8-2-2001

DATA EVALUATION RECORD

MESOTRIONE

Study Type: §83-3[a]; Developmental Toxicity of Mesotrione in Mice

Work Assignment No. 2-01-52AA (MRIDs 44920802 and 44901708)

Prepared for

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U.S. Environmental Protection Agency
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DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity in Mice with Rangefinding
OPPTS Number: 870.3700 OPP Guideline Number: §83-3a

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). ZA1296: Developmental Toxicity Study in Mice. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Report # CTL/P/6238, Laboratory Study # RM0793, June 29, 1999. MRID 44920802. Unpublished.

Diggins, G. (1997). ZA1296: Preliminary Developmental Toxicity Study in the Mouse. Quintiles Toxicology/Pathology Services, Quintiles England Limited, Ledbury, Herefordshire, UK. Laboratory Project ID ICL/022/97, September 5, 1997. MRID 44901708. Unpublished.

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

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44920802), mesotrione (96.8% a.i., Lot #: P17) in water was administered to pregnant Alpk:AP$_f$CD-1 mice (26/dose) at dose levels of 0, 10, 60, 150, or 600 mg/kg/day by gavage on gestation days (GDs) 5 through 18. All dams were sacrificed on GD 19. One 60 mg/kg female was sacrificed *in extremis* on GD 12 following the observation of a subcutaneous mass on the left anterior thorax on GD 9 and subsequent reduced body weight and food consumption. No other premature deaths occurred during the study.

When compared to concurrent controls, no treatment-related clinical signs, changes in body weight or adjusted body weights (using GD 5 body weight as a covariant), gravid uterine weight, food consumption, gross pathology, or reproductive parameters were noted at any dose level tested.

The maternal LOAEL was not observed.
The maternal NOAEL was ≥ 600 mg/kg/day.

At 600 mg/kg, a treatment-related pattern toward decreased ossification of the cervical vertebrae centra was observed. In addition, a number of other delays in ossification or variations were apparent at the high dose level (see Table 4c).

The developmental LOAEL is 600 mg/kg/day based on the pattern toward decreased ossification of the cervical vertebrae centra.
The developmental NOAEL is 150 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 mouse.

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

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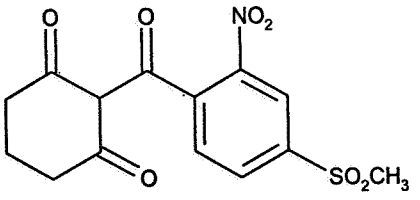
I. MATERIALS AND METHODS

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 for up to 23 days.
   CAS #: 104206-82-8
   Structure:



2. Vehicle: Water

3. Test animals: Species: Mouse
   Strain: Alpk:APfCD-1
   Age and mean weight range of females: Approximately 9 weeks old, 34.2-37.1 g on gestation day 1
   Source: Rodent Breeding Unit, Alderley Park, Cheshire, UK
   Housing: Individually; no further information provided
   Diet: R&M No. 3 diet, ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 22±3 °C
   Humidity: 30-70%
   Air changes: At least 15/hr
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period: 4 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 11/5/98 end: 3/5/99

2. Mating: Females were paired at the suppliers. The day on which a vaginal plug was detected was designated as gestation day (GD) 1. Successfully mated females were delivered to the laboratory on GD 1 over a 3 week period.

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

Table 1. Animal assignment [a]

Dose Group	Dose (mg/kg/day)	Number of Females
Control-1	0	30
Control-2 [b]	0	30
Low	10	30
Low-mid	60	30
Mid-high	150	30
High	600	30

a It was stated on page 25 of the study report that a number of mice from some of the first matings littered prior to terminal sacrifice. The reason for this was not fully ascertained although the use of virgin or inexperienced males for mating may have been the cause since the problem was not repeated when the same males were used later in the study. As a result of the atypical performance of the males at study start, a number of animals were excluded from statistical analysis of the data. Only 26 females/dose group were evaluated.

b No information was provided regarding the need for 2 control groups; when calculating the percent difference, reviewers averaged the data for the 2 control groups.

