DATA EVALUATION RECORD

3/14/01

MESOTRIONE

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

Work Assignment No. 2-02-95A (formerly 2-01-52Z) (MRID 44920801)

Prepared for

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MESOTRIONE (ZA1296)

Developmental Study (§83-3[a])

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DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity in Rats
OPPTS Number: 870.3700

OPP Guideline Number: §83-3a

DP BARCODE: D259369
P.C. CODE: 122990

SUBMISSION CODE: S541375
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 Rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Report # CTL/P/5034, Laboratory Study # RR0700, June 28, 1999. MRID 44920801 Unpublished.

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

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44920801), mesotrione (96.8% a.i., Lot#: P17) in deionized water was administered to pregnant Alpk:AP$_f$SD rats (24/dose) at dose levels of 0, 100, 300, or 1000 (limit dose) mg/kg/day by gavage on gestation days (GDs) 7 through 16. All dams were sacrificed on GD 22. No premature deaths occurred during the study.

Treatment-related toxicity was characterized by reduced body weight gains and food consumption at the mid- and high-dose levels. When compared to concurrent controls, no treatment-related changes in absolute body weight, gravid uterine weight, Cesarean section parameters, or gross pathology were noted at any dose level tested. Urine staining and colored feces were not considered adverse clinical effects.

At 1000 mg/kg, pink and/or purple colored feces (130 incidents in 22/24 animals vs 0/24 controls) and dry or wet staining with urine (29-39 observations in 6-8/24 animals vs 0/24 controls) were observed. Body weight gains, as calculated by reviewers, were reduced during the overall treatment interval (↓20%, GDs 8-16, not analyzed for statistical significance). In addition, decreases were noted in food consumption during GDs 7-16 (↓14-18%, $p \leq 0.01$). Body

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weights and food consumption increased in all dose groups including controls during the post-treatment interval.

At 300 mg/kg, an increased incidence of pink and/or purple colored feces was noted (35 observations in 10/24 animals vs 0/24 controls). Body weight gains were reduced during the overall treatment interval (↓17%, GDs 8-16). Decreases were noted in food consumption during GDs 7 through 16 (↓8-13%, $p \leq 0.01$).

At 100 mg/kg, no treatment-related adverse effects were noted.

The maternal LOAEL is 300 mg/kg/day, based upon decreased body weight gains during treatment and decreased food consumption. The maternal NOAEL is 100 mg/kg/day.

Developmental toxicity was characterized by decreased mean fetal weight (↓6%, $p \leq 0.01$) at the 1000 mg/kg/day level and a dose-dependent increase in incidences of decreased ossification of vertebral centra and of the *manus* and *pes* at all dose levels. Incidences of "non-ossification" of several cervical centra ranged from 2 - 87% (25 - 100%)[% fetal incidence (% litter incidence)] as compared to controls [0.2 - 22% (4.2 - 88%)]. Reduced ossification was seen in other skeletal structures such as cervical vertebra arches, vertebra transverse processes, otontoids, and calcaneum. When compared to mean scores for the controls, increased mean *manus* and *pes* scores/litter for all treatment groups were observed (↑4-11%). It should be noted that this shift toward reduced ossification was also observed in mice administered the test substance (MRID 44920802).

The developmental LOAEL is 100 mg/kg/day, based on delays in skeletal ossification and changes in *manus/pes* ossification assessments. The developmental NOAEL was not established.

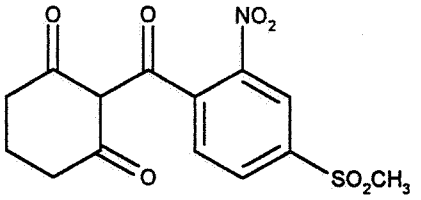
Even though a developmental NOAEL was not established and no dose rationale was provided, this developmental toxicity study is classified **acceptable/guideline (§83-3[a])**, and satisfies the guideline requirements for a developmental toxicity study in the rat as per the Hazard Identification Assessment Review Committee (March 13, 2001).

