US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

UNDATED

ZIRAM

Study Type: §83-3[b]; Developmental Toxicity of Ziram in Rabbits

Work Assignment No. 2-01-54 (MRID 00161316)

Prepared for

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SUPPLEMENTAL DATA EVALUATION RECORD FOR HED DOC.# 014277; MRID 00161316

STUDY TYPE: Developmental Toxicity in Rabbits
OPPTS Number: 870.3700 OPP Guideline Number: §83-3b

DP BARCODE: D215388 SUBMISSION CODE: S487026
P.C. CODE: 034805 TOX. CHEM. NO.: 931

TEST MATERIAL (PURITY): Ziram technical (98% a.i.)

SYNONYMS: Zinc bis(dimethyldithiocarbamate); (T-4)- bis(dimethyldithiocarbamato-S,S') zinc

CITATION(s): Barker, L., (1986). Ziram: Oral (Gavage) Teratology Study in the Rabbit. Hazleton Laboratories Europe Ltd., Harrogate, North Yorkshire, England, Laboratory Report No. 4913-508/2, July, 1986. MRID 00161316. Unpublished.

SPONSOR: Ziram Task Force, c/o Prochimie International, Inc., 488 Madison Avenue, New York, NY

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 00161316), ziram technical (98% a.i.) in 1% aqueous methyl cellulose was administered by gavage to pregnant New Zealand White rabbits (16/dose) at concentrations of 0, 3, 7.5, or 15 mg/kg/day on GDs 7 through 19. Does were sacrificed on GD 28.

One high-dose doe died on GD 23 and one mid-dose doe died on GD 13. Additionally, one control doe and one mid-dose doe were sacrificed *in extremis* on GDs 14 and 15, respectively; clinical signs observed prior to death in these two animals included weight loss, anorexia, and wheezing. These deaths were not considered to be the result of treatment due to the lack of a dose-response relationship. No other premature deaths occurred and no treatment-related clinical signs of toxicity were observed at any dose level.

At 15 mg/kg, decreased body weights were observed over GDs 0-28 (↓3-17%, $p \leq 0.01$ on GD 10 only). Additionally, for the overall treatment interval (GDs 7-19) and overall study interval (GDs 0-28) body weight gain, as calculated by the reviewers, were reduced as compared to the control (treatment, ↓81%; study, ↓30%, not analyzed for statistical significance). Decreases ($p \leq 0.01$) in absolute (g/animal/day) food consumption were observed beginning at the GDs 7-10 interval

(↓19%) and continuing throughout the GDs 13-16 interval (↓44-49%); decreased consumption was also observed for the GDs 16-19 interval (↓24%, not statistically significant [NS]). Food consumption was reduced for the overall treatment interval (↓34%, GDs 7-19) and for the overall study interval (↓18%, GDs 0-28).

At 7.5 mg/kg, decreased body weight gain was observed over GDs 7-19 (↓30%) and GDs 0-28 (↓19%). No other treatment-related maternal effects were noted at the mid-dose level.

No treatment-related findings were observed at gross necropsy of maternal animals.

The number of implantations/doe and percent male were similar between control and treated groups.

The maternal LOAEL is 7.5 mg/kg/day, based on decreased body weight gain. The maternal NOAEL is 3 mg/kg/day.

Reduced atrium/atria, a minor defect (variation), was observed at the mid- (fetal 2.9%; litter, 14.3%) and high-dose (fetal, 2.8%; litter, 20.0%) levels vs controls (fetal, 0.8%; litter, 6.7%). The OPP's Hazard Identification Review Committee (HIARC) did not consider this as an effect since there was no dose response, there were no statistically significant differences in the incidences at any dose, and the data on this parameter, which is a highly subjective observation, showed a wide-spread variation in the size of the atria (enlarged and reduced) among the control and the treatment groups.

At the high-dose level, increases (NS) as compared to the control were observed in the total number of resorptions/doe (↑88%) and the percent postimplantation loss (↑97%). Additionally, reductions (NS) in the number of live fetuses/doe (↓15%) were noted. Upon skeletal examination, absence of the interparietal bone, a major defect (malformation), was observed at the high-dose level only (fetal, 1.9%; litter, 13.3%) vs 0 controls; since this finding was only observed at the high-dose level and without the %fetal and %litter incidence ranges in the historical data, this malformation was considered equivocally treatment-related.

