(8-2-2001)

DATA EVALUATION RECORD

MESOTRIONE

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

Work Assignment No. 2-2-95A (formerly 2-01-52BB) (MRIDs 44901707 and 44505032)

Prepared for

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DATA EVALUATION RECORD

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

DP BARCODE: D259369 SUBMISSION CODE: S541375
P.C. CODE: 122990 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Mesotrione (96.8% a.i.)

SYNONYMS: ZA1296; 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione; 2-(4-mesyl-2-nitrobenzoyl)-cyclohexane-1,3-dione

CITATION: Moxon, M.E. (1999). First Revision to ZA1296: Developmental Toxicity Study in the Rabbit. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Report # CTL/P/4892, Laboratory Study # RB0684, June 28, 1999. MRID 44901707. Unpublished.

Moxon, M.E. (1997). ZA1296: Dose Range Finding Study in the Pregnant Rabbit. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Report # CTL/L/8024, Laboratory Study # RB0677, December 11, 1997. MRID 44505032. Unpublished.

SPONSOR: Zeneca Ag Products, Wilmington, DE 19850-5458

EXECUTIVE SUMMARY: In a developmental toxicity study with rangefinding (MRIDs 44901707 and 44505032), mesotrione (96.8% a.i., Lot #: P17) in deionized water was administered to pregnant New Zealand White rabbits (20/dose) at dose levels of 0, 100, 250, or 500 mg/kg/day by gavage on gestation days (GDs) 8 through 20. All does were sacrificed on GD 30.

A single 100 mg/kg animal was found dead on GD 4 and abnormal GI tract contents were observed at necropsy. One 250 mg/kg animal was sacrificed *in extremis* on GD 22 after displaying clinical signs including diarrhea, subdued behavior, thin appearance, and severe weight loss. Five does were sacrificed after showing signs of abortion as follows: one 100 mg/kg on GD 29; two 250 mg/kg on GD 25 or 28; and two 500 mg/kg on GD 23 or 25.

At 500 mg/kg/day, observations included the following: blood on the tray (6 observations in 4/20 animals vs 1 incidence in 1/20 controls); few feces on the tray (25 incidences in 7/20 animals vs 3 observations in 2/20 controls); no feces on the tray (6 observations in 3/20 animals vs 0/20 controls); and red to brown colored urine (41 observations in 12/20 animals vs 1 incident in 1/20 controls); decreases (NS) in food consumption during GDs 14-20 (↓12-24%) and increases during post-treatment (↑39%, GDs 26-30, $p \leq 0.01$); a decrease (NS) in gravid uterine weight (↓15%); and a decrease (NS) in the number of implantations/doe (↓13%). Food consumption was also reduced at both the 500 and 250 mg/kg/day dose levels with rebounds observed during the post dosing period.

At 250 mg/kg/day, findings observed included the following: few feces on the tray (11 observations in 4/20 animals vs 3 observations in 2/20 controls); no feces on the tray (4 observations in 3/20 animals vs 0/20 controls); red to brown colored urine (26 incidents in 12/20 animals vs 1 incident in 1/20 controls); and decreases (NS) in food consumption during GDs 14-20 (↓14-15%) and increases (NS) during the post-treatment interval (↑12%, GDs 26-30).

At 100 mg/kg/day, findings observed that were considered to be possibly treatment-related included few feces on the tray (6 observations in 5/20 animals vs 3 observations in 2/20 controls) and red to brown colored urine (14 incidents in 6/20 animals vs 1 incident in 1/20 controls).

When compared to concurrent controls, no treatment-related changes in body weight, adjusted body weight, or gross pathology were noted at any dose level tested.

NOTE: Due to the increase in preimplantation loss noted in the mid and high dose levels, these groups may have received test material prior to the completion of implantation. This would be a confounding factor which may interfere with the proper assessment of maternal toxicity in this study.

The maternal LOAEL is 250 mg/kg/day, based on abortions and clinical signs of toxicity. The maternal NOAEL is 100 mg/kg/day.

At 100, 250, and 500 mg/kg/day, skeletal examination (reported as [% fetal incidence (% litter incidence)]) revealed a shift toward a decreased degree of ossification of the 7th cervical vertebra transverse process, when compared to concurrent controls, as evidenced by i) a decreased ($p \leq 0.05$ or 0.01, fetal incidences only) incidence of a "partially ossified" transverse process at the low- [0.8 (7.1)], mid- [0.7 (5.9)], and high-dose levels [0 (0)] vs controls [6.7 (22.2)] and ii) a decreased (NS) incidence of a "fully ossified" transverse process in the 7th cervical vertebra in all treated groups [0 (0)] vs controls [3.3 (11.1)]. In addition, statistically significant decreases in the ossification (fetal incidence only) of the odontoid was observed in all dose groups as compared to controls. Conversely, an unexpected trend toward more complete ossification of the 5th sternebra was observed and was demonstrated by i) a dose-dependent decrease ($p \leq 0.01$, fetal incidences only) in the number of animals exhibiting a "partially ossified" 5th sternebra at