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 under unspecified conditions for up to 28 days.
   CAS #: 104206-82-8
   Structure:



2. Vehicle: Deionized water

3. Test animals: Species: Rat
   Strain: Alpk:AP$_f$SD (Wistar-derived)
   Age at delivery and mean weight range of females on gestation day 1: Approximately 11 weeks old, 261.6-271.0 g
   Source: Barriered Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park
   Housing: Individually in cages (type unspecified)
   Diet: CT1 Diet (Special Diets Services Limited, Witham, Essex, UK), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 21±2°C
   Humidity: 40-70%
   Air changes: Approximately 25-30/hr
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period: Not provided

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 2/6/96 end: 3/8/96

2. Mating: Females were paired at the suppliers with males of the same strain. On the morning following mating, vaginal smears were examined for the presence of sperm. The day sperm was observed was designated as gestation day (GD) 1.

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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	24
Low	100	24
Mid	300	24
High	1000 [a]	24

a Limit dose

4. Dose selection rationale: Doses were selected based on the findings of previously conducted laboratory rangefinding studies in the pregnant rat; no further information was provided.

5. Dosage preparation and analysis - Dosing solutions were prepared by mixing the appropriate amount of deionized water with the test substance. Each formulation was subdivided into aliquots and fresh aliquots were used daily. It was stated that further preparations were made at appropriate intervals during the study; no additional information was provided. Storage conditions were not provided. Prior to the start of the study, samples (start, middle, end) of 10 and 100 mg/mL formulations were analyzed for homogeneity. Also prior to the study, samples of 10 and 100 mg/mL concentrations were analyzed after storage under unspecified conditions for up to 28 days. Concentration analyses were performed to verify concentration twice during the study.

Results:

Homogeneity analyses (range as mean % of nominal): 90.6-92.0%

Stability analyses (range as mean % of Day 0): 95.7-104.6%

Concentration analyses (range as mean % of nominal): 90.0-97.5%

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; however, storage conditions corresponding to the stability data were not provided.

6. Dosage administration: All doses were administered once daily by gavage on GDs 7 through 16 in a volume of 1 mL/100 g 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 upon arrival, and on GDs 4, 7 through 16, 19, and 22. Food consumption was recorded for GDs 1-22. All does were sacrificed on GD 22. 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 apparently nonpregnant females were stained to ascertain 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 at least 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 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 skeletal ossification was 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: No historical control data were provided.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical observations: No premature deaths occurred during the study. When compared to concurrent controls, clinical signs (Table 2) were observed as follows: pink and/or purple colored feces (35 observations in 10/24 mid-dose animals and 130 incidents in 22/24 high-dose animals vs 0/24 controls); dry staining with urine (29 observations in 6/24 high-dose animals vs 0/24 controls); and wet staining with urine (39 observations in 8/24 high-dose animals vs 0/24 controls). Isolated incidents of vaginal bleeding and neurotoxic effects, such as piloerection and salivation, were observed at a low incidence in the high-dose animals.

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

Observation	Dose in mg/kg/day			
	0	100	300	1000
Pink and/or purple colored feces	0 (0)	0 (0)	35 (10)	130 (22)
Dry staining with urine	0 (0)	4 (1)	13 (1)	29 (6)
Wet staining with urine	0 (0)	8 (1)	1 (1)	39 (8)
Piloerection	0 (0)	0 (0)	0 (0)	1 (1)
Salivation	0 (0)	0 (0)	0 (0)	2 (2)
Vaginal bleeding	0 (0)	0 (0)	0 (0)	2 (1)

a Data extracted from the study report, Table 5, pages 35 and 36; n = 24.

2. Body weight: Using GD 7 body weight as a covariant, adjusted body weights (Table 3) were lower ($p \leq 0.05$ or 0.01) at the mid- and high-dose levels from GD 8 through 10 (↓2-3%) and in all treatment groups from GD 11 through 16 (↓1-5%). Body weights increased in all dose groups including controls during the post-treatment interval. Body weight gains, as calculated by reviewers and not analyzed for statistical significance, were reduced during the overall treatment interval (GDs 8-16) at the low- (↓12%), mid-

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(↓17%), and high-dose (↓20%) levels. Overall (GDs 1-22) body weight gains at all treatment levels were similar to controls.

