≈90% of control]. The 1000 mg/kg/day dose level resulted in severe maternal toxicity and therefore, the developmental findings at this dose level [lack of effect] are not considered reliable.

The developmental toxicity LOAEL is 400 mg/kg/day, based on a slight increase in visceral malformations and decreased fetal body weight. The developmental toxicity NOAEL is 200 mg/kg/day.

The developmental toxicity study in the rabbit is classified Acceptable/Guideline, and it satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3*(b)*) in rabbits.

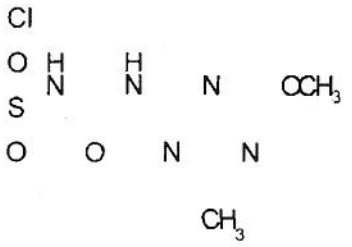
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

2

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Chlorsulfuron
Description: off-white solid
Lot/Batch #: Lot 12-51 Drum 14; Batch 12-51-88
Purity: 98.2% a.i.
CAS #: 64902-72-3
[Structure]:



1. Vehicle: aqueous solution of methyl cellulose
Description: 0.5% (w/v) aqueous solution of methyl cellulose (4000 centripoises)
Lot/Batch #: Lot # 883057
Purity: not provided
CAS #: 9004-67-5

1. Test animals: Species: rabbit **[female]**
Strain: Hra: (NZW)SPF
Age at mating: artificial insemination = 5-5.5 months old
Weight at mating: mean 3200-3300 g
Source: Hazleton Research Products, Inc. Denver, PA
Housing: individual stain-less steel, wire-mesh cages
Diet: Purina® Certified Rabbit Chow® #5325 ad libitum
Water: suburban ad libitum
Environmental conditions:
Temperature: 18-23 °C
Humidity: 40-60%
Air changes: not provided/hr
Photoperiod: 12 hrs dark/12 hrs light
Acclimation period: 20 days
Species: rabbit **[male]** - semen
Strain: Hra: (NZW)SPF
Age: 9 months to 4.5 years old [1.5-3.5 years supplemental study]
Weight at mating: 2999-4710 g [3612-5118 g [supplemental study]
Source: Hazleton Research Products, Inc. Denver, PA
Acclimation period: none; in-house colony [semen collected]

B. PROCEDURES AND STUDY DESIGN

1. In life dates - original start: 4/8/90; end: ≈5/11/90
supplemental start: 2/4 or 5/91; end: ≈3/8/91

2. Mating: Nineteen days [original study] and 28 days [supplemental study] prior to artificial insemination, the females were injected intravenously [i.v.] with 50 U.S.P. units of chorionic gonadotropin. On the day of artificial insemination, each female was injected i.v. with 100 U.S.P. units of chorionic gonadotropin and artificially inseminated with semen [diluted with 0.9% sodium chloride] collected that day from a male of proven fertility [same strain and supplier as females]. Semen from 8 males was used in the original study and semen from 5 males was used in the supplemental study.

1. Animal Assignment: The females were ranked, prior to insemination, by their body weights and assigned to the control and treatment groups by random sampling from strata established within the ranked list. The randomization resulted in a distribution in which the mean body weights for all groups were not statistically different. They were then divided into 5 daily insemination lots. The day of insemination was designated day 0 of gestation. The same procedure was used in the supplemental study with the exception of being divided into 3 daily insemination lots. Animals were assigned to dose groups as indicated in Table 1.

TABLE 1 Animal Assignment

Test Group	Dose (mg/kg/day)	Number of Females
original study		
Control	0	20
Low	25	20
Mid	75	20
Mid-high	200	20
High I	400	20
supplemental study		
Control	0	20
High I duplicate	400	20
High II	1000	20

1. Dose selection rationale: *Original study*: In a previous developmental toxicity study [HLO-0534-80, 1980, no MRID] in which dose levels of 10, 25, and 75mg/kg/day were administered to 16-17 artificially-inseminated females on days 6-19 of gestation, a significant decrease in fetal viability was observed at 75 mg/kg/day. In the pilot study [no MRID or study #], dose levels of 50, 75, 100, and 150 mg/kg/day were administered to 8 artificially-inseminated females. Although no overt maternal or fetal toxicity was evident, a slight decrease in maternal body-weight gain and food consumption was observed during the latter part of the dosing period at the highest dose level. Dose levels of 25-400 mg/kg/day were selected for the original study.

