(8-2-2001)

DATA EVALUATION RECORD

MESOTRIONE

Study Type: §83-4b; Multigeneration Reproduction Study of Mesotrione Administered in the Diet to CD-1 Mice

Work Assignment No. 2-01-52DD (MRID 44505034)

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - Mouse
OPPTS Number: 870.3800 OPP Guideline Number: §83-4b

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. (1997) ZA1296: Multigeneration Study in the Mouse. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Study Nos. RM0728/F0, RM0728/F1, and RM0728/F2. December 4, 1997. MRID 44505034. Unpublished.

SPONSOR: Zeneca Ag Products, 1800 Concord Pike, Wilmington, DE

EXECUTIVE SUMMARY: In a 2-generation reproduction study (MRID 44505034), mesotrione (96.8% a.i., lot # P17) was administered in the diet continuously to Alpk:AP$_f$CD-1 mice (26 mice/sex/dose) at dose levels of 0, 10, 50, 350, 1500, or 7000 ppm (equivalent to 0, 2.1/2.4, 10.1/11.7, 71.4/82.5, 306.7/362.7, or 1455.5/1652.3 mg/kg/day [M/F] in the P and F_1 animals). The P animals were exposed to the test substance beginning at approximately 3 weeks of age and exposure lasted for approximately 8 weeks prior to mating. F_1 pups selected (26/sex/dose) to produce the F_2 generation were exposed to the same dosage as their parents beginning on postnatal day (PND) 29 and continuously throughout the rest of the study. F_1 animals were administered the test article for approximately 8 weeks prior to mating to produce the F_2 animals. Mating to produce an F_{2b} generation was not performed. Exposure of all animals to the test material was continuous throughout the study. The analytical data indicated that the mixing procedure was adequate and the variation between nominal and actual dosage to the study animals was acceptable.

There were no statistically significant and treatment-related changes in mortality, or reproductive performance observed in the P or F_1 adults. Adjusted (to LD 1) body weights were decreased

during lactation in 7000 ppm P dams on LDs 5, 8, and 15 (↓3-13%). Body weights were decreased (↓9-12%) in 7000 ppm F_1 dams on LDs 1 (absolute) and 15 (adjusted). Food consumption was decreased in the 7000 ppm P dams throughout lactation (↓15-28%) and in 7000 ppm F_1 dams during weeks 2, 3, and 4 of lactation (↓10-33%).

An increase in the incidence of opaque eyes in 7000 ppm F_1 males (4/26 treated vs 0/26 controls) and females (6/26 treated vs 0/26 controls) was observed. Opaque eyes were also observed in a single animal from each of the following groups: 7000 ppm P males, 10 and 350 ppm F_1 males, and 1500 ppm F_1 females. At necropsy, an associated increase was observed in the incidence of grossly visible opaque/cloudy eyes (4 - 20% treated vs. 0 - 4% controls) in 7000 ppm P males, P females, F_1 males and F_1 females. In addition, upon histological examination, an increase was observed in the incidence of minimal to marked unilateral and bilateral ocular cataractous change in the 7000 ppm P males (unilateral - 3/26 treated vs 0/26 controls), F_1 males (unilateral - 7/25 treated, bilateral 1/25 treated vs 0/26 controls), and F_1 females (unilateral - 4/26 treated, bilateral 1/26 treated vs 0/26 controls). Retinal detachment with marked cataractous change was also observed in one of the 7000 ppm F_1 males and females; whether this lesion was a primary effect of mesotrione on the eye is unclear.

Absolute kidney weights were statistically significantly increased in F_1 males at 350 ppm and above. Percent increases above controls were 11, 8, and 13% for 350, 1500, and 7000 ppm, respectively. Relative kidney weights were also statistically significantly increased at 350 ppm and above in males. Percent increases above controls were 6, 8 and 17% for 350, 1500, and 7000 ppm, respectively. In females, relative kidney weights were statistically significantly increased at 50 ppm and above, but percent increases on ranged from 4 to 9% above controls.

The LOAEL for parental toxicity is 350 ppm (equivalent to 71.4/82.5 mg/kg/day [M/F]) based upon dose-related and statistically significant increases in kidney weights in F_1 males. The increased kidney weights may be associated with tyrosyluria since tyrosinemia was observed at all dose levels in F_1 animals (exposed in utero) in this study. Ocular lesions in both sexes and decreased body weights in females were observed at higher dose levels. The NOAEL for parental toxicity is 50 ppm (equivalent to 10.1/11.7 mg/kg/day [M/F]).