4. Dose selection rationale: Doses were selected based on the findings of a rangefinding study submitted with the current study (MRID 44901708) in which Crl:CD-1(ICR)BR mice were dosed at 0, 300, or 600 mg/kg/day during GDs 7-16. Does were sacrificed on GD 19. No premature deaths or clinical signs of toxicity were observed. No treatment-related effects were noted in body weights, food consumption, or gross pathology. Additionally, no effects were observed in the numbers of implantations/doe, live fetuses/doe, pre- and postimplantation losses, fetal weights, or percent male. No major treatment-related defects were observed at fetal external, visceral, or skeletal examination. At 600 mg/kg, the following minor skeletal defects were observed [% fetal incidence (% litter incidence)]: one or more cervical vertebra centra incompletely ossified [12.0 (50.0) vs controls 7.6 (25.0)]; one or more cervical vertebra centra not ossified [4.5 (30.0) vs controls 3.5 (16.7)]; one or more additional ossified sternum centra [3.1 (20.0) vs 1.0 (8.3) controls]; and one or more bilobed, bipartite, misshapen, or misaligned sternum [6.2 (30.0) vs 3.1 (16.7) controls]. The following skeletal variants were noted at 600 mg/kg [% fetal incidence (% litter incidence)]: incomplete ossification of the occipital bone [26.5 (80.0) vs 15.6 (41.7) controls]; 14 thoracic vertebrae [28.5 (70.0) vs 20.7 (50.0) controls]; 5 lumbar vertebrae [28.5 (70.0) vs 20.7 (50.0) controls]; 14th uni- or bilateral extra rib [28.5 (70.0) vs 20.7 (50.0) controls]; and astragalus uni- or bilateral not ossified hindlimb [6.0 (40.0) vs 1.4 (8.3) controls]. None of these findings were statistically significant. The maternal LOAEL was not established. It was stated that this study was not designed to provide a definitive view of the potential of the test

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substance to induce changes in fetal development; there were no treatment-related major abnormalities and no statistically significant changes for any of the specific minor abnormalities or variants. A developmental LOAEL was not provided.

Also cited in the study report was a rangefinding study (CTL/P/6231) in which mice (5/group) were dosed at concentrations of 0, 600, 800, or 1000 mg/kg/day. It was stated that two 1000 mg/kg mice were found dead after 3/4 doses and 1 female receiving 800 mg/kg was found dead after 8 doses. No further information was provided.

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 twice during the study by mixing the appropriate amount of water with the test substance. Following preparation, the formulations were subdivided into aliquots and fresh aliquots were used daily. Dose formulations were stored at room temperature. Prior to the start of the study, samples (top, middle, bottom) of 1 and 60 mg/mL formulations were analyzed for homogeneity. Also prior to the study, samples of 1 and 60 mg/mL concentrations were analyzed after storage at room temperature for up to 23 days. Concentration analyses were performed twice.

   Results:

   Homogeneity analyses (range as mean % of nominal): 96.8-108.0%

   Stability analyses (range as mean % of Day 0): 95.4-103.6%

   Concentration analyses (range as mean % of nominal): 88.0-108.0%

   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 5 through 18 in a volume of 1 mL/100 g body weight. Dosing was based on the GD 5 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 1, 5 through 18, and 19. Food consumption was recorded for the GDs 1-3, 3-5, 5-8, 8-11, 11-14, 14-17, and 17-19 periods. All does were sacrificed on GD 19.

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
- gravid uterine weight
- number of corpora lutea
- number and location of implantation sites
- number of live fetuses
- number of resorptions (early and late)

The uteri of all apparently nonpregnant females were stained to confirm pregnancy status.

2. Fetal evaluations - Each fetus was weighed and examined for external abnormalities including an examination of the oral cavity. All fetuses were then examined for visceral abnormalities, sexed, eviscerated, and fixed in 70% industrial methylated spirits. Following approximately 24 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 and Alcian Blue for skeletal assessment. Observations were classified as major defects, minor defects, or variants. The degree of skeletal ossification was analyzed, including the *manus/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: No indices calculations were provided in the study report.