Table 3. Selected mean maternal adjusted body weights (using GD 7 body weight as a covariant, g) and body weight gains [a]

Interval	Dose in mg/kg/day			
	0 n = 24	100 n = 24	300 n = 24	1000 n = 24
Adjusted body weights				
Treatment:				
GD 8	301.6	299.9	296.3** (↓2%)	294.0** (↓3%)
GD 12	322.9	318.5* (↓1%)	313.5** (↓3%)	310.4** (↓4%)
GD 16	351.6	343.9** (↓2%)	337.8** (↓4%)	333.8** (↓5%)
Post-treatment:				
GD 19	391.7	384.6	376.3**	374.4**
GD 22 [b]	423.4 [↑21%]	421.1 [↑22%]	415.7 [↑23%]	407.8** [↑22%]
Gravid uterine weight	96.3±14.5	94.2±20.5	91.6±19.2	90.9±20.6
Body weight gains [c]				
Overall treatment:				
GDs 8-16	50	44 (↓12%)	41.5 (↓17%)	39.8 (↓20%)
Overall study: [d]				
GDs 1-22	156.4	153.0	148.4	139.7

a Data extracted from the study report, Tables 6 and 9, pages 38, 39, and 43. Percent difference from controls is presented parenthetically, unless otherwise noted.
b Increases relative to GD 16 body weights presented in brackets.
c Calculated by reviewers from previously cited data.
d Calculated using the absolute, not adjusted, mean.
* or ** Significantly different from controls at $p \leq 0.05$ or 0.01.

3. Food consumption - When compared to concurrent controls, dose-dependent decreases ($p \leq 0.05$ or 0.01) were noted in food consumption (Table 4) at the low-, mid-, and high-dose levels, respectively, during dosing as follows: GDs 7-10 (↓7, 8, and 17%), GDs 10-13 (↓8, 13, and 18%), and GDs 13-16 (↓7, 10, and 14%). Food consumption increased in all dose groups including controls during GDs 16-19; food consumption in all dose groups was increased (not statistically significant) relative to controls (↑1-4%) during GDs 19-22.

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

Interval	Dose in mg/kg/day			
	0 n = 24	100 n = 24	300 n = 24	1000 n = 24
Pretreatment:				
GDs 4-7	24.0	24.1	24.2	23.2
Treatment:				
GDs 7-10	28.5	26.7* (↓7%)	26.2** (↓8%)	23.6** (↓17%)
GDs 10-13	32.7	30.0** (↓8%)	28.5** (↓13%)	26.9** (↓18%)
GDs 13-16	35.2	32.7** (↓7%)	31.7** (↓10%)	30.3** (↓14%)
Post-treatment:				
GDs 16-19	38.5	36.8	36.5*	35.8**
GDs 19-22	32.8	33.4 (↑2%)	34.0 (↑4%)	33.0 (↑1%)

a Data extracted from the study report, Table 7, page 40. Percent difference from controls is presented parenthetically.

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

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 5. The number of implantations/dam, resorptions/dam, pre- and postimplantation losses, and percent male were similar between control and treated groups.

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Table 5. Cesarean section observations [a]

Observation	Dose (mg/kg/day)			
	0	100	300	1000
# Animals Assigned (Mated)	24	24	24	24
# Animals Pregnant Pregnancy Rate (%) [b]	24 (100)	24 (100)	24 (100)	24 (100)
# Nonpregnant	0	0	0	0
# Total Dams Died # Died Pregnant # Died Nonpregnant # Aborted # Premature Delivery	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0
Total # Corpora Lutea Corpora Lutea/Dam	366 15.3	386 16.1	373 15.5	358 14.9
Total # Implantations Implantations/Dam	340 14.2	343 14.3	322 13.4	327 13.6
Total # Litters Examined	24	24	24	24
Total # Live Fetuses Live Fetuses/Dam	329 13.7	320 13.3	312 13.0	324 13.5
Total # Dead Fetuses Dead Fetuses/Dam	NR NR	NR NR	NR NR	NR NR
Total # Resorptions Early Late Total Resorptions/Dam Early Late Litters with Total Resorptions	11 9 2 0.5±0.6 0.4±0.6 0.1±0.3 0	23 20 3 1.0±1.0 0.8±1.0 0.1±0.3 0	10 9 1 0.4±0.8 0.4±0.8 0.0±0.2 0	3 2 1 0.1±0.3 0.1±0.3 0.0±0.2 0
Mean Fetal Weight (g) Males Females	4.95 NR NR	4.89 NR NR	4.91 NR NR	4.66** (↓6%) NR NR
Sex Ratio (% Male)	48.7	47.1	55.2	55.1
Preimplantation Loss (%)	7.1	11.1	13.7	8.7
Postimplantation Loss (%)	3.2	6.7	3.1	0.9

a Data extracted from the study report, Tables 4, 9, and Appendix 5, pages 33, 43, and 219 through 222. Percent difference from controls is presented parenthetically.
b Calculated by reviewers.
NR Not reported
** Significantly different from controls at $p \leq 0.01$.