No treatment-related effects on mortality or viability were observed at any time in the F_1 and F_2 litters. An increase in the number of pups with opaque eyes (6 pups in 1 litter vs 0 controls) and pups with eye(s) shut (7 pups in 4 litters vs 0 controls) was observed in the 7000 ppm F_2 pups. An increase in the number of pups with ocular discharge was observed in the 7000 ppm dose groups (F_1 and F_2 - 11 pups in 5 litters each vs 0 controls). A slight increase in the number of pups with ocular discharge was also observed in the other F_1 (1-5 pups/group in 1-3 litters/group in the 10, 50, 350, and 1500 ppm groups) and F_2 litters (2-3 pups/group in 2-3 litters in the 10, 350, and 1500 ppm groups). At necropsy, an increase was observed in the incidence of opaque or cloudy eyes in 7000 ppm (10/33 treated vs 0/30 controls) and 1500 ppm (4/30 treated) F_2 males. Upon histological examination, an associated increase was observed in the incidence of microscopic minimal to marked unilateral and/or bilateral cataractous changes in all 7000 ppm

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groups, with the severity ranging from minimal to marked: F_1 males - 4/30 treated vs 0/37 controls; F_1 females - 2/30 treated vs 0/40 controls; F_2 males - 11/33 treated vs 0/30 controls; F_2 females - 2/31 treated vs 0/32 controls. Minimal unilateral cataractous change was also observed in the 1500 ppm F_2 males (2/18 treated vs 0/30 controls).

Body weights were decreased in all 7000 ppm groups: F_1 males from PND 8 to weaning (↓6-24%); F_1 females from PND 15 to weaning (↓14-22%); F_2 males from PND 15 to weaning (↓9-20%); F_2 females from PND 22 to weaning (↓12-17%). Body weights were also decreased in 1500 ppm groups on PND 22 and 29 (↓6-14%). Body weight gains, as calculated by the reviewers, were decreased in both generations in the 7000 ppm groups (↓16-24%).

Plasma tyrosine levels were elevated in a dose related manner in all F_1 treatment groups (exposed in utero). A statistical assessment of these data was not presented by the study investigators. However, increases ranged from 269-586% of controls in males and from 159-542% of controls in females. In addition, plasma tyrosine levels were even more dramatically elevated in F_2 pups. At the 10 ppm level, male tyrosine levels were 794% of controls and females levels were 665% of controls. At the 7000 ppm level, male tyrosine levels were 5009% of controls and females were 3945% of control levels.

The LOAEL for offspring toxicity for males and females is 10 ppm (LDT) (equivalent to 2.1/2.4 mg/kg/day [M/F]) based on tyrosinemia at all dose levels in F_{2a} pups (exposed in utero). In addition, ocular discharge was observed at all dose levels in F_1 pups (and in nearly all F_2 pup dose groups) with a 0 incidence in controls and cataractous changes were observed histologically at the high dose level. An offspring toxicity NOAEL was not observed.

Even though a NOAEL for effects observed in offspring was not determined, this reproductive study in the mouse is determined to be **acceptable/guideline (§83-4(b), reproduction)** and satisfies the requirements for a multigenerational reproductive toxicity study in mice as per the Hazard Identification Assessment Review Committee (March 13, 2001).

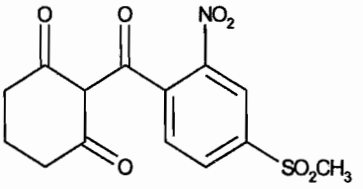
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

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 for up to 14 days.
CAS #: 104206-82-8
Structure:



2. Vehicle: Diet

3. Test animals: Species: Mouse
Strain: Alpk:AP$_f$CD-1
Age at start of dosing: P - approximately 3 weeks; F_1 - 29 days;
Weight at start of dosing:
(P, group means) Males: 22.1-23.2 g; Females: 20.4-21.1 g
(F_{1A}, group means) Males: 21.6-25.8 g; Females: 21.1-25.5 g
Source: Rodent Breeding Unit (RBU), Alderley, Park, Macclesfield, Cheshire, UK
Housing: Two males or two females/cage during premating, 1 male and 1 female/cage during mating, and 1 female/cage in solid bottom polycarbonate cages during gestation and lactation. The cage type used during premating and mating was not specifed.
Diet: Rat and Mouse No. 3 diet (RM3) Special Diets Services Limited, Stepfield, Witham, Essex, UK ad libitum
Water: Tap water, ad libitum
Environmental conditions:
Temperature: 21±2°C
Humidity: 30-70%
Air changes: ≥15/hour
Photoperiod: 12 h dark/12 h light
Acclimation period: Approximately 7 days
Study Duration (in life dates): start - 11/4/96 end - 7/2/97

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: One female was caged with one male from the same test group (P and F_1 generations) for 21 days. The day a vaginal plug was observed was designated gestation day (GD) 1.