Since no toxicity was observed in the study, a second pilot study [no MRID or study #] was performed in 3 artificially-inseminated rabbits/dose [daily doses of 1000, 1500, and 2000 mg/kg/day; apparently during gestation days 6-19]. All rabbits died at the 2 highest dose levels, and one died at the low-dose level. There were also reductions in maternal body-weight changes and food consumption. Dose levels of 400 and 1000 mg/kg/day were chosen for the supplemental study. Neither pilot study was submitted.

2 Dosage preparation and analysis: Test substance formulations were prepared on the morning of each dosing day by suspending appropriate amounts of DPX-W4189-165 in 0.5% solution of methyl cellulose [prepared every two weeks]. Formulations of 25, 75, 200, and 400 mg/ml, [corrected for purity] were prepared during the original study and concentrations of 100 and 250 mg/mL [corrected for purity] were prepared for the supplemental study. Stability of the formulations was evaluated on the fourth day of dosing by retaining a sample at room temperature for 5 hours before analysis. Concentration and uniformity of the formulation were evaluated by analysis of samples taken on the 4th, 10th, and 17th days of dosing [original study] and on samples taken on the 1st, 8th, and 15th days of dosing [supplemental study].

Results - Homogeneity Analysis: The test material was shown to be suspended homogeneously [104-105%].

Stability Analysis: The percent of nominal concentrations ranged from 91% to 101%, indicating that the test material was stable in the vehicle up to 5 hours.

Concentration Analysis: The percent of nominal concentrations were 87%-106% [25 mg/kg/day]; 89%-110% [75 mg/kg/day]; 93%-104% [200 mg/kg/day], 95%-110% [400 mg/kg/day in the original study and 97%-105% [400 mg/kg/day], 92%-103% [1000 mg/kg/day]

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

3. Dosage administration: All doses were administered once daily *via* gavage on gestation days 7 through 19, in a volume of 1 mL/kg of body weight/day [original] or 4 mL/kg/day

[supplemental]. Dosing was based on the body weight obtained on the day of dosing.

C. OBSERVATIONS

1. Maternal observations and evaluations - For both studies, body weights and clinical signs were recorded on the day after arrival and each week during quarantine. Observations for morbidity and mortality were made daily. Individual clinical signs were recorded each morning on gestation days 0-29 and each afternoon during the dosing period. Body weight were recorded on days 0, 7-20, 24, and 29 of gestation, and food consumption was monitored during quarantine and on gestation days 0-29. Dams were sacrificed [injection of T-61® euthanasia solution/V-Pento® or Bio-Tal®] on day 29 of gestation and examined for gross anatomical abnormalities. The gravid uterus was removed, weighed, and opened, and live and dead fetuses and resorptions and their relative positions were recorded. The empty uterine weight was then recorded. The uterus of apparently non-pregnant rabbits was opened and stained with ammonium sulfide to detect very early resorptions [used to determine the incidence of pregnancy and the number of females with total resorptions only]. Corpora lutea in females with live fetuses were counted and the number was recorded for each ovary.

Females dying prior to scheduled sacrifice were examined for gross anatomical alterations and pregnancy status [presence or absence of implanations]. When possible, implantations were counted and their contents described and classified. Females that aborted were maintained until their scheduled sacrifice and examined for gross anatomical alterations and pregnancy status. Fetuses from these does were examined for gross anatomical alterations and discarded.

2. Fetal Evaluations - Live fetuses of females surviving to day 29 of gestation were weighed, examined for external alterations, and sacrificed [i.p. injection of V-Pento®]. The fetuses were then examined for visceral alterations. The sex of each fetus was determined by examination of the internal reproductive organs. The brain was examined by making a transverse section between the parietal and frontal bones of the unfixed fetal head. The eyelids of each fetus were removed, and the eyes were examined for alterations. All fetuses were fixed in 70% ethanol, macerated in aqueous potassium hydroxide solution, stained with alizarin red S, and examined for skeletal alterations.