B. DEVELOPMENTAL TOXICITY: At 1000 mg/kg, a decrease in mean fetal weight (Table 5) was noted (↓6%, $p \leq 0.01$) when compared to controls. 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	300	1000
#Fetuses (#litters) examined	329 (24)	320 (24)	312 (24)	324 (24)
Cleft palate	0 (0)	0 (0)	0.3 (4.2)	0 (0)
Microphthalmia	0.3 (4.2)	0 (0)	0 (0)	0 (0)

a Data extracted from the study report, Tables 10 and 12, pages 45 and 48. 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	300	1000
#Fetuses (#litters) examined	329 (24)	320 (24)	312 (24)	324 (24)
Hernia	0 (0)	0.3 (4.2)	0 (0)	0 (0)
Encephalocoele	0 (0)	0 (0)	0.3 (4.2)	0 (0)
Slightly dilated ureter	3.6 (25.0)	2.8 (25.0)	1.9 (20.8)	2.5 (25.0)
Kinked ureter	8.8 (50.0)	8.1 (41.7)	7.7 (50.0)	10.2 (66.7)

a Data extracted from the study report, Tables 10 and 12, pages 45, 48, and 49. For individual observations, data are presented as % fetal incidence (% litter incidence).

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 Tables 6c and d.

A dose-dependent increase in the proportion of fetuses with "unossified" 2nd, 3rd, 4th, 5th, and 6th cervical centra was observed at all dose levels with the majority of the fetal and litter incidences attaining statistical significance ($p \leq 0.05$ or 0.01). It should be noted that this shift toward reduced ossification was also observed in mice administered the test substance (MRID 44920802). In addition, an increased incidence of 14th ribs of short ($p \leq 0.01$ at all dose levels) or full ($p \leq 0.05$, low-dose fetal incidence only) length were observed when compared to concurrent controls, but the incidences were not dose-related. A reduction ($p \leq 0.05$, all fetal incidences) in the number of animals displaying "partial ossification" of the 4th or 5th cervical arch was observed in all treated groups vs controls. Additionally, decreased numbers of treated fetuses exhibited "full ossification" of the 4th lumbar transverse process, "full ossification" of the 7th cervical vertebra transverse process, and "partial ossification" of the 7th cervical vertebra transverse process; the majority of these fetal incidences were statistically significant ($p \leq 0.05$ or 0.01), while litter incidences were statistically significant ($p \leq 0.05$ or 0.01) at the high-dose for the 4th lumbar transverse process and at the mid-dose for "partial ossification" of the 7th cervical vertebra transverse process. Reductions in ossification were further demonstrated by the dose-dependently increased numbers of treated fetuses exhibiting a "non-ossified" odontoid and calcaneum; fetal incidences were statistically significant ($p \leq 0.05$ or 0.01) at all dose levels.

A dose-dependent decrease in the ossification of the *manus* and *pes* was observed in all treated groups. Mean *manus* and *pes* scores/litter for all treatment groups were higher (↑4-11%, $p \leq 0.05$ or 0.01) when compared to mean scores for the concurrent controls; these increased scores in the treated animals verified the skeletal examination findings of reduced ossification. Regarding *manus* scores, a dose-dependent decrease ($p \leq 0.01$) was noted in the proportion of treated fetuses with a score of 3 (↓48-85%) when compared to concurrent controls with a *manus* score of 3. An increase ($p \leq 0.01$) was observed in the proportion of fetuses with a score of 5 at the mid- and high-dose levels (↑329 or 576%) when compared to controls with a *manus* score of 5. Regarding *pes* scores, a dose-dependent decrease was observed in the proportion of treated fetuses with a score of 3 (↓85-100%) when compared to concurrent controls with a *pes* score of 3. At the high-dose, a decrease ($p \leq 0.01$) was observed in the proportion of fetuses with a score of 4 (↓24%) when compared to controls with a *pes* score of 4. A dose-dependent increase ($p \leq 0.01$) was noted in the proportion of treated fetuses with a score of 5 (↑79-295%) when compared to controls with a *pes* score of 5.