2. Study schedule: The P animals (26 animals/sex/dose) were given test article diet formulations for 8 weeks prior to mating to produce the F_1 litters. The F_1 animals were reared to PND 29, at which time 26 animals/sex/dose were selected to become the F_1 parents of the F_2 generation. The F_1 animals were given test formulations for 8 weeks prior to mating to produce the F_2 litters. The F_2 animals were reared to PND 29, sacrificed, necropsied, and discarded. Male F_1 pups selected for reproduction and one randomly selected F_2 pup/litter were observed for preputial separation. The remaining

adults and pups were sacrificed and necropsied after weaning. Exposure of all animals to the test material was continuous throughout the study.

3. Animal assignment: The P animals were randomly assigned to test groups as shown in Table 1. Litters with unhealthy individuals and at the extreme of the weight range were not used to provide F_1 adults. Offspring used for mating were randomly chosen from the remaining litters using a shuffle card method.

Table 1. Animal assignment

Test Group	Dose (ppm)[a]	Achieved Dosage[b] (mg/kg/day)	Animals/group			
			P Males	P Females	F_1 Males	F_1 Females
Control	0	0	26	26	26	26
Low-dose	10	2.1/2.4	26	26	26	26
Mid-low-dose	50	10.1/11.7	26	26	26	26
Mid-dose	350	71.4/82.5	26	26	26	26
Mid-high-dose	1500	306.7/362.7	26	26	26	26
High-dose	7000	1455.5/1652.3	26	26	26	26

a Diets were administered from the beginning of study until sacrifice.
b Achieved dosage was calculated by the reviewers by averaging the achieved dosage from both generations as shown in Table 5 of this DER.

4. Dose selection rationale: It was stated that dose levels were based on the results of a preliminary reproduction study in the mouse, carried out in the same laboratory. No further information was provided.

5. Dosage preparation and analysis: The test diets were prepared from a premix. The frequency of preparation was not stated. Diets were stored at -20°C or at room temperature in plastic containers. Concentrations for all five dosages were analyzed four times after the start of the study. Homogeneity (top, middle, bottom) was determined on the low- and high-dose formulations. Stability was determined on diet maintained at room temperature for up to 14 days or at -20°C for up to 49 days.

   Results - Concentration analyses (range as % of nominal): 89.0-103.0%.

   Homogeneity analyses (range as % of nominal): 95-97.9%.

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Stability analyses (% of day 0): up to 14 days at room temperature - 93.8-100.8%; up to 49 days at -20°C - 87.5-101.0%

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

6. Dosage administration: Formulations were administered continuously in the diet.

C. OBSERVATIONS

1. Parental animals: Parental animals were observed daily for clinical signs, morbidity, and mortality and were given a detailed examination at the time of weighing. Animals were weighed weekly during the premating period. After the premating period, males were weighed at 2 week intervals. Females were weighed on GDs 1, 8, and 15; and on LDs 1, 5, 8, 22, and 29. All animals were weighed at termination. Food consumption was measured throughout the study period, but calculated on a weekly basis. These data were then used to calculate the food conversion ratios and the average test substance intake for individuals and treatment groups. Water consumption was not measured. Estrus cycles were not monitored. Semen counts were not measured.

2. Litter observations: Litters were examined daily for clinical signs, morbidity, and mortality. Pups were weighed on PND 1, 5, 8, 15, 22, and 29. Litters were not standardized. The following litter parameters (X) were determined (Table 2):

Table 2. Litter observations[a]

Observation	Time of observation (LD)					
	Day 1	Day 5	Day 8	Day 15	Day 22	Day 29
Number of live pups	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X
External alterations	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X
Sex of each pup	X	X	X	X	X	X

a Data extracted from the study report, page 23.

3. Developmental landmarks: Pups were not examined for the developmental landmarks.

4. Behavioral tests: Pups were not examined for behavioral endpoints.

5. Sexual development: The day of preputial separation was determined in F_1 males selected for reproduction and in one F_2 male pup/litter/dose.

6. Postmortem observations:

   1) Parental animals: All surviving P and F_1 adult males and females were sacrificed after weaning of their last litters. All parental animals were subjected to a complete external and internal postmortem examination. Blood for tyrosine determination was collected from 5 F_1 adult mice/sex/dose at termination. The uterus of each female was examined for implantation sites.

   2) Offspring: Pups that died or were sacrificed on or before PND 18 received an external, abdominal, and thoracic exam. Pups that died or were terminated prematurely after PND 18 received a cranial exam in addition to the previously mentioned exams. Blood for tyrosine determination was collected from 5 F_2 pups/sex/dose at termination. Selected F_1 and F_2 pups (10/sex/group, 1/sex/litter, and all clinically abnormal pups at weaning) were weighed and given a full necropsy.