3. Historical control data: No historical control data were provided to allow for comparison with treated groups.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical observations: One 60 mg/kg female was sacrificed *in extremis* on GD 12 following the observation of a subcutaneous mass on the left anterior thorax on GD 9 and reduced body weight and food consumption. No other premature deaths

occurred during the study. When compared to concurrent controls, no treatment-related clinical signs were observed at any dose level.

2. Body weight: When compared to concurrent controls, no treatment-related changes in body weight (Table 2), adjusted body weights (using GD 5 body weight as a covariant), or gravid uterine weight were noted at any dose level tested.

Table 2. Selected mean maternal body weights (g) [a]

Interval	Dose in mg/kg/day					
	0 n = 24	0 n = 26	10 n = 24	60 n = 25	150 n = 25	600 n = 23
Pre-treatment: GD 1	35.5	34.2	36.4	36.6	35.3	37.1
Treatment: GD 5 GD 11 GD 15 GD 18	37.7 41.7 50.9 61.6	36.4 40.4 49.3 59.7	38.8 43.5 52.9 64.2	38.3 43.1 52.4 64.0	37.6 42.2 51.4 62.9	39.1 43.4 52.8 64.8
Post-treatment: GD 19	65.4	63.5	68.4	68.2	67.3	69.2
Corrected body weight gain: [b] GD 19	44.2 (21.3)	42.7 (20.8)	45.2 (23.3)	45.8 (22.5)	44.7 (22.5)	45.5 (23.6)

a Data extracted from the study report, Tables 6 and 9, pages 38 through 40, and page 44. Nonpregnant females or does not surviving to scheduled termination were excluded from the mean by the sponsor.
b Corrected for gravid uterine weight; gravid uterine weight is presented parenthetically.

3. Food consumption - When compared to concurrent controls, no treatment-related changes in food consumption were observed at any dose level tested. Several statistically significant increases were noted (↑5-11%, $p \leq 0.05$ or 0.01) in all treatment groups throughout the study; these increases were minor and considered not to be of toxicological concern.

4. Gross pathology - When compared to concurrent controls, no treatment-related changes were noted in gross pathology.

5. Cesarean section data - Cesarean section findings are shown in Table 3. The number of implantations/dam, resorptions/dam, pre- and postimplantation losses, and percent male were similar between control and treated groups. Increases in mean fetal weight (males and females, separately and combined) were noted at 10 and 600 mg/kg (↑5-6%, $p \leq 0.05$) and in the females only at 60 mg/kg (↑5%, $p \leq 0.05$), however, these increases were minor

and not dose-dependent and considered not to be treatment-related.

Table 3. Cesarean section observations [a]

Observation	Dose (mg/kg/day)					
	0	0	10	60	150	600
# Animals Assigned (Mated) [b]	26	26	26	26	26	26
# Animals Pregnant Pregnancy Rate (%) [c]	24 (92)	26 (100)	24 (92)	25 (96)	25 (96)	23 (88)
# Nonpregnant	2	0	2	1	1	3
# Total Does Died # Died Pregnant # Died Nonpregnant # Aborted # Premature Delivery	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	1 0 1 0 0	0 0 0 0 0	0 0 0 0 0
Total # Corpora Lutea [c] Corpora Lutea/Doe	380 15.8	393 15.1	377 15.7	390 15.6	384 15.4	381 16.6
Total # Implantations [c] Implantations/Doe	327 13.6	338 13.0	320 13.3	347 13.9	340 13.6	320 13.9
Total # Litters Examined	24	26	24	25	25	23
Total # Live Fetuses [c] Live Fetuses/Doe	299 12.5	315 12.1	302 12.6	315 12.6	313 12.5	294 12.8
Total # Dead Fetuses Dead Fetuses/Doe	NR NR	NR NR	NR NR	NR NR	NR NR	NR NR
Total # Resorptions [c] Early [c] Late [c] Total Resorptions/Doe [c] Early [c] Late [c] Litters with Total Resorptions	28 22 6 1.17 0.92 0.25 0	23 18 5 0.88 0.69 0.19 0	18 15 3 0.75 0.63 0.13 0	32 25 7 1.28 1.0 0.28 0	27 20 7 1.08 0.80 0.28 0	26 21 5 1.13 0.91 0.22 0
Mean Fetal Weight (g) Males Females	1.27 1.30 1.25	1.29 1.32 1.26	1.34* (↑6%) 1.37* (↑5%) 1.31* (↑5%)	1.32 1.33 1.31* (↑5%)	1.32 1.33 1.30	1.34* (↑6%) 1.36* (↑5%) 1.32* (↑6%)
Sex Ratio (% Male)	58.6	48.3	51.1	56.6	58.7	54.4
Preimplantation Loss (%) [c]	13.9	14.0	15.1	11.0	11.5	16.0
Postimplantation Loss (%) [c]	8.6	6.8	5.6	9.2	7.9	8.1