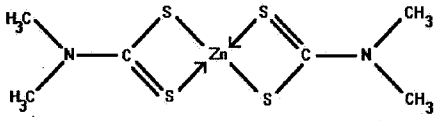
The developmental LOAEL is 15 mg/kg/day, based upon increased resorptions and post-implantation loss. The developmental NOAEL is 7.5 mg/kg/day.

This developmental toxicity study is classified **acceptable/guideline (§83-3[b])** and <u>does satisfy</u> the guideline requirement for a developmental toxicity study in the rabbit; it would be helpful if historical control data (% fetal and % litter incidences) were provided for reduced atrium/atria and absence of the interparietal bone.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Ziram Technical
   Description: White powder
   Lot/Batch #: P15/62,06-85
   Purity: 98% a.i.
   Storage stability: None provided; dose formulations were prepared fresh daily.
   CAS #: 137-30-4
   Structure:



2. Vehicle: 1% aqueous methyl cellulose

3. Test animals: Species: Rabbit
   Strain: New Zealand White
   Age and weight of females on gestation day 0: 16-28 weeks, 3.00-4.38 kg
   Source: Ranch Rabbits Ltd., Crawley
   Housing: Individually in anodized steel grid floor cages
   Diet: Ranch Rabbit Diet, pelleted (Grain Harvesters Ltd., Canterbury), small quantity given upon receipt; then were fed SQC Rabbit Diet (Special Diet Services, Ltd., Witham) ad libitum
   Water: Filtered tap water, ad libitum
   Environmental conditions:
   Temperature: 16-22°C
   Humidity: 40-70%
   Air changes: At least 15/hr
   Photoperiod: 10 hrs dark/14 hrs light
   Acclimation period: At least 32 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 7/1/85 (allocation to treatment groups) end: 8/2/85

2. Mating: Males and females were housed together (1 male and 1 female/cage) for breeding. Males were proven fertile, stock rabbits of the same source and strain. Does that successfully completed coitus were injected with 10 IU of chorionic gonadotropin to ensure ovulation. Mating was conducted over a 5-day period and the day of mating was designated as gestation day (GD) 0.

3. Animal assignment: Animals were randomly assigned (stratified by body weight) to dose groups as indicated in Table 1.

Table 1. Animal assignment

Dose Group	Dose (mg/kg/day)	Number of Females
Control	0	16
Low	3	16
Mid	7.5	16
High	15	16

4. Dose selection rationale: Doses were selected based on the findings of a rangefinding study in pregnant New Zealand White rabbits (5/dose) which received gavage doses of 0, 5, 10, or 20 mg/kg/day in 1% aqueous methyl cellulose from GDs 7-19. At 20 mg/kg, 2 does died and one aborted. One incidental death occurred at 5 mg/kg and one in the control group. No treatment-related changes were observed in clinical signs, body weight, or gross pathological findings. At 20 mg/kg, reduced body weight gains, as calculated by the reviewers, were noted (not analyzed for statistical significance) for the overall treatment period (↓95%, GDs 7-19); additionally, decreased (not statistically significant, [NS]) food consumption was observed during pretreatment (↓11-17, GDs 0-7), throughout the treatment period (↓30-40%, GDs 7-19), and during post-treatment days 19-25 (↓23-35%); consumption increased (NS) for the GDs 25-28 interval (↑58%). Also at 20 mg/kg, decreases (NS) were noted in percent preimplantation loss (↓48%), number of fetuses (↓38%), and mean litter weight (↓25%); percent postimplantation loss was increased (↑464%, NS). The maternal LOAEL was 20 mg/kg/day.

   Based on the results of the rangefinding study, the dose levels shown in Table 1 were selected for the subsequent full developmental toxicity study.

5. Dosage preparation and analysis - Test formulations in 1% aqueous methyl cellulose were prepared daily during the treatment period and stored at ambient temperature prior to dosing. During the study, concentration analyses in duplicate were performed on the week 1 and week 4 preparations to determine test substance content of all dose formulations.

   Results - Concentration analysis (range as mean % of nominal): 98-112%.