      The following organs or tissues, collected from all parental animals and from pups selected at weaning to receive a full necropsy, were weighed (XX) and/or preserved (X) in neutral buffered formalin for future examination:

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	DIGESTIVE		CARDIOVASC./HEMAT		NEUROLOGIC
	Tongue		Aorta		Brain (medulla, cerebellum, cortex)
	Salivary glands		Heart		Peripheral nerve
	Esophagus		Bone marrow		Spinal cord (3 levels)
	Stomach		Lymph nodes	X	Pituitary
	Duodenum		Spleen	X	Eyes
	Jejunum		Thymus		GLANDULAR
	Ileum		UROGENITAL		Adrenal glands
	Cecum	XX	Kidneys		Harderian gland
	Colon (mid)		Urinary bladder		Mammary gland
	Rectum	XX	Testes		Thyroid
XX	Liver	XX	Epididymis		
	Gall bladder	X	Prostate		OTHER
	Pancreas	X	Seminal vesicle/coag.		Bone
	RESPIRATORY	X	Ovaries		Skeletal muscle
	Trachea		Oviducts		Smooth muscle
	Lungs	X	Uterus		Lacrimal gland
	Nasal cavity	X	Vagina		Zymbal gland
	Pharynx		Ureter	X	All gross lesions and masses
	Larynx		Urethra		Skin
		X	Cervix		Teeth

Slides prepared from these tissues were examined for control and high-dose adults and pups only. The eyes and abnormal tissues from all animals and reproductive organs and pituitary gland from infertile animals (adults only) in the 10, 350, and 1500 ppm groups were also examined.

D. DATA ANALYSIS

1. Statistical analyses: F_1 adult week 1 body weight, food consumption and utilization, GD1 and LD1 body weights, adult and pup organ weights, litter size, gestation length, precoital interval, PND1 pup body weight, total litter weight, and day of preputial separation were tested for significant differences by analysis of variance. In addition, the following were tested by analysis of covariance: adult premating body weights (week 1 body weight as covariant), adult gestation and lactation body weights (GD1 and LD1 body weights as covariants), pup body weights (PND1 as covariant), and adult and pup organ weights (final body weight as covariant).

   The following proportions were analyzed by Fisher's Exact Test: successful matings, whole litter losses, litters with precoital interval of 1, 2, 3, 4, and >4 days, females with gestation periods of 17-22 days, male pups with preputial separation on each day, pups born live, pups surviving, litters with all pups born live, and litters with all pups surviving.

The percentage of live born pups and percentage of pup survival for days 1-22 were double arcsine transformed, then considered by analysis of variance.

Females with total litter loss were excluded from lactation analyses. Females not giving birth to at least one live pup were excluded from the analysis of gestation length. Litters with total litter loss were excluded from pup survival and litter size analyses.

2. Indices:

Reproductive indices: The following reproductive indices as presented in the study report were calculated for the P, F_{1B}, and F_2 adults:

female parturition index (%) = # of females producing at least one live pup/# of females paired x 100

Offspring viability indices: The following viability indices as presented in the study report were calculated for the F_1 and F_2 litters:

livebirth index (%) = # of pups born live/total pups born
day 22 viability index (%) = # of live pups at day 22/ # of pups born alive x 100%

3. Historical control data: Historical control data were not provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs: There was an increase in the incidence of opaque eyes (Table 3) in 7000 ppm F_1 males (4/26 treated vs 0/26 controls) and females (6/26 treated vs 0/26 controls). Opaque eyes were also observed in a single animal from each of the following groups: 7000 ppm P males, 10 and 350 ppm F_1 males, and 1500 ppm F_1 females. There was no treatment-related mortality observed in the P or F_1 adults. No P males died prematurely. Five P females died or were euthanized during the study: one 50 ppm female was euthanized during week 3 upon observation of a swollen head, piloerection, hunched posture, dyspnea, pallor, and urine-stained fur; a second 50 ppm female was euthanized at week 9 because it lacked a vaginal opening and was unable to mate; one control and one 10 ppm dam were found dead during lactation (LDs 19 and 23, respectively); and one 1500 ppm dam was found dead on the day prior to scheduled termination. One F_1 male was euthanized during week 13 after observation of subcutaneous shoulder mass, paleness, hunched posture, and dyspnea. Six F_1 adult females died or were euthanized during the study: one 50 ppm and one 350 ppm female were euthanized during week 6 because they lacked a vaginal opening; two 350 ppm females were found dead on GD 21 or LD 29, respectively; one 10 ppm female was found dead on LD 20; and one 1500 ppm female was found dead on LD 19. The cause of death for animals found dead was not provided.

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Table 3. Incidence (# of animals) of opaque eyes in P and F_1 generation adults.[a]

Observation	Dose Group (ppm)					
	0	10	50	350	1500	7000
P males						
# Animals examined	NR	NR	NR	NR	NR	NR
Opaque eyes	0	0	0	0	0	1
P females						
# Animals examined	NR	NR	NR	25	NR	NR
Opaque eyes	0	0	0	0	0	0
F_1 males						
# Animals examined	NR	NR	NR	NR	NR	NR
Opaque eyes	0	1	0	1	0	4
F_1 females						
# Animals examined	NR	NR	NR	NR	NR	NR
Opaque eyes	0	0	0	0	1	6

a Data extracted from the study report Tables 9 and 10, pages 89, 94, and 98.
NR Not reported. n=26 at study start for all groups.