D. DATA ANALYSIS

1. Statistical analyses: The litter was considered the experimental unit for statistical analyses.

Parameter	test for linear trend	pair-wise test between groups
incidence of pregnancy clinical observations maternal mortality does with total resorptions	Cochran-Armitage	Fischer's exact

4

Parameter	test for linear trend	pair-wise test between groups
maternal body weight maternal body-weight change maternal feed intake	Linear combination of dose ranks from ANOVA	Dunnett's when one-way ANOVA was significant
live fetuses dead fetuses resorptions nidations corpora lutea fetal body weight incidence of fetal alterations	Jonckheere's	Mann-Whitney U

When more than 75% ties occurred in reproductive and fetal parameters, the Cochran-Armitage test replaced Jonckheere's test to detect trend and the Fischer's exact test was applied instead of the Mann-Whitney U test to detect a significant difference between groups. When Bartlett's test for homogeneity of variances was significant, analyses of maternal body weights and feed consumption were conducted on the ranks of the original values.

2. Indices: No indices were provided in the study reports. The following indices were calculated by the reviewer: pre- and post-implantation losses using the individual data in Appendix I [pages 161-168].

$$\text{pre-implantation loss} = \frac{\#\text{ corpora lutea} - \#\text{ nidations}}{\#\text{ corpora lutea}}$$

$$\text{post-implantation loss} = \frac{\#\text{ nidations} - \#\text{ live fetuses}}{\#\text{ nidations}}$$

3. Historical control data: Historical control data were referred to but were not provided in either report.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical observations: No treatment-related deaths occurred in the original study. In the supplemental study, six of the high-dose [1000 mg/kg/day] does were found dead on gestation days 17 [two], 19, 20, 23, and 25. Another high-dose doe was sacrificed *in extremis* on gestation day 19. Four does [one in each control group, one at 75, and one at 1000 mg/kg/day] were injured during dosing, and one of these controls was sacrificed *in extremis*. No treatment-related clinical signs were observed in the original study. In the supplemental study, the high-dose does displayed a higher incidence of the presence of red-

stained tail, red discharge or stain on cageboard and a reduction in size or absence of fecal pellets during the postdosing period. The staining was associated with the five abortions, but it was also observed in other rabbits prior to death.

2. Body Weight/Body-Weight Gain - **Original study**: There was an initial [days 7-10], dose-related, body-weight loss [-5.3 grams and -19.8 grams] at the two highest dose levels [Table 2a], and a dose-related decrease in body-weight gain during the same time period at the two lower dose levels [69% and 31% of control]. Body-weight gains were decreased at the three highest dose levels during the day 13-16 interval also [90%, 73%, and 53% of control at 75, 200, and 400 mg/kg/day, respectively]. A decrease in body-weight gain over the entire dosing period [days 7-20] was displayed at the highest dose level [73% of control]. **Supplemental study**: At 1000 mg/kg/day, body-weight gains [Table 2b] were decreased at all intervals during the dosing period [days 7-10 (33% of control); days 10-13 (22% of control); 7-20 (12% of control)]. At 400 mg/kg/day, body-weight gain was decreased during the initial [days 7-10] dosing period [59% of control] and again during the day 13-16 interval [67% of control]. Body weights were comparable among the groups, however, throughout each study [Table 2c]. Gravid uterine weights were not provided, although adjusted body weights [maternal body weight minus the products of conception] were reported. Adjusted maternal body-weight gain was substantially lower than control at the 200 [original study], 400 [original and supplemental studies], and 1000 mg/kg/day [supplemental study] dose levels [days 0-29: 78%, 54%, 43%, and 43% of control, respectively; days 7-29: 24% of control, -24 grams, -25 grams, -67 grams, respectively].