a Data extracted from the study report, Tables 4, 9, and Appendix 5, pages 35, 44, 45, and 125 through 130. Percent difference from controls is presented parenthetically.

b Recall that as a result of the atypical performance of the males at study start, a number of animals were excluded from statistical analysis of the data. Individual data were provided for 30 does/group. Summary data were provided for 23-26 does/group. It was deduced by reviewers which does were to be excluded from the individual data by comparing these data with the fetal defect data in Appendix 6, pages 132 through 137.

c Calculated by reviewers.

B. DEVELOPMENTAL TOXICITY: 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 4a.

Table 4a. External observations [a]

Observations	Dose (mg/kg/day)					
	0	0	10	60	150	600
#Fetuses (#litters) examined	299 (24)	315 (26)	302 (24)	315 (25)	313 (25)	294 (23)
Major defects						
Encephalocoele	0.3 (4.2)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Pinna malformed	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Thoracogastroschisis	0 (0)	0 (0)	0.3 (4.2)	0 (0)	0 (0)	0 (0)
Gastroschisis	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0.7 (8.7)
Omphalocoele	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.3)
Umbilical hernia	0.3 (4.2)	0 (0)	0.3 (4.2)	0 (0)	0.3 (4.0)	0 (0)
Extra digit(s) on forelimb or paw	0 (0)	1.0 (3.8)	0 (0)	0 (0)	0 (0)	0 (0)
Fused digit(s) on forelimb or paw	0.3 (4.2)	0.3 (3.8)	0 (0)	0 (0)	0 (0)	0.3 (4.3)
Shortened digit(s) on forelimb or paw	0.3 (4.2)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0.7 (8.7)
Forelimb shortened	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.3)
Extra digit(s) on hindlimb or paw	0.3 (4.2)	1.0 (3.8)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Fused digit(s) on hindlimb or paw	0 (0)	1.0 (3.8)	0 (0)	0 (0)	0 (0)	0 (0)
Shortened digit(s) on hindlimb or paw	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.3)
Hindlimb malrotated	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.7 (8.7)
Hindpaw extremely flexed	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0 (0)
Microphthalmia	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lower jaw shortened	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Jaw stiffened	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Cleft palate	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0.7 (8.7)
Minor defects						
Hindpaw slightly flexed	0 (0)	0.3 (3.8)	0.3 (4.2)	1.6* (20.0)*	0.6 (8.0)	1.4 (8.7)

a Data extracted from the study report, Tables 10 and 12, pages 46, and 52 through 54. For individual observations, data are presented as % fetal incidence (% litter incidence).

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

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 4b.

Table 4b. Visceral observations [a]

Observations	Dose (mg/kg/day)					
	0	0	10	60	150	600
#Fetuses (#litters) examined	299 (24)	315 (26)	302 (24)	315 (25)	313 (25)	294 (23)
Major defects						
Situs inversus	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pulmonary artery fused to the right subclavian	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Encephalocoele	0.3 (4.2)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Thoracogastroschisis	0 (0)	0 (0)	0.3 (4.2)	0 (0)	0 (0)	0 (0)
Gastroschisis	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0.7 (8.7)
Omphalocoele	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.3)
Umbilical hernia	0.3 (4.2)	0 (0)	0.3 (4.2)	0 (0)	0.3 (4.0)	0 (0)
Lung lobe(s) absent	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Ureter extremely dilated	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Minor defects						
Thymus discolored	1.0 (12.5)	0.3 (3.8)	2.3 (29.2)*	1.6 (16.0)	1.6 (20.0)	1.0 (13.0)
Umbilical artery left of bladder	3.0 (33.3)	3.8 (19.2)	2.3 (16.7)	2.2 (24.0)	4.2 (28.0)	5.4 (30.4)

a Data extracted from the study report, Tables 10 and 12, pages 46, 52, and 54 through 56. For individual observations, data are presented as % fetal incidence (% litter incidence).