the low- [32.3 (92.9)], mid- [28.9 (76.5)], and high-dose levels [24.6 (62.5)] vs controls [52.0 (94.4)] and ii) a decrease ($p \leq 0.05$ or 0.01, fetal incidences only) in the number of animals exhibiting a "nonossified" 5th sternebra at the low- [3.2 (14.3)], mid- [3.4 (11.8)], and high-dose levels [4.2 (25.0)] vs controls [12.7 (33.3)]. In addition, statistically significant ($p \leq 0.01$) increases in the fetal incidence of 13 full ribs and 27 pre-sacral vertebra were noted at all dose levels.

An increase in preimplantation loss ($p \leq 0.01$) was noted at the high-dose level in the submitted report, but errors were observed in the calculation, and therefore, the percent losses were recalculated by reviewers. As a result of the Agency contractor re-calculation, increases were observed at both the mid- (↑42%) and high-dose (↑152%) levels. The observation of preimplantion loss in several dose groups would suggest dosing began in these groups prior to the completion of the implantation process. This introduces a confounding factor into study interpretation.

At 500 mg/kg, a decrease (NS) in the number of live fetuses/doe (↓11%) was observed. This may be associated with the preimplantation loss noted in this dose group.

There were no treatment-related external or visceral effects noted at any dose level. Treatment-related changes observed in the *manus* ossification data included statistically significant increases in the proportion of fetuses with scores of 5 at the mid and high dose levels.

The developmental LOAEL is 100 mg/kg/day, based on delayed ossification of the 7th cervical transverse process and odontoid and increases in extra full 13th ribs and 27 pre-sacral vertebra. Hence, a developmental NOAEL was not established.

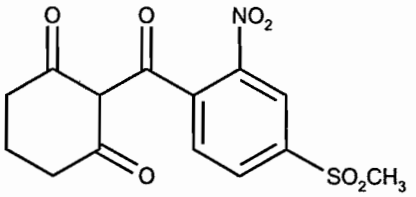
Since a developmental NOAEL was not established and because dosing was apparently initiated in several dose groups prior to the completion of implantation (resulting in increased preimplantation loss), this developmental toxicity study is classified **unacceptable/ not upgradeable** (§83-3[b]) and does not satisfy the guideline requirement for a developmental toxicity study in the rabbit.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Mesotrione
   Description: Light beige solid
   Lot/Batch #: P17
   Purity: 96.8% a.i.
   Storage stability: Formulations were stable at room temperature (assumed by reviewers) for up to 14 days.
   CAS #: 104206-82-8
   Structure:



2. Vehicle: Deionized water

3. Test animals: Species: Rabbit
   Strain: New Zealand White
   Age and mean weight range of females: Approximately 18-25 weeks old at receipt, 3812-3845 g on gestation day 4
   Source: Interfauna (UK) Limited, Huntingdon, Cambridgeshire, UK
   Housing: Individually in mobile rabbit units
   Diet: STANRAB SQC (Special Diets Services Limited, Witham, Essex, England), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 17±2°C
   Humidity: 40-70%
   Air changes: 25-30/hr
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period: Not provided

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 8/9/95 end: 9/15/95

2. Mating: Females were paired at the suppliers with males of the same strain. The day mating was observed was designated as gestation day (GD) 1. Successfully mated females were delivered to the laboratory on GD 2 or 3 over a 2 week period.

3. Animal assignment: Females were randomly assigned to dose groups as indicated in Table 1.

Table 1. Animal assignment

Dose Group	Dose (mg/kg/day)	Number of Females
Control	0	20
Low	100	20
Mid	250	20
High	500	20

4. Dose selection rationale: Doses were selected based on the findings of a rangefinding study (MRID 44505032) reviewed with the current study in which New Zealand White rabbits (10/dose) were dosed at 0, 450, 600, or 750 mg/kg/day during GDs 8-20. No data were provided; however, summary information was provided in the results section of the study report. At 750 mg/kg, 5/10 females displayed severe body weight loss and reduced food consumption by the termination of dosing. Two of these animals aborted on GD 22 or 24; a third 750 mg/kg female aborted on GD 24. The remaining 4 animals at 750 mg/kg were sacrificed prior to scheduled termination due to the extreme maternal toxicity at this dose level. It was stated that at 600 mg/kg 3/10 animals showed severe body weight loss and reduced food consumption; two were sacrificed following signs of abortion (GD 22 or 25) and another sacrificed *in extremis* on GD 24. All other treated animals survived to scheduled termination. An increase in percent postimplantation loss was noted at 600 mg/kg (↑194%) when compared to concurrent controls; however, the significance of this finding was equivocal as only 4 pregnant animals survived to C-sectioning. It was stated that at the necropsy of several animals that died prematurely, changes in the stomach including abnormal content and red/black spots or areas were observed.