TABLE 2a Maternal Body Weight Gain (g)[a] **Original Study**

	Dose in mg/kg/day (# of Does)				
Interval	0	25	75	200	400
N	16	17	15	16	16
Pretreatment:					
Days 0-7	90.4±53.6	82.4±48.3	106.4±52.0	88.7±58.1	91.5±38.6
Treatment:					
Days 7-10	13.4±38.2	9.2±31.5 [69]	4.1±41.1 [31]	-5.3±65.2	-19.8±36.6
Days 10-13	67.8±81.5	74.8±62.9	81.7±28.0	102.5±83.9	87.3±55.7
Days 13-16	92.6±91.1	97.4±63.1	83.2±45.1 [90]	67.7±71.8 [73]	48.7±68.0 [53]
Days 16-20	12.3±46.8	-9.0±53.6	8.7±38.7	4.7±66.6	19.3±39.5
Days 7-20	186.0±48.5	172.3±71.5	177.6±70.0	169.6±74.3 [94]	135.7±75.3 [73]
Posttreatment:					
Days 20-29	160.3±75.0	134.6±78.0	178.7±69.3	194.9±55.5	180.9±50.6
Days 0-29√	125±110	124±107	169±75	97±80 [78]	67±100 [54]
Days 7-29√	34±88	42±96	62±78	8±63 [24]	-24±94

a Data extracted from Appendix C, pages 92-97 and Appendix E, pages 108-112 of the report;
[% of control]; √maternal adjusted body weight

TABLE 2b Maternal Body Weight Gain (g)[a] **Supplemental Study**

Interval	Dose in mg/kg/day (# of Does)		
	0	400	1000
N=	10	13	4
Pretreatment:			
Days 0-7	111.6±44.2	110.6±102.0	152.5±14.1
Treatment:			
Days 7-10	90.0±62.2	53.4±75.5 [59]	29.6±31.0 [33]
Days 10-13	100.7±140.9	109.4±76.2	22.4±59.3 [22]
Days 13-16	68.8±155.2	46.2±84.5 [67]	45.3±113.5 [66]
Days 16-20	-35.1±76.7	9.7±45.7	-71.1±64.8
Days 7-20	224.5±82.0	218.8±107.3	26.2±91.2 [12]
Posttreatment:			
Days 20-29	116.9±49.9	146.3±83.4	165.9±94.5
Days 0-29√	202±94	86±108 [43]	86±62 [43]
Days 7-29√	90±94	-25±75	-67±55

a Data extracted from Appendix C, pages 93, 98, 99 and Appendix E, pages 108-115 of the report; [% of control]; √maternal adjusted body weight

TABLE 2c Maternal Body Weight (g)[a] - **Original and Supplemental Studies**

Day	Dose in mg/kg/day (# of Does)					
	0	25	75	200	400	1000
Original Study						
N=	16	17	15	16	16	-
Day 0	3217±193	3265±200	3243±170	3216±208	3232±180	
Day 7	3307±211	3348±188	3350±179	3305±206	3323±175	
Day 13	3388±242	3431±198	3435±186	3402±241	3391±146	
Day 20	3493±225	3520±160	3527±188	3475±249	3459±146	
Day 29	3653±248	3654±194	3706±177	3670±260	3640±142	
Day 29√	3341±253	3389±192	3412±162	3313±207	3299±176	
Supplemental Study						
N=	10				13	4
Day 0	3324±143	-	-	-	3274±264	3297±109
Day 7	3435±153				3384±251	3449±115
Day 13	3626±179				3547±289	3501±109
Day 20	3660±184				3603±327	3475±176
Day 29	3777±191				3749±328	3641±131
Day 29√	3526±197				3360±284 [95]	3382±142 [96]

a Data extracted from Appendix C, pages 100, 101, 103-107 and Appendix E, pages 108-115 of the report; [% of control]; √adjusted maternal body weight

3. Food Consumption - Food consumption was comparable among the groups in both studies, although a slight decrease in food consumption was observed during the dosing period of days 13-16 in both studies [original at 400 mg/kg/day (93% of control) and supplemental at 1000 mg/kg/day (92% of control)].

4. Gross Pathology - No treatment-related gross pathological findings were reported. However, more numerous entries were noted in the supplemental study report than in the original study report. The most frequent finding was staining of the hair and alopecia [Table 3]. In the supplemental study, there was an apparent dose-related increase in enlarged gall bladders and misshapen gall bladders.