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

3. Skeletal examination -When compared to concurrent controls, no treatment-related major skeletal defects were observed at any dose level tested. The most common observations and the *manus/pes* data are shown in Table 4c. At 600 mg/kg, a treatment-related pattern toward decreased ossification of the cervical vertebrae centra was observed; the decreases in ossification were classified as minor skeletal defects or variants. At the high-dose level, the findings were as follows [% fetal incidence (% litter incidence)]: cervical centrum 2 not ossified [6.1 (34.8) treated vs 3.0 (29.2) controls; $p \leq 0.01$ for the fetal incidence]; cervical centrum 3 not ossified [17.0 (65.2) treated vs 13.0 (54.2); $p \leq 0.01$ for the fetal incidence]; cervical centrum 4 not ossified [21.1 (73.9) treated vs 15.1 (54.2) controls; $p \leq 0.05$ or 0.01 for the fetal and litter incidences]; cervical centrum 5 not

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ossified [19.7 (73.9) treated vs 12.4 (54.2) controls; $p \leq 0.05$ or 0.01 for the fetal and litter incidences]; cervical centrum 6 not ossified [10.2 (52.2) treated vs 7.4 (29.2) controls; $p \leq 0.01$ for the fetal incidence]; and cervical centrum 7 not ossified [5.1 (30.4) treated vs 1.3 (12.5) controls; $p \leq 0.01$ for the fetal incidence]. It should be noted that this pattern was also observed in rats administered the test substance (MRID 44920801). No treatment-related changes were noted in the *manus/pes* ossification data.

Table 4c. Skeletal observations [a]

Observations	Dose (mg/kg/day)					
	0	0	10	60	150	600
#Fetuses (#litters) examined	299 (24)	315 (26)	302 (24)	315 (25)	313 (25)	294 (23)
Major defects						
Frontals, extreme incomplete ossification	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Interparietal absent	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ventral jugal displaced	0.3 (4.2)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Mandibles fused	0.3 (4.2)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Mandible misshapen ossification	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Mandible shortened, ventral	0.3 (4.2)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Nasal extreme incomplete ossification	0 (0)	0 (0)	0 (0)	1.3* (8.0)	0 (0)	0 (0)
Premaxillae fused	0.3 (4.2)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Premaxilla shortened	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Pterygoid processes widespread	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.3)
Gross skull malformation	0.3 (4.2)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Ventral palate fused to cartilage of arch 5	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0 (0)
Misaligned cervical vertebrae	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Unidentified vertebra(e) absent	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Thoracic arch 1 fused to cervical arch 7	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0 (0)
Thoracic arch 4 fused to arch 3	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Widespread thoracic arches	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.3)
Cartilaginous sacral arch 1 cleft	0 (0)	0 (0)	0.3 (4.2)	0 (0)	0 (0)	0 (0)
Misaligned sacral vertebrae	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Sternebra 4 cleft	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.3)
Sternal cartilage cleft between 4 and 3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.3)
Sternebra 5 cleft	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.3)
Sternal cartilage cleft between 5 and 4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.3)
Sternebra 6 cleft	0.3 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0.7 (8.7)
Sternal cartilage cleft between 6 and 5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (4.3)
All sternebra cleft	0 (0)	0 (0)	0.3 (4.2)	0 (0)	0 (0)	0 (0)
Rib 13 absent	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.7 (4.3)
Rib 4 costal cartilage fused to rib 3, extreme	0 (0)	0.3 (3.8)	0 (0)	0 (0)	0 (0)	0 (0)
Extra phalange(s) present on hindpaw	0.3 (4.2)	1.0 (3.8)	0 (0)	0.3 (4.0)	0 (0)	0 (0)
Extra metatarsal(s) present on hindpaw	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	0 (0)

(continued on next page, footnotes follow)

Table 4c. Skeletal observations (con't.)