   Based on these results, the doses shown in Table 1 were chosen for the subsequent developmental study.

5. Dosage preparation and analysis - Dosing solutions were prepared four times during the study by mixing the appropriate amount of deionized water with the test substance. Dose formulations were stored at room temperature. Prior to the start of the study, samples (top, middle, bottom) of 10 and 60 mg/mL formulations were analyzed for homogeneity. Also prior to the study, samples of 10 and 50 mg/mL concentrations were analyzed after storage at room temperature (assumed by reviewers) for up to 14 days. Concentration

analyses were performed to verify concentration of each new preparation.

Results:

Homogeneity analyses (range as mean % of nominal): 93-98%

Stability analyses (range as mean % of Day 0): 97.7-102.2%

Concentration analyses (range as mean % of nominal): 88.0-99.6%

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 8 through 20 in a volume of 10 mL/kg body weight. Dosing was based on the daily body weight. Control animals received the vehicle only.

C. OBSERVATIONS

1. Maternal observations and evaluations - The animals were observed daily for mortality and clinical signs of toxicity. Cage side observations were also made as soon as possible after dosing and daily at the end of the working day. Body weights were recorded on GDs 4, 8 through 20, 23, 26, and 30. Food consumption was recorded on GDs 4 through 30. All does were sacrificed on GD 30. Examinations at sacrifice consisted of a gross exam of the thoracic and abdominal cavities. The reproductive tract was removed, examined, and the following were recorded:
   - pregnancy status (except for the single 100 mg/kg animal found dead on GD 4)
   - gravid uterine weight
   - number and location of corpora lutea
   - number and location of implantation sites
   - number and location of live fetuses
   - number of resorptions (early and late)

   The uteri of apparently nonpregnant females were stained to ascertain pregnancy status. For animals that died prior to scheduled termination, neither uterine weight nor individual fetal details were recorded.

2. Fetal evaluations - Each fetus was weighed and examined for external abnormalities. All fetuses were then examined for visceral abnormalities, sexed, eviscerated, and fixed in 70% industrial methylated spirits. Following at least 18 hours of fixation, the head of each fetus was cut along the fronto-parietal suture line and the brain was examined macroscopically. The carcasses were then returned to the fixative for subsequent processing and staining with Alizarin Red S for skeletal assessment. Observations were

classified as i) major defects, defined by the sponsor as permanent structural or functional deviations that are considered likely to be incompatible with survival or are rarely seen, and ii) minor defects or variants, defined by the sponsor as small, generally transient deviations that are considered not to be incompatible with survival and which frequently represent a manifestation of delayed development. Further, the minor defect classification is used for observations which generally occur at a low frequency, while the variant classification is used for observations which consistently occur at a frequency greater than 10%. The degree of fore and hindlimb skeletal ossification was further analyzed by assessment of the *manus* and *pes*. The assessment scale for the *manus/pes* data is found in Appendix 1.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures.

2. Indices: The following indices calculations were provided in the study report:

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

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

3. Historical control data: Historical control data for the incidence of abortion were provided to allow for comparison with treated groups.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical observations: A single 100 mg/kg animal was found dead on GD 4. One 250 mg/kg animal was sacrificed *in extremis* on GD 22 after displaying clinical signs of toxicity including diarrhea and subdued behavior; additionally, this animal appeared thin and showed severe weight loss. Five does were sacrificed after showing signs of abortion as follows: one 100 mg/kg on GD 29; two 250 mg/kg on GD 25 or 28; and two 500 mg/kg on GD 23 or 25. The single abortion at 100 mg/kg was within the historical control range (abortion incidence at 100 mg/kg, 6.7% vs historical control range of 0.0-6.7%), and therefore, was considered not to be treatment-related. The remaining abortions at the mid- and high-dose levels were considered to be treatment-related. When compared to concurrent controls, clinical signs (Table 2) were observed as follows: blood on the tray (6 observations in 4/20 high-dose animals vs 1 incidence in 1/20 controls); few feces on the tray (6 observations in 5/20 low-dose animals; 11 observations in 4/20 mid-dose animals; and 25 incidences in 7/20 high-dose animals vs 3 observations in 2/20 controls); no feces on the tray (4 observations in 3/20 mid-dose animals and 6

observations in 3/20 high-dose animals vs 0/20 controls); and red to brown colored urine (14 incidents in 6/20 low-dose animals; 26 incidents in 12/20 mid-dose animals; and 41 observations in 12/20 high-dose animals vs 1 incident in 1/20 controls). The observations of few feces in the tray and red to brown colored urine increased in a dose-dependent manner and were considered to be treatment-related at the mid- and high-dose levels; additionally, these findings at the low-dose level are considered to be possibly treatment-related.