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

6. Dosage administration: All doses were administered once daily by gavage on GDs 7

through 19 in a volume of 2 mL/kg body weight. Dosing was based on the most recent body weight. Control animals received the vehicle only.

C. OBSERVATIONS

1. Maternal observations and evaluations - The animals were observed for moribundity and mortality twice daily and observed at least once daily for clinical signs of toxicity. Body weight data were recorded on GDs 0, 7, 8, 9, 10, 13, 16, 19, 23, and 28. Food consumption was measured on GDs 0-3, 3-7, 7-10, 10-13, 13-16, 16-19, 19-23, 23-25, and 25-28 and was reported as g/animal/day. Does were sacrificed on GD 28, then dissected and examined macroscopically. The reproductive tract was removed, examined, and the following were recorded:
   - pregnancy status
   - gravid uterine weight
   - number of corpora lutea
   - number and location of implantation sites
   - number and location of fetuses (live and dead)
   - number and location of resorptions (early and late)

2. Fetal evaluations - All fetuses were weighed, sexed, and examined for external abnormalities. Crown/rump lengths (mm) were measured. Fetuses were skinned, dissected, and examined viscerally. Following the visceral examination, the fetuses were eviscerated and stored in 70% industrial methylated spirits for approximately 24 hours; the head was then sliced through the fronto-parietal suture line and the brain was examined macroscopically. Subsequently, the fetuses were macerated in 2% (w/v) aqueous KOH and stained with KOH and Alizarin Red S to allow for skeletal examination. Fetal abnormalities were recorded as major, minor, or variants.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures. Statistical evaluation of maternal body weight gain and food consumption, gravid uterus weight, litter and fetal weights, crown/rump length, sex ratio, minor skeletal defects and variants used ANOVA and "t" test for normally distributed errors and non-parametric testing (e.g. Kruskal-Wallis, Wilcoxon rank sum test) for non-normally distributed errors. Evaluation of pre- and post-implantation loss, implantations, external/visceral defects (major and minor), and major skeletal defects used the Fisher's two-sum randomization test with a Monte Carlo simulation for computation of significance levels.

2. Indices: The following indices were calculated by the sponsor for cesarean section findings:

   Preimplantation loss (%) = # corpora lutea - # implantations/ # corpora lutea x 100
   Postimplantation loss (%) = # implantations - # live fetuses/ # implantations x 100

Sex ratio = 1: # of females/ # of males

3. Historical control data: Historical control data were provided to allow for comparisons. Data were presented as absolute fetal number and percent affected; no litter data were provided.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical observations: One high-dose doe died on GD 23 and one mid-dose doe died on GD 13. Additionally, one control doe and one mid-dose doe were sacrificed *in extremis* on GDs 14 and 15, respectively; clinical signs observed prior to death in these two animals included weight loss, anorexia, and wheezing. No other premature deaths occurred and no treatment-related clinical signs of toxicity were observed at any dose level.

2. Body weight: When compared to concurrent controls, body weights (Table 2) at the high-dose level were decreased on GDs 0-28 (↓3-17%, $p \leq 0.01$ on GD 10 only). Overall treatment interval (GDs 7-19) and overall study interval (GDs 0-28) body weight gain, as calculated by the reviewers, were reduced (not analyzed for statistical significance) at the mid- (treatment, ↓30%; study, ↓19%) and high-dose levels (treatment, ↓81%; study, ↓30%).

Table 2. Mean maternal body weight and overall body weight gains (kg) [a]

Interval	Dose in mg/kg/day			
	0	3	7.5	15
Pretreatment:				
Day 0	3.76	3.71	3.78	3.64 (↓3%)
Treatment:				
Day 7	3.86	3.80	3.87	3.74 (↓3%)
Day 10	3.92	3.86	3.94	3.72** (↓5%)
Day 13	4.00	3.94	3.90	3.69 (↓8%)
Day 16	4.11	4.03	3.98	3.73 (↓17%)
Day 19	4.13	4.06	4.06	3.79 (↓8%)
Post-treatment:				
Day 23	4.17	4.12	4.10	3.86 (↓7%)
Day 28	4.23	4.18	4.16	3.97 (↓6%)
Corrected body weight: [b]				
Days 0-28	3.78	3.74	3.78	3.60 (↓5%)
	0.46	**0.44**	**0.39** (↓15%)	**0.37** (↓20%)
Overall body weight gain: [c]				
Days 7-19	0.27	0.26	0.19 (↓30%)	0.05 (↓81%)
Overall body weight gain: [c]				
Days 0-28	0.47	0.47	0.38 (↓19%)	0.33 (↓30%)