2. Body weight, body weight gains, and food consumption: Adjusted (to LD 1) body weights (Tables 4a and b) were decreased during lactation in 7000 ppm P dams on LDs 5, 8, and 15 (↓3-13%, $p \leq 0.05$ or 0.01). Body weights were decreased in 7000 ppm F_1 dams on LDs 1 and 15 (↓9-12%, $p \leq 0.01$). Body weights were not different on LDs 22 or 29 in either generation. There were no differences of toxicological concern during the premating or gestation periods. There were only minor, sporadic differences (↓5-↑7%, $p \leq 0.05$ or 0.01) in the P adult adjusted (to week 1) body weights during premating. Absolute body weights were decreased ($p \leq 0.01$) during week 1 of premating in 7000 ppm F_1 males (↓16%) and females (↓17%). These differences, although large, were brief. Differences (↓7-↑7%, $p \leq 0.05$ or 0.01) occurred in adjusted (to week 1) body weights at other timepoints and in other dose groups. There were no differences of toxicological concern during gestation for either generation.

Table 4a. Selected mean body weights (g) and body weight gains (g) during premating for P and F_1 generation males and females.[a]

Study Period	Dose Group (ppm)					
	0	10	50	350	1500	7000
P Generation Males-Premating						
Week 1	22.3	22.8	23.2	22.6	22.1	22.2
Week 9	42.1	42.4	44.0	42.8	40.6	41.1
Gain (Week 1-9)	19.8	19.6	20.8	20.2	18.5	18.9
P Generation Females-Premating						
Week 1	20.5	20.4	20.4	20.7	20.8	21.1
Week 9	33.6	34.3	35.2	35.2	34.8	35.0
Gain (Week 1-9)	13.1	13.9	14.8	14.5	14.0	13.9
F_1 Generation Males-Premating						
Week 1	25.8	24.5	25.1	25.3	24.4	21.6**(↓16)
Week 9	43.9	43.7	44.2	45.1	43.7	42.1
Gain (Week 1-9)	18.1	19.2	19.1	19.8	19.3	20.5
F_1 Generation Females-Premating						
Week 1	25.5	23.7*(↓7)	24.9	24.1	23.7*(↓7)	21.1**(↓17)
Week 9	38.1	37.2	37.6	37.7	37.2	35.4
Gain (Week 1-9)	12.6	13.5	12.7	13.6	13.5	14.3

a Body weight data were extracted from the study report, Tables 11 and 12, pages 101 through 104. Body weight gains were calculated from this data by the reviewers. Percent difference from controls is listed parenthetically.

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

Table 4b. Selected mean body weights (g) and body weight gains (g) during gestation and lactation for P and F_1 generation males and females.[a]

Study Period	Dose Group (ppm)					
	0	10	50	350	1500	7000
P Generation Females-Gestation						
Day 1	33.9	34.1	34.7	35.5*(↑5)	35.3	34.3
Day 15	50.0	50.3	51.9	52.2	53.3	51.3
Gain 1-15	16.1	16.2	17.2	16.7	18.0	17.0
P Generation Females-Lactation						
Day 1	43.0	43.1	43.4	44.1	44.0	43.5
Day 5[b]	47.9	48.6	48.7	48.8	48.0	46.6*(↓3)
Day 8[b]	50.2	50.5	51.0	51.3	50.3	46.9**(↓7)
Day 15[b]	54.0	55.2	55.3	54.4	53.3	46.9**(↓13)
Day 29	41.9	42.7	42.8	43.9	43.5	41.9
Gain 1-29	-1.1	-0.4	-0.6	-0.2	-0.5	-1.6
F_1 Generation Females-Gestation						
Day 1	38.7	37.1	38.3	37.3	36.7	35.4**(↓9)
Day 15	55.1	54.3	54.9	53.1	53.1	52.3
Gain 1-15	16.4	17.2	16.6	15.8	16.4	16.9
F_1 Generation Females-Lactation						
Day 1	48.1	46.7	47.8	47.0	46.8	44.0**(↓9)
Day 15[b]	58.9	58.9	60.0	58.1	57.6	52.1**(↓12)
Day 29	47.8	45.6	47.2	47.4	45.2	43.4
Gain 1-29	-0.3	-1.1	-0.6	0.4	-1.6	-0.6

a Body weight data were extracted from the study report, Tables 13 through 16, pages 105 through 108. Body weight gains were calculated from this data by the reviewers. Data reported are absolute body weights, except where noted. Percent difference from controls is listed parenthetically.

b Adjusted (to day 1) body weights.