TABLE 3. Gross Pathological Findings - **Original and Supplemental Studies**

	Dose in mg/kg/day (# of Does)					
Finding	0	25	75	200	400	1000
original study						
N=	20	20	20	20	20	
Hair						
staining	2	0	1	3	1	
alopecia	3	3	2	3	1	
supplemental study						
N=	20				20	20
Hair						
staining	3				2	7
alopecia	5				7	5
Gall bladder						
enlarged	0				2	4
misshapen	0				0	2

Data extracted from Appendix II, pages 148-160

5. Cesarean Section Data - **Original Study**: Pregnancy rate was comparable among the groups [Table 3]. One doe at 200 mg/kg/day [day 24] and one doe at 400 mg/kg/day [day 24] aborted. There were comparable numbers of corpora lutea, implantations, live fetuses, and resorptions among the groups [total number and per litter basis]. There was one dead fetus [control]. Pre- and post-implantation losses were comparable among the groups. Two control, one 25 mg/kg/day, two 75 mg/kg/day, and one 400 mg/kg/day does had 100% resorptions. The sex ratios were comparable among the groups. **Supplemental Study**: The control group had a low pregnancy rate [65%] compared to the treated groups [70% at 400 mg/kg/day and 85% at 1000 mg/kg/day]. Because six of the high-dose does aborted, and there were 8 deaths (one of these aborted also) at this dose level, there were only 4 litters available for assessment. Additionally, there were only 10 control litters and 13 litters at 400 mg/kg/day. Two control litters, 1 400 mg/kg/day litter, and 1 1000 mg/kg/day litter had 100% resorptions. There were comparable numbers of corpora lutea [on a litter basis] among the three groups, a comparable number of resorption, and there were no dead fetuses. The 400 mg/kg/day group displayed a significant increase 7.5±1.9] in the number of implantations/litter compared to the control [5.5±2.8] and an increased number of live fetuses/litter [7.2±2.0] compared to the control [5.3±2.7]. The sex ratio was comparable between the control [45% male] and 400 mg/kg/day [47% male] groups. The sex ratio at the 1000 mg/kg/day dose level was 65% males [4 litters]. Pre- and post-implantation losses were low in the 400 mg/kg/day dose group and high in both the control and 1000 mg/kg/day groups.

TABLE 3 Cesarean Section Observations[a] - **Original and Supplemental Studies**

Observation	Dose (mg/kg/day)						
	0	25	75	200	400	400	1000
# Animals Assigned (inseminated)	20	20	20	20	20	20	20
# Animals Pregnant Pregnancy Rate (%)	19 (95) [13 (65)]'	18 (90)	18 (90)	17 (85)	18 (90)	14 (70)	17 (85)
# Nonpregnant	1 [7]	2	2	3	2	6	3
Maternal Wastage							
# Died	1 [1]	0	1	0	0	0	8*
# Died Pregnant	[1]						7
# Died Nonpregnant	1		1				1
# Aborted	0 [0]	0	0	1	1	0	6*
# Premature Delivery	-	-	-	-	-	-	-
Total # Corpora Lutea♪	142 [108]	135	143	140	158	115	37
Corpora Lutea/Dam	8.9±1.5 [10.8±4.5]	7.9±2.1	9.5±2.1	8.8±2.9	9.9±2.5	8.8±2.5	9.3±2.6
Total # Implantations♪	88 [57]	84	85	111	105	97	19
Implantations/Dam	5.5±2.8 [4.8±2.9]	4.9±2.5	5.7±2.3	6.9±3.1	6.6±2.8	7.5±1.9*	4.8±2.2
Total # Litters	16 [10]	17	15	16	16	13	4
Total # Live Fetuses♪	84 [44]	76	80	103	101	93	17
Live Fetuses/Dam	5.3±2.7 [4.4±2.9]	4.5±2.5	5.3±2.5	6.4±3.3	6.3±2.8	7.2±2.0	4.3±1.9
Total # Dead Fetuses	1 [0]	0	0	0	0	0	0
Dead Fetuses/Dam	0.1±0.3	0	0	0	0	0	0
Total # Resorptions♪	3 [4]	8	5	8	4	4	2
Early♪	3 [3]	7	5	7	3	3	0
Late♪	0 [1]	1	0	1	1	1	2
Resorptions/Dam	0.2±0.4 [0.4±0.7]	0.5±0.6	0.3±0.6	0.5±0.7	0.3±0.5	0.3±0.5	0.5±0.6
Early	0.2±0.4 [0.3±0.7]	0.4±0.6	0.3±0.6	0.4±0.7	0.2±0.4	0.2±0.4	0
Late	0 [0.1±0.3]	0.1±0.2	0	0.1±0.3	0.1±0.3	0.1±0.3	0.5±0.6
Litters w/ Total Resorptions♪ [b]	2 [2]	1	2	0	1	1	1
Mean Fetal Weight (g)√	49.4±6.2 [48.5±7.0]	49.7±6.7	45.9±4.3	46.9±6.4	45.2±5.6 (91)	43.5±3.0 (90)	46.1±7.3
Males✖	46.5 [47.1]	48.7	46.9	45.5	45.6 (98)	44.7 (95)	46.8
Females✖	49.2 [46.9]	48.7	44.5	46.6	44.0* (90)	42.2 (90)	43.7
Sex Ratio (% Male)	55 [45]	51	53	48	54	47	65