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

Observation	Dose in mg/kg/day			
	0	100	250	500
Blood on tray	1 (1)	0 (0)	3 (1)	6 (4)
Few feces on tray	3 (2)	6 (5)	11 (4)	25 (7)
No feces on tray	0 (0)	0 (0)	4 (3)	6 (3)
Red to brown colored urine	1 (1)	14 (6)	26 (12)	41 (12)

a Data extracted from the study report, Table 5, pages 35 through 37; n = 20.

2. Body weight: When compared to concurrent controls, no treatment-related changes in body weight (Table 3) were noted at any dose level tested. Using day 8 body weight as a covariant, GD 18 adjusted high-dose body weight was lower (↓2%, $p \leq 0.05$), but this decrease was minor and isolated and was considered not to be treatment-related. However, note that gravid uterine weight is reduced at the high dose level. This might be associated with significant preimplantation loss also noted at this dose level.

Table 3. Selected mean maternal body weights (g) [a]

Interval	Dose in mg/kg/day			
	0 n = 18	100 n = 14	250 n = 17	500 n = 16
Pretreatment:				
GD 4	3818	3814	3812	3845
GD 8	3899	3899	3881	3950
Treatment:				
GD 10	3912	3930	3925	3953
GD 15	4060	4033	4058	4077
GD 20	4186	4134	4162	4138
Post-treatment:				
GD 23	4261	4213	4231	4170
GD 30	4376	4382	4393	4416
Gravid uterine weight	548	564	580	467 (↓15%)

a Data extracted from the study report, Tables 6 and 9, pages 38 through 40 and page 45. Non-pregnant animals, those that aborted, and those that failed to survive to scheduled sacrifice were excluded from the mean by the sponsor.

3. Food consumption - When compared to concurrent controls, treatment-related decreases (not statistically significant [NS]) were noted in food consumption (Table 4) at the mid- and high-dose levels during the GDs 14-17 (↓12-15%) and GDs 17-20 (↓14-24%) intervals. In addition, increases were noted in the post-treatment interval (GDs 26-30) in the mid- and high-dose animals (↑12-39%; $p \leq 0.01$ at the high-dose level only). No other treatment-related changes were observed.

Table 4. Selected mean maternal food consumption (g/animal/day) [a].

Interval	Dose in mg/kg/day			
	0	100	250	500
Pretreatment:				
GDs 4-8	155 [18]	141 [14]	174 [17]	171 [16]
Treatment:				
GDs 11-14	155 [18]	145 [14]	160 [17]	153 [16]
GDs 14-17	155 [18]	158 [14]	132 [17] (↓15%)	136 [16] (↓12%)
GDs 17-20	198 [18]	159 [13]	170 [17] (↓14%)	150 [15] (↓24%)
Post-treatment:				
GDs 20-23	176 [17]	161 [13]	187 [16]	175 [15]
GDs 26-30	122 [18]	133 [14]	137 [16] (↑12%)	169** [16] (↑39%)

a Data extracted from the study report, Table 7, page 41. Non-pregnant animals, those that aborted, and those that failed to survive to scheduled sacrifice were excluded from the mean by the sponsor. Number of animals measured at each interval is presented in brackets. It was assumed by the reviewers that animals with excess spillage were also excluded by the sponsor, thereby resulting in the various number of animals measured at each interval. Percent difference from controls is presented parenthetically.

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

4. Gross pathology - When compared to concurrent controls, no treatment-related changes were noted in gross pathology. At necropsy, the single 100 mg/kg female that died on GD 4 displayed abnormal contents in the gastrointestinal tract. At the high-dose, a treatment-related decrease (NS) was observed in gravid uterine weight (↓15%) when compared to concurrent controls (Table 3).

5. Cesarean section data - Cesarean section findings are shown in Table 5. At 500 mg/kg, a decrease (NS) in the number of implantations/doe (↓13%) and the number of live fetuses/doe (↓11%) were noted. In addition, an increase in preimplantation loss ($p \leq 0.01$) was noted at the high-dose in the study. However, the Agency contract reviewers noted that the data provided in the report table were incorrect and the percent preimplantation loss was recalculated. The recalculation demonstrated that preimplantation loss was also observed at the mid- (↑42%) as well as the high-dose level (↑152%). No other treatment-related Cesarean section observations were noted.

Table 5. Cesarean section observations [a]