a Data extracted from the study report, Tables 1 and 2, pages 29 and 30; n= 14-16 does/dose. Percent difference from controls is presented parenthetically.
b Corrected for gravid uterine weight; gravid uterine weight (kg) presented in bold.
c Calculated by reviewers.
** Significantly different from controls at $p \leq 0.01$.

3. Food consumption - When compared to concurrent controls, treatment-related decreases ($p \leq 0.01$) in absolute (g/animal/day) food consumption (Table 3) were observed in the high-dose does beginning at the GDs 7-10 interval (↓19%) and continuing throughout the GDs 13-16 interval (↓44-49%); decreased consumption was also observed for the GDs 16-19 interval (↓24%, not statistically significant [NS]). Food consumption was reduced for the overall treatment interval (↓34%, GDs 7-19) and for the overall study interval (↓18%, GDs 0-28) at the high-dose level. No further treatment-related differences were observed in food consumption.

Table 3. Mean maternal food consumption (g/day). [a]

Interval	Dose (mg/kg/day)			
	0	3	7.5	15
Pretreatment:				
Days 0-3	196	209	202	190
Days 3-7	188	191	211	187
Treatment:				
Days 7-10	203	206	218	164** (↓19%)
Days 10-13	197	206	163	110** (↓44%)
Days 13-16	192	200	175	98** (↓49%)
Days 16-19	190	196	211	144 (↓24%)
Post-treatment:				
Days 19-23	170	175	197	157
Days 25-28	144	130	148	134
Overall treatment:				
Days 7-19	196	202	192	129** (↓34%)
Overall interval:				
Days 0-28	183	188	193	150 (↓18%)

a Data extracted from the study report, Table 3, page 31; n = 14-16 does/dose. Percent difference from controls is presented parenthetically.

** Significantly different from controls at $p \leq 0.01$.

4. Gross pathology - No treatment-related changes were observed upon gross pathological examination. At necropsy, the high-dose doe that was found dead on GD 23 displayed a thick, beige "pus" substance adhering to all lung lobes and a thickened pericardium. In the mid-dose doe found dead on GD 13, all lung lobes were red and diffuse. The mid-dose doe sacrificed *in extremis* on GD 15 exhibited pale kidneys, moderate gaseous distension of the gastrointestinal tract, and mottled, firm, beige lung lobes.

5. Cesarean section data - At the high-dose level, treatment-related increases (NS) were observed in the total number of resorptions/doe (↑88%) and the percent postimplantation loss (↑97%). Additionally, reductions (NS) in the number of live fetuses/doe (↓15%) were noted. No other dose-dependent, treatment-related Cesarean section findings were noted in the treated animals (Table 4); percent preimplantation loss was increased (↑124%, NS) at the mid-dose level, but implantation should occur prior to dosing on GD 7. The number of implantations/doe and percent male were similar between control and treated groups. Fetal weights were unaffected by treatment.

Table 4. Cesarean section observations [a]

Observation	Dose (mg/kg/day)			
	0	3	7.5	15
# Animals Assigned (Mated)	16	16	16	16
# Animals Pregnant	16	16	16	16
Pregnancy Rate (%)	(100)	(100)	(100)	(100)
# Nonpregnant	0	0	0	0
# Total Does Died	1	0	2	1
# Died or Sacrificed *in extremis* Pregnant	1	0	2	1
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	153	159	137	139
Corpora Lutea/Doe	10.2	9.9	9.8	9.3
Total # Implantations	140	146	111	130
Implantations/Doe	9.3	9.1	7.9	8.7
Total # Litters Examined	15	16	14	15
Total # Live Fetuses	128	140	103	108
Live Fetuses/Doe	8.5±2.2	8.8±2.0	7.4±2.8	7.2±2.7
Total # Dead Fetuses	NR	NR	NR	NR
Dead Fetuses/Doe	NR	NR	NR	NR
Total # Resorptions	12	6	8	22
Early	12	3*	4	5
Late	0	3	4	17
Total Resorptions/Doe [b]	0.8	0.4	0.6	1.5
Early	0.8	0.2	0.3	0.3
Late	0	0.2	0.3	1.1
Litters with Total Resorptions	0	0	0	0
Mean Fetal Weight (g)	37.6	37.0	37.8	36.2
Males	37.8	37.0	38.3	36.9
Females	37.5	37.5	37.5	34.5
Sex Ratio (% Male) [b]	48.4	55.7	47.6	43.5
Preimplantation Loss (%)	8.5	8.2	19.0	6.5
Postimplantation Loss (%)	8.6	4.1	7.2	16.9