Observation	Dose (mg/kg/day)						
	0	25	75	200	400	400	1000
Preimplantation Loss (%)♪♬	38±31 [53±25]	39±26	38±27	21±22	33±25	13±16	45±32
Postimplantation Loss (%)♬	3.4±8.0 [9.6±21.2]	10.5±15.8	8.8±16.2	9.6±17.9	5.6±13.0	4.1±6.5	9.4±12.0

aData extracted from Tables 4A and 4B, and Appendix I, pages 161-168; [a] supplemental study control; [b] total resorption determined by uterine stain [data omitted from calculations] or grossly visible implants [data omitted from calculations]; ♪ calculated by reviewer; ♬ calculated by reviewer using individual data from Appendix I, pages 161-168; ✖ individual fetal body weights were provided but sex not identified; therefore s.d. could not be calculated by reviewer [✖NOTE: In several cases, the total mean exceeds both individual means], which may be due to the following: √ For each litter, the maximum stunted weight was calculated by subtracting the lightest weight from the total weight, dividing by the remaining number of fetuses and multiplying by 0.666. A fetus weighing the same or less than the maximum stunted weight would be considered stunted and its weight omitted when the mean litter weight was calculated. The procedure was repeated until all remaining weights were greater than the maximum stunted weight]; * $p<0.05$; (% of control)

B. DEVELOPMENTAL TOXICITY Fetal body weights on a per litter basis [Table 3] were lower than the control at 400 mg/kg/day in both studies [≈90% of control], and the decrease can be attributed to the female fetuses. However, in both cases, the size of the litter was larger than the control, which might account for the lower body weight [original study 6.3 *vs* 5.3; supplemental study 7.2 *vs* 4.4]. Since the individual fetal body-weight data were not identified as to the sex of the fetus, a further assessment was not possible. Additionally, the procedure used by the author of removing "stunted" fetuses from the calculations further complicates assessment, since there was no discussion of how many fetuses were stunted and in which dose groups they occurred.

There were no malformations in the control in the original study [Tables 4a-4d], but there were two fetuses with malformations in the supplemental control group [both with hemivertebra]. In the original study, two 25 mg/kg/day [fused rib, hydrocephaly], one 75 mg/kg/day [hydrocephaly], one 200 mg/kg/day [hemivertebra], and five 400 mg/kg/day [ventricular septal defect of the heart, doubled aorta and hydrocephaly, absent gall bladder, and two with hemivertebra] fetuses displayed malformations. No malformations were observed at 400 mg/kg/day and 1000 mg/kg/day in the supplemental study. Small gallbladders were found in each of the treatment groups in the original study, with the highest number being observed at the high-dose level [4 fetuses in two litters]. One 400 mg/kg/day fetus was missing a gallbladder, which is considered a malformation. At necropsy, two does at the 400 mg/kg/day dose level [supplemental study] and four does at 1000 mg/kg/day [supplemental study] displayed enlarged gallbladders, and two does at1000 mg/kg/day [supplemental study] displayed misshapen gallbladders. Skeletal variations included partially and unossified sternebrae, fused sternebrae, partially ossified vertebra centrum, bent hyoid, and partially ossified skull. Only unossified sternebrae showed a slight increase compared to the concurrent control [0, 0, 1, 2, 3] in the original study. There were no external malformations or variations reported in either study.