Observations	Dose (mg/kg/day)					
	0	0	10	60	150	600
#Fetuses (#litters) examined	299 (24)	315 (26)	302 (24)	315 (25)	313 (25)	294 (23)
Minor defects						
Cervical centrum 2 not ossified	3.0 (29.2)	1.6 (11.5)	4.3 (20.8)	6.7** (28.0)	3.5 (32.0)	6.1** (34.8)
Cervical centrum 6 not ossified	7.4 (29.2)	2.5 (23.1)	9.6* (41.7)	8.3 (44.0)	6.1 (40.0)	10.2** (52.2)
Cervical centrum 7 not ossified	1.3 (12.5)	0.6 (7.7)	5.3** (29.2)	5.7** (20.0)	0.6 (8.0)	5.1** (30.4)
Odontoid not ossified	2.7 (25.0)	2.5 (23.1)	5.0 (29.2)	6.7** (28.0)	4.5 (32.0)	7.5** (47.8)
Cervical arch 2 narrowed with ossification centre	5.7 (41.7)	4.4 (34.6)	2.3 (25.0)	2.2 (28.0)	1.9* (16.0)	1.0** (8.7)*
Sternebra 2 slightly offset hemicentres	1.3 (12.5)	0.6 (7.7)	2.6 (25.0)	2.2 (20.0)	2.2 (28.0)	3.4* (30.4)
Sternebra 4 slightly offset hemicentres	3.0 (29.2)	5.1 (50.0)	5.3 (54.2)	8.3* (56.0)	6.4 (52.0)	8.2* (56.5)
Sternebra 5 incompletely cleft	6.0 (41.7)	4.8 (26.9)	4.3 (37.5)	4.8 (24.0)	3.2 (32.0)	9.9* (56.5)
Sternebra 6 hemicentres incompletely fused	7.4 (41.7)	3.5 (23.1)	9.9* (45.8)	7.3 (32.0)	6.7 (44.0)	15.0** (69.6)**
Sternebra 6 incompletely ossified	2.3 (16.7)	0.3 (3.8)	3.6* (29.2)	5.1** (12.0)	3.2 (28.0)	4.8** (34.8)*
Rib 13 shortened	0 (0)	0 (0)	0 (0)	0.3 (4.0)	0 (0)	1.4* (4.3)
Rib 14, short length	1.0 (8.3)	1.0 (11.5)	3.0 (8.3)	3.2* (16.0)	1.6 (12.0)	5.4** (30.4)
Rib 7, long length	5.7 (33.3)	5.1 (34.6)	8.3 (37.5)	3.2 (28.0)	3.2 (32.0)	2.0* (21.7)
Variants						
Interfrontal suture bone(s) not ossified	31.4 (87.5)	17.1 (73.1)	24.8 (83.3)	24.1 (88.0)	27.2 (88.0)	33.3** (100)*
Cervical centrum 3 not ossified	13.0 (54.2)	3.5 (26.9)	11.9 (50.0)	11.4 (56.0)	12.5* (52.0)	17.0** (65.2)
Cervical centrum 4 not ossified	15.1 (54.2)	5.4 (26.9)	14.6 (58.3)	12.1 (52.0)	14.7 (56.0)	21.1** (73.9)*
Cervical centrum 5 not ossified	12.4 (54.2)	3.5 (26.9)	11.9 (54.2)	11.1 (52.0)	12.8* (48.0)	19.7** (73.9)*
Calcaneum ossified	28.8 (58.3)	36.2 (84.6)	31.1 (62.5)	18.4** (60.0)	22.0** (64.0)	11.2** (47.8)

a Data extracted from the study report, Tables 10 and 12, pages 47, and 57 through 73. For individual observations, data are presented as % fetal incidence (% litter incidence).
* or ** Significantly different from controls at $p \leq 0.05$ or 0.01.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS - Administration of the test substance at dose levels of up to 600 mg/kg/day resulted in no maternal toxicity. The maternal LOAEL was not established and the NOAEL was ≥600 mg/kg/day.