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Table 6c. Skeletal observations [a]

Observations	Dose (mg/kg/day)			
	0	100	300	1000
#Fetuses (#litters) examined	329 (24)	320 (24)	312 (24)	324 (24)
Gross skull malformation	0 (0)	0 (0)	0.3 (4.2)	0 (0)
Maxillae fused	0 (0)	0 (0)	0.3 (4.2)	0 (0)
Arch not ossified in 2nd cervical vertebra	0 (0)	0.3 (4.2)	0 (0)	0 (0)
Arch partially ossified in 4th cervical vertebra	2.4 (20.8)	0.3* (4.2)	0.3* (4.2)	0.3* (4.2)
Arch partially ossified in 5th cervical vertebra	3.0 (16.7)	0.3* (4.2)	0.6* (8.3)	0.3* (4.2)
Centrum not ossified, 3rd cervical vertebra	3.6 (29.2)	19.1** (66.7)*	30.1** (70.8)**	58.3** (100)**
Centrum not ossified, 4th cervical vertebra	2.4 (29.2)	8.1** (54.2)	14.1** (54.2)	34.3** (83.3)**
Centrum not ossified, 5th cervical vertebra	0.3 (4.2)	2.2 (25.0)	6.7** (41.7)**	15.1** (62.5)**
Centrum not ossified, 6th cervical vertebra	0.9 (12.5)	0.6 (4.2)	2.6 (20.8)	6.2** (45.8)*
Centrum bipartite, 12th thoracic vertebra	0.6 (8.3)	0.9 (12.5)	1.6 (12.5)	2.5 (29.2)
Transverse processes of the 4th lumbar fully ossified	7.0 (41.7)	2.2** (16.7)	2.6* (16.7)	1.2** (12.5)*
Transverse process of the 7th cervical vertebra fully ossified	2.4 (16.7)	0.3* (4.2)	0** (0)	0.9 (12.5)
Transverse process of the 7th cervical vertebra partially ossified	21.9 (87.5)	9.4** (66.7)	4.5** (41.7)**	12.7** (70.8)
Bipartite, 3rd sternebra	0 (0)	0.3 (4.2)	1.0 (8.3)	0.9 (12.5)
Not ossified, 5th sternebra	1.8 (25.0)	0.6 (8.3)	1.9 (12.5)	1.5 (16.7)
Misaligned slightly, 5th sternebra	5.2 (54.2)	5.0 (41.7)	3.5 (29.2)	2.5 (25.0)
14th short length extra rib	3.0 (29.2)	23.8** (83.3)**	20.5** (70.8)**	19.4** (62.5)**
14th normal length extra rib	0 (0)	1.6 (20.8)*	1.6 (8.3)	1.2 (12.5)
Odontoid not ossified	19.5 (95.8)	40.9** (100)	48.4** (95.8)	67.3** (100)
Centrum not ossified, 2nd cervical vertebra	22.2 (87.5)	59.4* (100)	69.9** (95.8)	86.7** (100)
Partially ossified 5th sternebra	28.0 (91.7)	10.9** (70.8)	24.4 (87.5)	36.4* (91.7)
Calcaneum not ossified	25.5 (87.5)	50.6** (100)	71.2** (95.8)	89.8** (100)

a Data extracted from the study report. Tables 10 and 12, pages 46, and 50 through 58. For individual observations, data are presented as % fetal incidence (% litter incidence).

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

Thus, at this point there is no practical enforcement method for residues to be included in the tolerance. The SAC suggested that HED's recommendation be changed to state that we recommend for temporary tolerances pending validation of a practical enforcement method.

b. Processed commodities and MMPE:

For the rice processed commodities, in the absence of any information, temporary tolerances should be established for polished rice, hulls, and bran, based on the maximum theoretical concentration factors for these commodities (???). The SAC agreed with the conclusion that there is no need for meat/milk/poultry/eggs tolerances and further suggested that HED not include any of these food commodities in its dietary exposure assessment.

c. Rotational Crop Issues:

The SAC suggested that the reviewer determine if this chemical is persistent in soil. If it is persistent then the review should recommend setting a 12 month PBI for rotational crops.