a Data extracted from the study report, Table 4, pages 32 and 33, Appendix 7, pages 70 though 73, and Appendix 8, pages 82 through 97.
b Calculated by reviewers.
NR Not reported
* Significantly different from controls at $p \leq 0.05$.

B. DEVELOPMENTAL TOXICITY: Fetal examinations included external, visceral, and skeletal observations at necropsy.

1. External examination - There were no treatment-related external malformations or

variations detected at any dose level. The most common findings are shown in Table 5a.

Table 5a. External examinations [a]

Observations	Dose (mg/kg/day)			
	0	3	7.5	15
#Fetuses (#litters) examined	128 (15)	140 (16)	103 (14)	107 (15)
Major				
Head, multiple defects [b]	0 (0)	0.7 (6.3)	0 (0)	0 (0)
Eyes, slight unilateral microphthalmia	0 (0)	0.7 (6.3)	0 (0)	0 (0)
Minor				
Eye, right hemorrhagic	0 (0)	0.7 (6.3)	0 (0)	0 (0)

a Data extracted from the study report, Tables 4 and 5, pages 34 and 35. For individual observations, data are presented as % fetal incidence (% litter incidence). Litter incidences were calculated by reviewers from data in Appendix 8, pages 82 through 97.

b Defects included dome-shaped head; microstomia; cleft palate; brachygnathia; upper jaw malformed and reduced; cyclopia (open eye); external nares absent; and hydrocephaly.

2. Visceral examination - There were no treatment-related visceral malformations observed at any dose level (Table 5b). Reduced atrium/atria, a variation, was observed at the mid- (fetal 2.9%; litter, 14.3%) and high-dose (fetal, 2.8%; litter, 20.0%) levels vs controls (fetal, 0.8%; litter, 6.7%); this finding was observed in a dose-dependent manner and without the %fetal and %litter incidence ranges in the historical data, this variation was considered to be equivocally treatment-related.

Table 5b. Visceral examinations [a]

Observations	Dose (mg/kg/day)			
	0	3	7.5	15
#Fetuses (#litters) examined	128 (15)	140 (16)	103 (14)	107 (15)
Minor				
Atrium/atria enlarged	6.3 (26.7)	0.7 (6.3)	2.9 (21.4)	0 (0)
Atrium/atria reduced	0.8 (6.7)	0 (0)	2.9 (14.3)	2.8 (20.0)
Variants				
Abnormal common carotid	19.5 (73.3)	12.1 (62.5)	13.6 (50.0)	12.1 (46.7)

a Data extracted from the study report, Tables 4 and 5, pages 34 and 35. For individual observations, data are presented as % fetal incidence (% litter incidence). Litter incidences were calculated by reviewers from data in Appendix 8, pages 82 through 97.

3. Skeletal examination - When compared to concurrent controls, absence of the interparietal bone, a malformation (Table 5c), was observed at the high-dose level only (fetal, 1.9%; litter, 13.3%) vs 0 controls; this finding was only observed at the high-dose level and without the %fetal and %litter incidence ranges in the historical data, this malformation was considered to be equivocally treatment-related. Unossified thoracic vertebral centrum, a malformation, was noted at the low- (fetal, 0.7%; litter, 6.3%) and high-dose (fetal, 0.9%; litter, 6.7%) levels vs 0 concurrent controls, but this finding was not observed in a dose-dependent manner and was considered not to be treatment-related. Interparietal bipartite, a variation, was observed in a single high-dose fetus (fetal, 2.6%; litter, 6.7%) vs 0 controls. It was stated that the observed increased incidence of skeletal malformations may be attributed to 2 males which sired 5 of the 8 fetuses exhibiting major vertebral/rib defects; no further explanation was provided.