Observation	Dose (mg/kg/day)			
	0	100	250	500
# Animals Assigned (Mated)	20	20	20	20
# Animals Pregnant Pregnancy Rate (%) [b]	18 (90)	15 (75)	20 (100)	18 (90)
# Nonpregnant	2	4	0	2
# Total Does Died # Died Pregnant # Died Nonpregnant # Aborted [d] # Premature Delivery	0 0 0 0 0	1 [c] 0 0 1 0	1 1 0 2 0	0 0 0 2 0
Total # Corpora Lutea Corpora Lutea/Doe	185 10.3	151 10.8	195 11.5	172 10.8
Total # Implantations Implantations/Doe	167 9.3	135 9.6	168 9.9	130 8.1 (↓13%)
Total # Litters Examined	18	14	17	16
Total # Live Fetuses Live Fetuses/Doe	150 8.3	124 8.9	149 8.8	118 7.4 (↓11%)
Total # Dead Fetuses Dead Fetuses/Doe	NR NR	NR NR	NR NR	NR NR
Total # Resorptions Early Late Total Resorptions/Doe Early Late Litters with Total Resorptions	17 7 10 0.9 0.4 0.6 0	11 7 4 0.8 0.5 0.3 0	19 4 15 1.1 0.2 0.9 0	12 7 5 0.8 0.4 0.3 0
Mean Fetal Weight (g) Males Females	43.0 NR NR	42.6 NR NR	42.2 NR NR	41.0 NR NR
Sex Ratio (% Male)	56.1	57.8	43.1*	57.6
Preimplantation Loss (%) [b]	9.7	10.6	13.8 (↑42%)	24.4 [e] (↑152%)
Postimplantation Loss (%) [b]	10.2	8.1	11.3	9.2

a Data extracted from the study report, Tables 4, 9, and Appendix 5, pages 34, 45, 46, and 252 through 255.
b Calculated by reviewers.
c One 100 mg/kg animal died on GD 4 and pregnancy status was not determined.
d All five does were sacrificed after showing signs of abortion, but were only reported as abortions and were not reported as does that died.
e Reported as statistically significant ($p \leq 0.05$) in the MRID, but when calculated by reviewers many of the numbers were incorrect, and therefore, all percent pre- and postimplantation losses were recalculated by the reviewers.
NR Not reported.
* Significantly different from controls at $p \leq 0.05$.

B. DEVELOPMENTAL TOXICITY: Fetal weights were reduced at the high dose level although

no statistical significance was reported by the investigators. No changes were observed in fetal weights. Fetal examinations included external, visceral, and skeletal observations at necropsy.

1. External examination - When compared to concurrent controls, no treatment-related external observations were noted. The most common external findings are shown in Table 6a.

Table 6a. External observations [a]

Observations	Dose (mg/kg/day)			
	0	100	250	500
#Fetuses (#litters) examined	150 (18)	124 (14)	149 (17)	118 (16)
Spina bifida meningocoele	0.7 (5.6)	0 (0)	0 (0)	0.8 (6.3)
Pollex absent on forepaw	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Encephalocoele	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Lower jaws shortened	0 (0)	0.8 (7.1)	0 (0)	0 (0)

a Data extracted from the study report, Tables 10 and 12, pages 47 and 51. For individual observations, data are presented as % fetal incidence (% litter incidence).

2. Visceral examination - When compared to concurrent controls, no treatment-related visceral findings were observed at any dose level tested. The most common observations are shown in Table 6b.

Table 6b. Visceral observations [a]

Observations	Dose (mg/kg/day)			
	0	100	250	500
#Fetuses (#litters) examined	150 (18)	124 (14)	149 (17)	118 (16)
Internal hydrocephaly	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Enlarged aorta	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Reduced pulmonary artery	0 (0)	0.8 (7.1)	0 (0)	0 (0)
All lung lobes extremely reduced in size	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Cyst(s) attached to liver	8.0 (38.9)	6.5 (35.7)	4.0 (23.5)	3.4 (18.8)
Secondary spleen	2.7 (16.7)	2.4 (21.4)	0.7 (5.9)	0.8 (6.3)

a Data extracted from the study report, Tables 10 and 12, pages 47, 51, and 52. For individual observations, data are presented as % fetal incidence (% litter incidence).

3. Skeletal examination - Note that the following data are reported as [% fetal incidence (% litter incidence)].

Decreased ($p \leq 0.05$ or 0.01, fetal incidences only) incidence of a "partially ossified" transverse process in the 7th cervical vertebra was observed at the low- [0.8 (7.1)], mid- [0.7 (5.9)], and high-dose levels [0 (0)] vs controls [6.7 (22.2)]. Also noted was a decreased (not statistically significant) incidence of a "fully ossified" transverse process in the 7th cervical vertebra in all treated groups [0 (0)] vs controls [3.3 (11.1)]. These findings may indicate a general decrease in ossification of the 7th cervical vertebra transverse process. An increased incidence ($p \leq 0.01$, fetal incidences only) of a "partially ossified" odontoid was observed at the low- [64.5 (100)], mid- [61.7 (100)], and high-dose levels [72.0 (100)] vs controls [40.0 (72.2)]. This finding was further supported by an increase in odontoid (not ossified) at the low [0.8 (7.14)], mid [3.14 (11.8)] and high dose levels [2.5 (12.5)]. As well, the incidence of 27 pre-sacral vertebrae was statistically significantly increased at all dose levels [28 (66.7)] in the controls vs [58.9(100)] in the low dose, [65.1(88.2)] at the mid dose and [69.5 (93.8)] at the high dose level.