1. External Examination - There were no reported external malformations or variations in either study.

2. Visceral Examination - Visceral malformations were observed in the original study only and only at the high-dose [400 mg/kg/day] level. Three fetuses in three litters were affected. One fetus displayed a double aorta, one a ventricular septal defect, and one was absent a gall bladder. The overall incidence of visceral variations was comparable among the groups in both studies. The variations were categorized by the study author as developmental variations or variations due to retarded development.

3. Skeletal Examination - Skeletal variations included partially and unossified sternebrae, fused sternebrae, partially ossified vertebra centrum, bent hyoid, and partially ossified skull. Only unossified sternebrae showed a slight increase compared to the concurrent control [0, 0, 1, 2, 3]. Although there were no malformations observed at the 1000 mg/kg/day dose level in the supplemental study, there were only 17 live fetuses in 4 litters. However, this dose level is the limit dose, and there was severe maternal toxicity at this dose level. A dose level between 400 mg/kg/day and 1000 mg/kg/day might have been more informative as to whether these findings are treatment-related.

TABLE 4a. External Examinations[a] - **Original and Supplemental Studies**

Observations[b]	Dose (mg/kg/day)							
	0	0	25	75	200	400	400	1000
#Fetuses (litters) examined	84 (16)	44 (10)	76 (17)	80 (15)	103 (16)	101 (16)	93 (13)	17 (4)
#fetuses (litters) affected	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

a Data extracted from Tables 5a and 5b, pages 32-35 of MRID 41983101
b Some observations may be grouped together
c Fetal (litter) incidence

TABLE 4b. Visceral Examinations[a] - **Original and Supplemental Studies**

Observations[b]	Dose (mg/kg day)							
	0	0	25	75	200	400	400	1000
#Fetuses (litters) examined	84 (16)	44 (10)	76 (17)	80 (15)	103 (16)	101 (16)	93 (13)	17 (4)
malformations #fetuses (litters) affected mean % affected	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (3) 2.2+4.8	0 (0)	0 (0)
Finding - malformations *gallbladder absent* *doubled aorta* *ventricular septal defect*	 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 (0) 0 (0) 0 (0)	 1 (1) 1 (1) 1 (1)	 0 (0) 0 (0) 0 (0)	 0 (0) 0 (0) 0 (0)
Finding - variations gallbladder "Y" shaped, lobes normal[d] gallbladder small[d] kidney small papilla[d]	 0 (0) 0 (0) 1 (1)	 1 (1) 0 (0) 1 (1)	 0 (0) 2 (2) 1 (1)	 0 (0) 2 (2) 0 (0)	 0 (0) 1 (1) 1 (1)	 0 (0) 4 (2) 1 (1)	 0 (0) 0 (0) 0 (0)	 0 (0) 0 (0) 0 (0)

a Data extracted from Tables 5a, 5b, 6a, and 6b [pages 32-43] of MRID 41983101
b Some observations may be grouped together
c Fetal (litter) incidence
d tabulated by reviewer

TABLE 4c.. Head Examinations[a] - **Original and Supplemental Studies**

Observations	Dose (mg/kg/day)							
	0	0	25	75	200	400	400	1000
#Fetuses (litters) examined	84 (16)	44 (10)	76 (17)	80 (15)	103 (16)	101 (16)	93 (13)	17 (4)
Malformations hydrocephaly #fetuses (litters) affected mean % affected	0 (0) 0	0 (0) 0	1 (1) 1.5+6.1	1 (1) 1.3+5.2	0 (0) 0	1 (1) 0.6-2.5	0 (0) 0	0 (0) 0