Table 5c. Skeletal examinations [a]

Observations	Dose (mg/kg/day)			
	0	3	7.5	15
#Fetuses (#litters) examined	128 (15)	140 (16)	103 (14)	107 (15)
Major				
Skull, multiple defects [b]	0 (0)	0.7 (6.3)	0 (0)	0 (0)
Interparietal absent	0 (0)	0 (0)	0 (0)	1.9 (13.3)
Thoracic vertebral arch(es) fused and/or asymmetrically ossified	1.6 (13.3)	1.4 (6.3)	0 (0)	1.9 (13.3)
Thoracic vertebral arch not ossified	0 (0)	1.4 (12.5)	0 (0)	0 (0)
Thoracic vertebral centra fused, bipartite, asymmetrically ossified, and/or incompletely ossified	0.8 (6.7)	1.4 (12.5)	0 (0)	0.9 (6.7)
Thoracic vertebral centrum not ossified	0 (0)	0.7 (6.3)	0 (0)	0.9 (6.7)
Ribs fused and/or unattached	1.6 (13.3)	1.4 (6.3)	0 (0)	1.9 (13.3)
Cervical vertebral centrum not ossified	0.8 (6.7)	0 (0)	0 (0)	0 (0)
Lumbar vertebral centra asymmetrically ossified, fused, and/or incompletely ossified	0.8 (6.7)	0.7 (6.3)	0 (0)	0 (0)
Lumbar vertebral arch not ossified	0 (0)	0.7 (6.3)	0 (0)	0 (0)
Sternebra bipartite, fused, and/or malformed	0 (0)	0.7 (6.3)	0 (0)	0 (0)
Minor				
Interparietal bipartite	0 (0)	0 (0)	0 (0)	2.6 (6.7)
Spatulate rib(s)	2.3 (13.3)	0.7 (6.3)	0 (0)	1.9 (13.3)
Metacarpals incompletely ossified	7.0 (46.7)	10.0 (18.8)	2.9 (14.3)	15.0 (46.7)
Variants				
Cleft in frontal(s)	36.7 (86.7)	30.7 (93.8)	38.8 (92.9)	33.6 (93.3)
Extra pair of ribs	61.7 (100)	34.3 (75.0)	59.2 (92.9)	43.9 (93.3)

a Data extracted from the study report, Tables 4 and 5, pages 34 and 36 through 38. For individual observations, data are presented as % fetal incidence (% litter incidence). Litter incidences were calculated by reviewers from data found in Appendix 8, pages 82 through 97.

b Defects included interparietal and parietal fused; right parietal incompletely ossified; left and right zygomatic arches fused/asymmetrically ossified/malformed; frontals greatly reduced in size/malformed; upper and lower jaws greatly reduced in size malformed; nasals and hyoid malformed.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS - Administration of the test substance at 15 mg/kg resulted in maternal toxicity characterized by reductions in body weight gain and food intake. The maternal LOAEL is 15 mg/kg/day and the NOAEL is 7.5 mg/kg/day.

Reduced fetal body weights and increased percent postimplantation loss were observed at 15 mg/kg. The developmental LOAEL is 15 mg/kg/day and the NOAEL is 7.5 mg/kg/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Ziram Technical (98% a.i.) in 1% aqueous methyl cellulose was administered to pregnant New Zealand White rabbits (16/dose) at concentrations of 0, 3, 7.5, or 15 mg/kg/day by gavage on GDs 7 through 19. Does were sacrificed on GD 28. The analytical data indicated that the mixing procedure was adequate and that the variability between nominal and actual dosage to the study animals was acceptable.

One high-dose doe died on GD 23 and one mid-dose doe died on GD 13. Additionally, one control doe and one mid-dose doe were sacrificed *in extremis* on GDs 14 and 15, respectively; clinical signs observed prior to death in these two animals included weight loss, anorexia, and wheezing. These deaths were not considered to be the result of treatment due to the lack of a dose-response relationship. No other premature deaths occurred and no treatment-related clinical signs of toxicity were observed at any dose level.