In addition, a shift toward complete ossification of 5th sternebra was observed and was demonstrated by i) a dose-dependent decrease ($p \leq 0.01$, fetal incidences only) in the number of animals exhibiting a "partially ossified" 5th sternebra at the low- [32.3 (92.9)], mid- [28.9 (76.5)], and high-dose levels [24.6 (62.5)] vs controls [52.0 (94.4)] and ii) a decrease in the number of animals exhibiting a "nonossified" 5th sternebra at the low- [3.2 (14.3)], mid- [3.4 (11.8)], and high-dose levels [4.2 (25.0)] vs controls [12.7 (33.3)]. All fetal incidences of a partially ossified 5th sternebra and the 5th sternebra not ossified were statistically significant ($p \leq 0.05$ or 0.01). Finally, the percentage of fetuses with 13th full ribs was statistically significantly increased ($p \leq 0.01$) at all dose levels(see table 6c). Collectively, the increases in

skeletal variations at all dose levels do not support the establishment of a NOAEL in this study.

In the *manus* assessment, an increase ($p \leq 0.05$ or 0.01) was observed in the proportion of fetuses with a score of 5 at the mid- (5.4%) and high-dose (4.2%) vs 0.0% concurrent controls with a score of 5; however, this increased proportion was not dose-dependent and the mean *manus* scores/litter at the mid- and high-dose level were comparable to the mean *manus* scores of the controls (mid- 2.87% and high-dose 2.94% vs 2.83% concurrent controls), and therefore, this finding was considered not to be treatment-related. Regarding *pes* scores, a decrease ($p \leq 0.01$) was noted in the proportion of high-dose fetuses with a score of 1 (88.1% treated vs 97.3% controls with a score of 1) and an increase was noted in the proportion of fetuses with a score of 2 (11.9% treated vs 2.7% controls with a score of 2). The mean *pes* score/litter at the high-dose level was similar to mean scores for the controls (1.11% treated vs 1.02% controls), and therefore, the decreased or increased number of fetuses with scores of 1 or 2 were considered not to be treatment-related.

Table 6c. Skeletal observations [a]

Observations	Dose (mg/kg/day)			
	0	100	250	500
#Fetuses (#litters) examined	150 (18)	124 (14)	149 (17)	118 (16)
Gross skeletal malformation	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Skull, Cebocephaly	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Fenestration in parietal(s)	0 (0)	0.8 (7.1)	0.7 (5.9)	0 (0)
Gross malformation	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Maxillae fused	0 (0)	1.6 (14.3)	0 (0)	0 (0)
Arch absent in 4th thoracic vertebra	0 (0)	0 (0)	0 (0)	0.8 (6.3)
Arch not ossified in 2nd lumbar vertebra	0.7 (5.6)	0 (0)	0 (0)	0 (0)
Extra arch between 3rd and 4th sacral vertebra	0 (0)	0.8 (7.1)	0 (0)	0 (0)
4th to 6th lumbar arches widespread in vertebral column	0.7 (5.6)	0 (0)	0 (0)	0 (0)
Fused arches, 5th and 6th thoracic	0 (0)	0 (0)	0 (0)	0.8 (6.3)
8th and 9th thoracic	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Ribs, Absent 4th rib	0 (0)	0 (0)	0 (0)	0.8 (6.3)
5th and 6th ribs, extremely fused	0 (0)	0 (0)	0 (0)	0.8 (6.3)
Forepaw, Pollex absent	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Pollex reduced	0 (0)	0.8 (7.1)	0 (0)	0 (0)
Transverse process partially ossified 7th cervical vertebra	6.7 (22.2)	0.8* (7.1)	0.7* (5.9)	0** (0)
Transverse process fully ossified 7th cervical vertebra	3.3 (11.1)	0 (0)	0 (0)	0 (0)
5th sternebra not ossified	12.7 (33.3)	3.2** (14.3)	3.4** (11.8)	4.2* (25.0)
13th short length and floating extra rib	14.0 (61.1)	5.6* (35.7)	2.7** (17.6)*	5.9* (37.5)
Partially ossified odontoid	40.0 (72.2)	64.5** (100)	61.7** (100)	72.0** (100)
Asymmetrical development in 1st sacral vertebra	18.7 (83.8)	16.9 (78.6)	14.8 (58.8)	14.4 (68.8)
27 pre-sacral vertebra in vertebral column	28.0 (66.7)	58.9** (100)*	65.1** (88.2)	69.5** (93.8)
Partially ossified 5th sternebra	52.0 (94.4)	32.3** (92.9)	28.9** (76.5)	24.6** (62.5)
13th normal length extra rib	42.0 (83.3)	78.2** (100)	82.6** (100)	81.4** (100)

a Data extracted from the study report, Tables 10 and 12, pages 48, and 53 through 59. For individual observations, data are presented as % fetal incidence (% litter incidence).

* or ** Significantly different from controls at $p \leq 0.05$ or 0.01.