Data extracted from Tables 5a and 5b, pages 32-35 of MRID 41983101

TABLE 4d. Skeletal Examinations[a] - **Original and Supplemental Studies**

Observations [b]	Dose (mg/kg/day)							
	0	0	25	75	200	400	400	1000
#Fetuses(litters) examined	84 (16)	44 (10)	76 (17)	80 (15)	103 (16)	101 (16)	93 (13)	17 (4)
Finding malformations								
#Fetuses(litters) affected	0 (0)	2 (2)	1 (1)	0 (0)	1 (1)	2 (2)	0 (0)	0 (0)
mean % affected/litter	0	4.8±11.0	2.0±8.1	0	1.6±6.3	1.6±4.4	0	0
rib - fused	-	-	X	-	-	-	-	-
hemivertebra		X	-	-	X	X	-	-
Finding [c] variations								
partially ossified sternebra (#6) [d]	13 (6)	5 (1)	5 (4)	10 (7)	7 (4)	14 (5)	12 (5)	4 (2)
unossified sternebra [d]	0	0	0	1 (1)	2 (1)	3 (3)	2 (2)	0
fused sternebra [d]	0	0	0	2 (2)	0	0	1 (10	0
partially ossified vertebra centrum [d]	1 (1)	1 (1)	0	9 (9)	0	0	0	0
hyoid bent [d]	0 (0)	0 (0)	3 (3)	2 (1)	2 (2)	0	0	0
partially ossified skull [d]	0 (0)	0 (0)	1 (1)	0 (0)	1 (1)	0	0	0

a Data extracted from Tables 5A, 5B, 6A, 6B [pages 32-43] and Appendix K [pages 174-195] of report
b Some observations may be grouped together
c Fetal (litter) incidence
d tabulated by reviewer

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: Results of both studies indicated than maternal toxicity was evident at dose levels of 400 mg/kg/day and 1000 mg/kg/day. Fetal toxicity was not observed. The author concluded that the NOAEL for maternal toxicity was 200 mg/kg/day and the maternal toxicity LOAEL was 400 mg/kg/day. The developmental toxicity NOAEL was set at 1000 mg/kg/day.

B. REVIEWER'S DISCUSSION:

1. MATERNAL TOXICITY: In the supplemental study, maternal toxicity was evident at the 1000 mg/kg/day dose level, as evidenced by the death of 8 of the 20 does and 6 abortions. Additionally, there was a negative body-weight gain during the initial 3 days of dosing at two highest dose levels [200 mg/kg/day and 400 mg/kg/day] in the original study and a substantial decrease in body-weight gain in the supplemental study at both dose levels [400 mg/kg/day and 1000 mg/kg/day]. Adjusted maternal body weight gain was substantially lower than control at the 400 [both studies] and 1000 [supplemental study] mg/kg/day dose levels; in fact, there was a negative body-weight gain at these dose levels during the days 7-29 interval. The adjusted body-weight gain at 200 mg/kg/day in the original study was substantially lower [24% of the control during days 7-29 interval] than the control also. The maternal toxicity NOAEL is 75 mg/kg/day, based on decreased body-weight gain at the maternal toxicity LOAEL of 200 mg/kg/day.

2. DEVELOPMENTAL TOXICITY: Developmental toxicity was observed at the 400 mg/kg/day dose level, as evidenced by the decrease [90% of control] in female fetal body weight [both studies] and the slight increase in the incidence of visceral malformations [original study] compared to the control. The 1000 mg/kg/day dose level resulted in severe maternal toxicity and therefore, the findings at this dose level [lack of effect] are not considered reliable. The developmental toxicity NOAEL is 200 mg/kg/day, based on decreased female fetal body weight and a slight increase in visceral malformations at the developmental toxicity LOAEL of 400 mg/kg/day.

a. Deaths/Resorptions: There was only one dead fetus, which occurred in one of the control groups. Resorptions were comparable among the groups, and there were no differences in either pre- or post-implantations losses among the groups.
b. Altered Growth: There was a slight decrease in fetal body weight, mainly in the females, at the 400 mg/kg/day dose level in both the original and supplemental studies. The incidence of variations due to retarded development were comparable among the groups [18%, 12%, 16%, 14%, and 19% at the 0, 25, 75, 200, and 400 mg/kg/day dose levels, respectively].
c. Developmental Variations: There was a slight increase in the incidence of small gallbladders at the 400 mg/kg/day dose level.
d. Malformations: Visceral malformations [absent gallbladder, doubled aorta, and a ventricular septal defect] were observed only at the 400 mg/kg/day dose level in the original study. Skeletal malformations were observed at comparable rates among the groups. Hydrocephaly was observed [one fetus in each] at the 25, 75, and 400 mg/kg/day dose levels.

C. STUDY DEFICIENCIES There were fewer than 20 does/group at necropsy at all dose levels in both studies. The selection of 1000 mg/kg/day as the high-dose level is considered a poor decision, based on the fact that death was observed at this dose level in the second pilot study [one doe out of three] and, therefore, 7 does could have been expected to die in the definitive study and eight does did die.