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

Differences (↓8-↑11%, $p \leq 0.05$ or 0.01) occurred in all treatment groups during premating or gestation, but these differences were not strictly dose-dependent. However, food consumption was decreased in the 7000 ppm P dams throughout lactation (↓15-28%, $p \leq 0.05$ or 0.01) (Table 4c) and in F_1 dams during weeks 2, 3, and 4 of lactation (↓10-33%, $p \leq 0.05$ or 0.01). At 1500 ppm, reductions in food consumption were also apparent during weeks 3 and 4 reaching statistical significance during several intervals $p \leq 0.05$ in P and F1 females.

Table 4c. Selected mean food consumption (g) during lactation for P and F_1 generation dams.[a]

Study Period	Dose Group (ppm)					
	0	10	50	350	1500	7000
P Generation Females						
Week 1	38.5	35.5	37.4	37.7	38.4	32.6*(↓15)
Week 2	48.8	43.5*(↓11)	47.0	47.5	47.1	35.0**(↓28)
Week 3	66.3	64.9	69.6	67.1	61.6	56.3*(↓15)
Week 4	115.8	110.3	111.6	111.9	107.9*(↓7)	98.7**(↓15)
F_1 Generation Females						
Week 1	47.6	44.9	46.7	44.4	49.9	42.6
Week 2	49.8	45.0*(↓10)	48.5	44.6*(↓10)	45.6	33.6**(↓33)
Week 3	67.2	71.9	72.6*(↑8)	61.1	59.3*(↓12)	52.5**(↓22)
Week 4	103.8	106.2	107.1	98.9	98.2	93.3*(↓10)

a Data were extracted from the study report, Tables 23 and 24, pages 117 and 118. Percent difference from controls is listed parenthetically.

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

3. Test substance intake: Based on food consumption, body weight, and nominal diet concentration, the doses expressed as mean daily mg test substance/kg body weight during the 8 week premating period are presented in Table 5. The values for the P or F_1 generation were considered to be representative of the test substance intake for the entire study.

Table 5. Test substance intake (mean mg/kg body weight/day).[a]

Male					Female				
10	50	350	1500	7000	10	50	350	1500	7000
P Generation									
2.1	10.2	71.4	311.8	1471.9	2.4	12.0	84.4	371.6	1631.5
F_1 Generation									
2.1	10.0	71.3	301.6	1439.1	2.4	11.4	80.5	353.8	1673.0

a Data extracted from the study report, Appendix H, pages 245 and 246

4. Food efficiency: Mean food efficiencies (body weight gain [g]/100 g food consumption) during the 8-week premating period were calculated by the investigators and ranged as follows: P males 4.56 - 4.86; P females 3.20-3.65; F_1 males 4.25-4.92; F_1 females 2.97-

3.46. Increases ($p \leq 0.05$ or 0.01) in food efficiency, which were considered not of toxicological concern, were observed between 7000 ppm F_1 males and females vs controls during weeks 1-4 and for the overall (weeks 1-8) premating period (↑13-19%). Increases ($p \leq 0.05$ or 0.01) in food efficiency were also observed in the 50 and 350 ppm P females (↑11-17%) during weeks 1-4 and for the overall (weeks 108) premating period, but these were also considered not of toxicological concern. A decrease in food efficiency was observed in the 50 and 350 ppm F_1 females during weeks 5-8 (↓27-29%), but these decreases were not dose-dependent and not observed at any other times; therefore, these differences were considered not of toxicological concern.

5. Reproductive function:

   a. Estrus cycle length and periodicity: No observations were made pertaining to estrus cycle length and periodicity.
   b. Sperm and male reproductive organ measures in males: No observations were made pertaining to sperm and male reproductive organ measures.
   c. Sexual maturation: Preputial separation (Table 6) was delayed ($p \leq 0.05$ or 0.01) in 10, 1500, and 7000 ppm F_1 males (↑6, 3, and 7%, respectively) and in the 7000 ppm F_2 males (↑10%). These differences appear to demonstrate a dose response relationship in both the F1 and F2 and are considered effects associated with administration of the test material and resulted in delays of up to 1 to 3 days.

Table 6. Mean day of preputial separation.[a]

Dosage (ppm)					
0	10	50	350	1500	7000
F_1 Generation					
30.2	31.9**(↑6)	31.0	30.7	31.2*(↑3)	32.2**(↑7)
F_2 Generation					
30.5	30.6	30.3	31.2	31.3	33.5**(↑10)

a Data extracted from the study report, Tables 38 and 39, pages 135 and 136. Percent difference from controls is listed parenthetically. N = 26 for F_1 generation and 17-20 for F_2 generation.