Table 6d. *Manus/pes* assessment (% fetal incidence) [a]

Observations	Dose (mg/kg/day)			
	0	100	250	500
#Fetuses (#litters) examined	150 (18)	124 (14)	149 (17)	118 (16)
***Manus* scores**				
Proportion with score 2	17.3	10.5	20.1	13.6
Proportion with score 3	80.7	83.9	72.5	77.1
Proportion with score 4	2.0	3.2	2.0	5.1
Proportion with score 5	0.0	2.4	5.4**	4.2*
Mean *manus* score/litter	2.83	2.97	2.87	2.94
***Pes* scores**				
Proportion with score 1	97.3	92.7	92.6	88.1** (↓9%)
Proportion with score 2	2.7	7.3	7.4	11.9** (↑341%)
Mean *pes* score/litter	1.02	1.08	1.06	1.11

a Data extracted from the study report, Table 13, page 60.
* or ** Significantly different from controls at $p \leq 0.05$ or 0.01.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS - 1) Maternal toxicity: Administration of the test substance at 250 and 500 mg/kg yielded abortions that were considered to be equivocally treatment-related.

2) Developmental toxicity: The investigators noted that at dose levels of ≥100 mg/kg resulted in changes in the ossification (either reduced or additional ossification centers) of the fetal skeleton, but that no increases in major malformations were observed. Based on their assessment of the data (which apparently excludes all (?) developmental anomalies except major malformations, it was concluded that a developmental LOAEL was not observed and the NOAEL was ≥500 mg/kg/day.

It was concluded that the "overall" NOAEL for this study is 100 mg/kg/day. This apparently included maternal toxicity (abortions).

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Mesotrione (96.8% a.i.) in deionized water was administered to pregnant New Zealand White rabbits (20/dose) at dose levels of 0, 100, 250, or 500 mg/kg/day by gavage on GDs 8 through 20. All does were sacrificed on GD 30. 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.

A single 100 mg/kg animal was found dead on GD 4 and abnormal GI tract contents were observed at necropsy. One 250 mg/kg animal was sacrificed *in extremis* on GD 22 after displaying clinical signs including diarrhea, subdued behavior, thin appearance, and severe weight loss. Five does were sacrificed after showing signs of abortion as follows: one 100 mg/kg on GD 29; two 250 mg/kg on GD 25 or 28; and two 500 mg/kg on GD 23 or 25.

At 100 mg/kg/day, findings observed that were considered to be possibly treatment-related included few feces on the tray (6 observations in 5/20 animals vs 3 observations in 2/20 controls) and red to brown colored urine (14 incidents in 6/20 animals vs 1 incident in 1/20 controls).

At 250 mg/kg/day, findings observed that were considered to be treatment-related included the following: few feces on the tray (11 observations in 4/20 animals vs 3 observations in 2/20 controls); no feces on the tray (4 observations in 3/20 animals vs 0/20 controls); red to brown colored urine (26 incidents in 12/20 animals vs 1 incident in 1/20 controls); and decreases (NS) in food consumption during GDs 14-20 (↓14-15%) and increases (NS) during the post-treatment interval (↑12%, GDs 26-30).

At 500 mg/kg/day, treatment-related observations included the following: blood on the tray (6 observations in 4/20 animals vs 1 incidence in 1/20 controls); few feces on the tray (25 incidences in 7/20 animals vs 3 observations in 2/20 controls); no feces on the tray (6 observations in 3/20 animals vs 0/20 controls); and red to brown colored urine (41 observations in 12/20 animals vs 1 incident in 1/20 controls); decreases (NS) in food consumption during GDs 14-20 (↓12-24%) and increases during post-treatment (↑39%, GDs 26-30, $p \leq 0.01$); a decrease (NS) in gravid uterine weight (↓15%); and a decrease (NS) in the number of implantations/doe (↓13%).

When compared to concurrent controls, no treatment-related changes in maternal body weight, adjusted body weight, or gross pathology were noted at any dose level tested. Due to the increased incidence of preimplantation loss at the mid and high dose levels (suggesting early administration of the test material prior to the completion of implantation), the interpretation of maternal toxicity in this study may be confounded.

Maternal LOAEL = 250 mg/kg/day, based on abortionsm and clinical signs of toxicity Maternal NOAEL = 100 mg/kg/day

2. DEVELOPMENTAL TOXICITY:

a. Deaths/resorptions: The numbers of resorptions/doe for the treatment groups were not

different from the concurrent controls. At 500 mg/kg, a decrease (NS) in the number of live fetuses/doe (↓11%) was noted. This may be associated with an increase in percent in preimplantation loss ($p \leq 0.01$ at the high-dose), but errors were observed in the calculation, and therefore, the percent losses were recalculated by reviewers. As a result of the Agency contractor re-calculation, increases were observed at both the mid- (↑42%) and high-dose (↑152%) levels. It was thought by the reviewers that these observations of increased preimplantation losses (which appeared to be associated with dose level) were the result of incorrect dosing initiation. If true, this error would result in a study that was compromised and invalid.