3. **Inadvertent Residues of Norflurazon in cereal grains resulting from growth as rotational crops.**

A question arose concerning the impending establishment of tolerances on grain crops based on extended rotational crop field trials. In these field trials rice grain and wheat grain have higher residues than corn grain/ears and sorghum grain. For corn and sorghum residues were <0.04 ppm to <0.043 ppm. For wheat grain, residues were <0.04 ppm in all samples except two, from the same trial, which were <0.077 and <0.240 ppm. For rice grain, residues were <0.04 to 0.237 ppm.

The SAC determined that, given these tolerances are to cover some hypothetical rotational scheme that is virtually impossible to capture in field trials, we should take the simplest approach, which is to set a single tolerance of 0.25 ppm for the cereal grain crop group.

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

Observations	Dose (mg/kg/day)			
	0	100	300	1000
#Fetuses (#litters) examined	329 (24)	320 (24)	312 (24)	324 (24)
***Manus* scores**				
Proportion with score 2	0.3	0.0	0.0	0.0
Proportion with score 3	18.8	9.7** (↓48%)	6.4** (↓66%)	2.8** (↓85%)
Proportion with score 4	78.7	85.3* (↑8%)	84.6	83.0
Proportion with score 5	2.1	4.7	9.0** (↑329%)	14.2** (↑576%)
Proportion with score 6	0.0	0.3	0.0	0.0
Mean *manus* score/litter	3.82	3.97* (↑4%)	4.03** (↑5%)	4.13** (↑8%)
***Pes* scores**				
Proportion with score 3	12.5	1.9** (↓85%)	0.3** (↓98%)	0.0** (↓100%)
Proportion with score 4	77.2	79.4	75.0	59.0** (↓24%)
Proportion with score 5	10.3	18.4** (↑79%)	24.7** (↑140%)	40.7** (↑295%)
Proportion with score 6	0.0	0.3	0.0	0.3
Mean *pes* score/litter	3.98	4.20** (↑6%)	4.23** (↑6%)	4.42** (↑11%)

a Data extracted from the study report, Table 13, page 59. Percent difference from controls is presented parenthetically.

* 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 100, 300, or 1000 mg/kg/day resulted in maternal toxicity characterized by reductions in body weight and food consumption. It was concluded that a maternal NOAEL was not observed.

2) Developmental toxicity: Decreased fetal weights were observed at 1000 mg/kg/day. Dose levels of ≥100 mg/kg resulted in changes in the ossification of the fetal skeleton and increased *manus* and *pes* scores, but no structural malformations were observed. These changes in ossification were transient in nature and considered not to be of toxicological significance in terms of postnatal development. The investigators concluded that the developmental LOAEL was 1000 mg/kg/day and the NOAEL was 300 mg/kg/day.

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B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Mesotrione (96.8% a.i.) in deionized water was administered to pregnant Alpk:AP$_f$SD rats (24/dose) at dose levels of 0, 100, 300, or 1000 (limit dose) mg/kg/day by gavage on GDs 7 through 16. All dams were sacrificed on GD 22. 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; however, storage conditions corresponding to the stability data were not provided. No premature deaths occurred during the study.

   Treatment-related toxicity was characterized by reduced body weight gains and food consumption at the mid- and high-dose levels. When compared to concurrent controls, no treatment-related changes in absolute body weight, gravid uterine weight, Cesarean section parameters, or gross pathology were noted at any dose level tested. Urine staining and colored feces were not considered adverse clinical effects.

   At 1000 mg/kg, pink and/or purple colored feces (130 incidents in 22/24 animals vs 0/24 controls), dry staining with urine (29 observations in 6/24 animals vs 0/24 controls), and wet staining with urine (39 observations in 8/24 animals vs 0/24 controls) were observed. Adjusted (using GD 7 body weight as a covariant) body weights were reduced ($p \leq 0.01$) from GDs 8-16 (↓2-5%). Body weight gains, as calculated by reviewers and not analyzed for statistical significance, were reduced during the overall treatment interval (↓20%, GDs 8-16). In addition, decreases ($p \leq 0.01$) were noted in food consumption during GDs 7-16 (↓14-18%). Body weights and food consumption increased in all dose groups including controls during the post-treatment interval. Overall (GDs 1-22) body weight gains were similar to controls at all dose levels.