6. Reproductive performance: There were no statistically significant differences observed in the reproductive performance of the P or F_1 adults (Table 7). However, there was a possible effect on the Parturition Index in the P dams producing the F1a litters and the F1 dams producing the F2 litters.

Table 7. Reproductive performance of P and F_1 dams.[a]

Observation	Dose Group (ppm)					
	0	10	50	350	1500	7000
P Dams - Litter F_{1A}						
Female Parturition Index-%	92.3	96.2	95.8	76.9	84.6	76.9
Mean days to mating	3.32	2.18	2.57	3.86	2.73	3.18
Gestation Length (days)	19.2	19.0	19.1	19.3	19.0	19.2
Number of Litters	24	25	23	20	22	20
F_1 Dams - Litter F_2						
Female Parturition Index-%	80.8	76.9	84.0	83.3	80.8	73.1
Mean days to mating	3.09	3.33	3.13	3.00	2.64	3.43
Gestation Length (days)	19.4	19.3	19.5	19.5	19.5	19.2
Number of Litters	21	20	21	20	21	19

a Data extracted from the study report, Tables 25, 26, and 27, pages 119, 120, and 121.

7. Parental postmortem results

a) Organ weights: There were differences ($p \leq 0.05$ or 0.01) in liver, kidney, and epididymides weights (↑4-17%) in P or F_1 males and females. In F1 adult males, relative epididymides weights were significantly increased at the 1500 and 2000 ppm dose levels. Absolute kidney weights were statistically significantly increased in F1 males at 350 ppm and above. Percent increases above controls were 11, 8, and 13% for 350, 1500, and 7000 ppm, respectively. Relative kidney weights were also statistically significantly increased at 350 ppm and above in males. Percent increases above controls were 6, 8 and 17% for 350, 1500, and 7000 ppm, respectively. In females, relative kidney weights were statistically significantly increased at 50 ppm and above, but percent increases on ranged from 4 to 9% above controls. In addition, F1 relative liver weights were also elevated at doses of 350 ppm ($p \leq 0.05$) and at $p \leq 0.01$ at 7000 ppm.

Few organs were weighed in this study and the reported organ weight changes are associated with in utero exposure at least in the F1 animals. Significant organ weight changes might potentially be associated with developmental effects. Therefore, although no histopathological changes suggestive of toxicity were reported, these organs weight effects which are dose related may be associated with adverse effects associated of the test material..

b) Pathology:

1) Macroscopic examination: The was an increase in the incidence of opaque/cloudy eyes (Table 8) in 7000 ppm P males (3/26 treated vs 0/26 controls), P females (1/26 treated vs 0/25 controls), F_1 males (5/25 treated vs 0/26 controls) and F_1

females (5/26 treated vs 1/26 controls).

Table 8. Incidence of opaque or cloudy eyes at necropsy in P and F_1 males and females.[a]

Observation	Dose Group (ppm)					
	0	10	50	350	1500	7000
P Males						
No. of animals examined	26	26	26	26	26	26
Opaque eyes	0	0	0	0	0	2
Cloudy eyes	0	0	0	1	0	1
P Females						
No. of animals examined	25	25	24	26	25	26
Opaque eyes	0	0	0	0	0	0
Cloudy eyes	0	0	1	0	0	1
F_1 Males						
No. of animals examined	26	26	26	26	26	25
Opaque eyes	0	1	0	1	0	4
Cloudy eyes	0	1	0	1	1	1
F_1 Females						
No. of animals examined	26	25	25	23	25	26
Opaque eyes	0	0	0	0	1	4
Cloudy eyes	1	0	0	0	0	1

a Data extracted from the study report, Tables 42 and 43, pages 148, 151, 155, and 158.

2) Microscopic examination: An increase in the incidence of minimal to marked unilateral and bilateral cataractous change in the eyes (Table 9) was observed in the 7000 ppm P males (unilateral - 3/26 treated vs 0/26 controls), F_1 males (unilateral - 7/25 treated, bilateral - 1/25 treated vs 0/26 controls), F_1 females (unilateral - 4/26 treated, bilateral - 2/26 treated vs 0/26 controls). Retinal detachment with marked cataractous change was also observed in one 7000 ppm F_1 male and female; whether this lesion was a primary effect of mesotrione on the eye is equivocal.