b. Altered growth: Although not reported as statistically significant by the investigators, there was a decrease in mean fetal body weight at the high dose level. In addition, a number of other alterations (including extra ribs and 27 pre-sacral vertebra) were noted at all dose levels.
c. Minor defects or variants: There were no treatment-related external or visceral minor defects noted at any dose level. When compared to controls at skeletal examination (reported as [% fetal incidence (% litter incidence)]), there was a shift toward a decreased degree of ossification of the 7th cervical vertebra transverse process as evidenced by i) a decreased ($p \leq 0.05$ or 0.01, fetal incidences only) incidence of a "partially ossified" transverse process at the low- [0.8 (7.1)], mid- [0.7 (5.9)], and high-dose levels [0 (0)] vs controls [6.7 (22.2)] and ii) a decreased (NS) incidence of a "fully ossified" transverse process in the 7th cervical vertebra in all treated groups [0 (0)] vs controls [3.3 (11.1)]. In addition, an unexpected and unusual shift toward complete ossification of 5th sternebra was observed and was demonstrated by i) a dose-dependent decrease ($p \leq 0.01$, fetal incidences only) in the number of animals exhibiting a "partially ossified" 5th sternebra at the low- [32.3 (92.9)], mid- [28.9 (76.5)], and high-dose levels [24.6 (62.5)] vs controls [52.0 (94.4)] and ii) a decrease ($p \leq 0.05$ or 0.01, fetal incidences only) in the number of animals exhibiting a "nonossified" 5th sternebra at the low- [3.2 (14.3)], mid- [3.4 (11.8)], and high-dose levels [4.2 (25.0)] vs controls [12.7 (33.3)]. As well, the incidence of fused sternebrae was increased between the 3rd and 4th [1.7(12.5)] and the 4th and 5th at the highest dose level. Other changes included an increase in the incidence of 13 full ribs and 27 pre-sacral vertebra at all dose levels,
d. *Manus/pes* skeletal assessment: In the manus assessment, an increase was observed in the proportion of fetuses with a score of 5 at both the mid and high dose levels.
e. Major defects: An increase in major malformations was not apparent.

Developmental LOAEL = 100 mg/kg/day, based on delays in ossification as evidenced by partially ossified transverse process and increases in 13 full ribs and 27 pre-sacral vertebra.
Developmental NOAEL = Not established

Because of the increase in preimplantation loss (in several dose groups) which appears to be a confounding factor and because of statistically significant alterations in skeletal ossification

noted at all test levels, this developmental toxicity study is classified **unacceptable/ not upgradeable** (§83-3[b]) and does not satisfy the guideline requirement for a developmental toxicity study in the rabbit.

C. STUDY DEFICIENCIES - The following deficiencies were noted:

- The contract reviewers believe that incorrect dosing initiation occurred as follows: 1) in this study, the day mating was observed was designated as GD 1, but by research, the day mating is observed may be categorized as early as GD -1 with the day of sperm in a vaginal smear being GD 0, etc.; 2) if this discrepancy proves true, then the dosing start on GD 8 would really be GD 6, the day of implantation in rabbits. Regardless, since there was an increase in preimplantation loss in the mid and high dose groups in this study, dosing may have begun prior to the completion of implantation. This may confound interpretation of study data.

- There is no NOAEL for developmental toxicity

- an insufficient number of females were mated to yield 20 animals/dose group with implantation sites

- As per the contractor reviewers, a number of calculation errors were noted.

- The study investigators did not properly include all of the developmental toxicity observed in this study in their interpretations/conclusions relative to the potential of the test material to induce developmental toxicity.

APPENDIX 1

APPENDIX D - SCALE FOR ASSESSMENT OF SKELETAL OSSIFICATION OF THE *MANUS* AND *PES*

Scale for *manus:*

1. (good) 2nd row of phalanges fully ossified.
2. 2nd row of phalanges one or more incompletely ossified, rest fully ossified.
3. 2nd row of phalanges one or more fully ossified, rest incompletely ossified with no more than one unossified.
4. 2nd row of phalanges incompletely ossified with one or more unossified.
5. 2nd row of phalanges unossified.
6. (poor) 2nd row of phalanges unossified, Ist and 3rd row phalanges with one or more not ossified.

Scale for *pes:*

1. (good) 2nd row of phalanges one or more incompletely or fully ossified.
2. 2nd row of phalanges unossified, Ist row of phalanges no more than one incompletely ossified, rest fully ossified.
3. 2nd row of phalanges unossified, Ist row of phalanges two or more incompletely ossified, rest fully ossified.
4. 2nd row of phalanges unossified, Ist row of phalanges no more than one unossified, one or more fully ossified, rest incompletely ossified.
5. 2nd row of phalanges unossified, Ist row of phalanges one or more unossified, rest incompletely ossified.
6. (poor) Ist and 2nd row of phalanges unossified.

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