At 15 mg/kg, decreased body weights were observed over GDs 0-28 (↓3-17%, $p \leq 0.01$ on GD 10 only). Additionally, for the overall treatment interval (GDs 7-19) and overall study interval (GDs 0-28) body weight gain, as calculated by the reviewers, were reduced as compared to the control (treatment, ↓81%; study, ↓30%, not analyzed for statistical significance). Decreases ($p \leq 0.01$) in absolute (g/animal/day) food consumption were observed beginning at the GDs 7-10 interval (↓19%) and continuing throughout the GDs 13-16 interval (↓44-49%); decreased consumption was also observed for the GDs 16-19 interval (↓24%, not statistically significant [NS]). Food consumption was reduced for the overall treatment interval (↓34%, GDs 7-19) and for the overall study interval (↓18%, GDs 0-28).

At 7.5 mg/kg, decreased body weight gain was observed over GDs 7-19 (↓30%) and GDs 0-28 (↓19%). No other treatment-related maternal effects were noted at the mid-dose level.

No treatment-related findings were observed at gross necropsy of maternal animals.

The number of implantations/doe and percent male were similar between control and treated groups.

The maternal LOAEL is 7.5 mg/kg/day, based on decreased body weight gain. The maternal NOAEL is 3 mg/kg/day.

2. DEVELOPMENTAL TOXICITY:

a. Deaths/Resorptions: At the high-dose level, treatment-related increases (NS) were observed in the total number of resorptions/doe (↑88%) and the percent postimplantation loss (↑97%). Additionally, reductions (NS) in the number of live fetuses/doe (↓15%) were noted.
b. Altered Growth: Fetal weights were not significantly different from the concurrent controls.
c. Developmental Minor Defects (variations): There were no treatment-related developmental external or skeletal variations noted at any dose level. At visceral examination, reduced atrium/atria was observed at the mid- (fetal 2.9%; litter, 14.3%) and high-dose (fetal, 2.8%; litter, 20.0%) levels vs controls (fetal, 0.8%; litter, 6.7%); this finding was observed in a dose-dependent manner and without the %fetal and %litter incidence ranges in the historical data, this finding was considered to be equivocally treatment-related.
d. Major Defects (malformations): There were no treatment-related external or visceral malformations detected at any dose level. Upon skeletal examination, absence of the interparietal bone was observed at the high-dose level only (fetal, 1.9%; litter, 13.3%) vs 0 controls; since this finding was only observed at the high-dose level and without the %fetal and %litter incidence ranges in the historical data, this finding was considered to be equivocally treatment-related.

Reduced atrium/atria, a minor defect (variation), was observed at the mid- (fetal 2.9%; litter, 14.3%) and high-dose (fetal, 2.8%; litter, 20.0%) levels vs controls (fetal, 0.8%; litter, 6.7%). The **HIARC** did not consider this as an effect since there was no dose response, there were no statistically significant differences in the incidences at any dose, and the data on this parameter, which is a highly subjective observation, showed a wide-spread variation in the size of the atria (enlarged and reduced) among the control and the treatment groups.

At the high-dose level, increases (NS) as compared to the control were observed in the total number of resorptions/doe (↑88%) and the percent postimplantation loss (↑97%). Additionally, reductions (NS) in the number of live fetuses/doe (↓15%) were noted. Upon skeletal examination, absence of the interparietal bone, a major defect (malformation), was observed at the high-dose level only (fetal, 1.9%; litter, 13.3%) vs 0 controls; since this finding was only observed at the high-dose level and without the %fetal and %litter incidence ranges in the historical data, this malformation was considered equivocally treatment-related.

The developmental LOAEL is 15 mg/kg/day, based upon increased resorptions and post-implantation loss. The developmental NOAEL is 7.5 mg/kg/day.

This developmental toxicity study is classified acceptable (§83-3[b]) and does satisfy the guideline requirement for a developmental toxicity study in the rabbit; it would be helpful if historical control data (% fetal and % litter incidences) were provided for reduced atrium/atria and absence of the interparietal bone.

C. STUDY DEFICIENCIES - The following deficiency was noted, but will not affect the conclusions of the study report:

- Summary tables were not provided for developmental anomalies.