   At 300 mg/kg, an increased incidence of pink and/or purple colored feces was noted (35 observations in 10/24 animals vs 0 controls). Adjusted body weights were lower ($p \leq 0.01$) from GDs 8 through 16 (↓2-4%). Body weight gains were reduced during the overall treatment interval (↓17%, GDs 8-16). Decreases ($p \leq 0.01$) were noted in food consumption during GDs 7 through 16 (↓8-13%).

   At 100 mg/kg, adjusted body weights were lower ($p \leq 0.05$) from GD 11 through 16 (↓1-3%). Body weight gains were reduced during the overall treatment interval (↓12%, GDs 8-16). Decreased ($p \leq 0.05$ or 0.01) food consumption was observed during GDs 7 through 16 (↓7-8%). These effects were considered minimal and not adverse.

   When compared to concurrent controls, no treatment-related changes in absolute body weight or gravid uterine weight were noted at any dose level tested. The number of implantations/dam, resorptions/dam, pre- and postimplantation losses, and percent male were similar between control and treated groups. When compared to concurrent controls, no treatment-related changes were noted in gross pathology.

Maternal LOAEL = 300 mg/kg/day, based upon decreased body weight gains during treatment and decreased food consumption.
Maternal NOAEL = 100 mg/kg/day

2. DEVELOPMENTAL TOXICITY: Developmental toxicity was characterized by decreased mean fetal weight at the high-dose level and decreased ossification of vertebral centra and of the *manus* and *pes* at all dose levels.

   a. Deaths/resorptions: The numbers of resorptions/dam or number of live fetuses/dam for the treatment groups were not different from the concurrent controls.
   b. Altered growth: At 1000 mg/kg, a decrease in mean fetal weight was noted (↓6%, $p \leq 0.01$).
   c. Minor defects/variants: At all three dose levels, reductions in ossification were observed in the treated fetuses when compared to concurrent controls. Reduced degrees of ossification were evidenced by dose-dependent increases in the proportion of fetuses exhibiting "unossified" 2nd, 3rd, 4th, 5th, and 6th cervical centra. It should be noted that this shift toward reduced ossification was also observed in mice administered the test substance (MRID 44920802). Reduced ossification was also demonstrated by a decreased number of fetuses exhibiting the following: "partially ossified" 4th or 5th cervical vertebra arch; "fully ossified" 4th lumbar transverse process; "fully ossified" 7th cervical vertebra transverse process; "partially ossified" 7th cervical vertebra transverse process; "non-ossified" odontoid; and "non-ossified" calcaneus. An increased incidence of an extra 14th rib of short or normal length were also noted, but were not dose-related and not considered of toxicological concern.
   d. *Manus/pes* skeletal assessment: A dose-dependent decrease in the ossification of the *manus* and *pes* was observed in all treated groups. When compared to mean scores for the controls, increased mean *manus* and *pes* scores/litter for all treatment groups were observed (↑4-11%, $p \leq 0.05$ or 0.01) indicating reduced ossification.
   e. Major defects: There were no treatment-related external, visceral, or skeletal major defects noted at any dose level.

   Developmental LOAEL = 100 mg/kg/day, based on delays in skeletal ossification and changes in *manus/pes* ossification assessments
   Developmental NOAEL = Not established

Even though a developmental NOAEL was not established and no dose rationale was provided, this developmental toxicity study is classified **acceptable/guideline (§83-3[a])**, and satisfies the guideline requirements for a developmental toxicity study in the rat as per the Hazard Identification Assessment Review Committee (March 13, 2001).

C. STUDY DEFICIENCIES - The following deficiencies were noted:

- No dose rationale was provided.
- No historical control data were provided.

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- Storage conditions corresponding to the stability data were not provided.
- Frequency of preparation of dosing formulations was not provided.
- Number of dead fetuses/dam was not reported.
- Acclimation period was not provided.
- Cage type was unspecified.

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