Dose levels of ≤600 mg/kg resulted in no developmental toxicity. A slight reduction in ossification of the centra of the cervical vertebrae, the sternebra, and the calcanea were noted, but were considered to be transient in nature, not of toxicological significance in terms

of post-natal development, and not treatment-related. The developmental LOAEL was not established and the NOAEL was ≥600 mg/kg/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Mesotrione (96.8% a.i.) in water was administered to pregnant Alpk:AP$_f$CD-1 mice (26/dose) at dose levels of 0, 10, 60, 150, or 600 mg/kg/day by gavage on GDs 5 through 18. All does were sacrificed on GD 19. A number of mice from some of the first matings littered prior to terminal sacrifice. The reason for this was not fully ascertained although the use of virgin or inexperienced males for mating may have been the cause since the problem was not repeated when the same males were used later in the study. As a result of atypical performance of stock males at study start, a number of animals were excluded from statistical analysis of the data and only 26 females/dose group were evaluated. 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 60 mg/kg female was sacrificed *in extremis* on GD 12 following the observation of a subcutaneous mass on the left anterior thorax on GD 9 and reduced body weight and food consumption. No other premature deaths occurred during the study.

   When compared to concurrent controls, no treatment-related clinical signs, changes in body weight, adjusted (to GD 5 body weight) body weights, gravid uterine weight, food consumption, or gross pathology were noted at any dose level tested. Additionally, the number of implantations/dam, resorptions/dam, pre- and postimplantation losses, and percent male were similar between control and treated groups.

   Maternal LOAEL = Not observed
   Maternal NOAEL ≥600 mg/kg/day

2. DEVELOPMENTAL TOXICITY:

   a. Deaths/Resorptions: The numbers of resorptions/doe or number of live fetuses/doe for the treatment groups were not different from the concurrent controls.
   b. Altered Growth: There were no treatment-related changes in fetal body weights. Increases in mean fetal weight (males and females, separately and combined) were noted at 10 and 600 mg/kg (↑5-6%, $p \leq 0.05$) and in the females only at 60 mg/kg (↑5%, $p \leq 0.05$); however, these increases were minor, not dose-dependent, and considered not of toxicological concern.
   c. Minor defects/Variants: There were no treatment-related external or visceral minor defects or variants noted at any dose level. At 600 mg/kg, a treatment-related pattern toward decreased ossification of the cervical vertebra centra was observed; the decreases in ossification were classified as minor skeletal defects or variants. At the

high-dose level, the findings were as follows [% fetal incidence (% litter incidence)]: cervical centrum 2 not ossified [6.1 (34.8) treated vs 3.0 (29.2) controls; $p \leq 0.01$ for the fetal incidence]; cervical centrum 3 not ossified [17.0 (65.2) treated vs 13.0 (54.2); $p \leq 0.01$ for the fetal incidence]; cervical centrum 4 not ossified [21.1 (73.9) treated vs 15.1 (54.2) controls; $p \leq 0.05$ or 0.01 for the fetal and litter incidences]; cervical centrum 5 not ossified [19.7 (73.9) treated vs 12.4 (54.2) controls; $p \leq 0.05$ or 0.01 for the fetal and litter incidences]; cervical centrum 6 not ossified [10.2 (52.2) treated vs 7.4 (29.2) controls; $p \leq 0.01$ for the fetal incidence]; and cervical centrum 7 not ossified [5.1 (30.4) treated vs 1.3 (12.5) controls; $p \leq 0.01$ for the fetal incidence]. It should be noted that this pattern was also observed in rats administered the test substance (MRID 44920801).

d. Major defects: There were no treatment-related external, visceral, or skeletal major defects noted at any dose level. Additionally, no treatment-related changes were observed in the *manus/pes* ossification data.

Developmental LOAEL = 600 mg/kg/day based on the pattern toward decreased ossification of the cervical vertebra centra
Developmental NOAEL = 150 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 mouse.

C. STUDY DEFICIENCIES - The following deficiencies were noted, but will not affect the conclusions of this report:

- The total number of corpora lutea, implantations, live fetuses, and resorptions were not provided in a summary table.

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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