Table 9. Select histological findings in P and F_1 males and females.[a]

Observation		Dose Group (ppm)					
		0	10	50	350	1500	7000
P Males							
No. of animals examined		26	22	25	26	26	26
Unilateral cataractous change	Slight	0	0	0	0	0	2
	Moderate	0	0	0	0	0	1
	Total	0	0	0	0	0	3
F_1 Males							
No. of animals examined		26	26	25	24	26	25
Unilateral cataractous change	Moderate	0	0	0	0	0	5
	Marked	0	0	0	0	0	2
	Total	0	0	0	0	0	7
Bilateral cataractous change	Marked	0	0	0	0	0	1
	Total	0	0	0	0	0	1
Retinal detachment	Slight	0	0	0	0	0	1
	Total	0	0	0	0	0	1
F_1 Females							
No. of animals examined		26	25	25	23	25	26
Unilateral cataractous change	Minimal	0	0	0	0	0	1
	Moderate	0	0	0	0	0	2
	Marked	0	0	0	0	0	1
	Total	0	0	0	0	0	4
Bilateral cataractous change	Slight	0	0	0	0	0	1
	Moderate	0	0	0	0	0	1
	Total	0	0	0	0	0	2
Retinal detachment	Slight	0	0	0	0	0	1
	Total	0	0	0	0	0	1

a Data extracted from the study report, Tables 44 and 45, ages 162, 173, and 177.

8. Plasma tyrosine levels: Plasma tyrosine levels (Table 10) were elevated in all treatment groups. The statistical significance of the differences were not determined. Increases ranged from 269-586% of controls in males and from 159-542% of controls in females.

Table 10. Mean plasma tyrosine levels (nmol/ml) in F_1 adults.[a]

Dosage (ppm)					
0	10	50	350	1500	7000
Males					
149.2	550.9(↑269)	704.2(↑372)	874.4(↑486)	905.5(↑507)	1022.9(↑586)
Females					
124.4	322.0(↑159)	369.0(↑197)	634.0(↑410)	710.2(↑471)	798.2(↑542)

a Data extracted from the study report, Table 52, page 227.

The toxicological significance of the increases in plasma tyrosine levels is not completely understood as it relates to other effects in this mouse study. It is possible that the increased tyrosine levels are associated with the cataractous or other eye effects or other toxicological findings observed in this study. Assuming that there would be a threshold for these other effects, one also must consider the duration of the effect over the course of the life-span of the exposed animal and that over time, more eye effects might be observed in the population of test animals at still lower dose levels. In addition, the potential effect of in utero exposure needs to be considered. In assessment of developmental toxicants, it is known that developmental toxicity may not be apparent until much later in life.

Clearly, the above changes in tyrosine levels are dramatic as compared to the controls. However, as noted above, the effects on the eye may be at much greater incidences and at lower dose levels if examinations were performed near the end of the normal life-span of these animals. If increases in eye pathology are not apparent in chronic mouse studies, it might be assumed that this is a manifestation of developmental toxicity since exposure of F_1 animals was in utero.

B. OFFSPRING

1. Viability and clinical signs: An increase in the number of pups with ocular discharge (Table 11a) was observed in the 7000 ppm dose groups (F_1 and F_2 - 11 pups in 5 litters each vs 0 controls). A slight increase in the number of pups with ocular discharge was also observed in the other F_1 (1-5 pups/group in 1-3 litters/group in the 10, 50, 350, and 1500 ppm groups) and F_2 litters (2-3 pups/group in 2-3 litters in the 10, 350, and 1500 ppm groups). The toxicological significance of these findings is unclear at this time. However, no controls were affected suggesting that the effect is certainly compound related. In addition, an increase in the number of pups with opaque eyes (6 pups in 1 litter vs 0 controls) and pups with eye(s) shut (7 pups in 4 litters vs 0 controls) was observed in the 7000 ppm F_2 pups. No treatment-related effects on mortality or viability

were observed at any time in the F_1 and F_2 litters (Tables 11b and c).

Table 11a. Select clinical signs [# of pups(# of litters)] in F_1 and F_2 pups.[a]

Observation	Dose Group (ppm)					
	0	10	50	350	1500	7000
F_1 Generation						
No. of live pups at PND 22	258(15)	307(17)	287(15)	240(11)	256(7)	221(10)
Ocular discharge	0	4(3)	1(1)	4(3)	5(3)	11(5)
F_2 Generation						
No. of live pups at PND 22	235(12)	223(9)	236(12)	207(12)	218(13)	211(10)
Ocular discharge	0	3(3)	0(0)	3(3)	2(2)	11(5)
Opaque eye	0	0	0	0	0	6(1)
Eyes shut	0	2(1)	0	0	0	7(4)

a Data extracted from the study report, Tables 31, 32 and 33, pages 126, 127, and 128.

Table 11b. F_1 generation mean litter size and viability.[a]

Observation	Dose Group (ppm)					
	0	10	50	350	1500	7000
Mean litter size						
Day 1	11.7	12.8	13.0	12.6	12.7	11.8
Day 5	11.3	12.4	12.5	12.3	11.9	11.1
Day 8	11.2	12.4	12.5	12.3	11.8	11.1
Day 15	11.2	12.4	12.5	12.1	11.7	11.1
Day 22	11.2	12.3	12.5	12.0	11.6	11.1
Day 29	11.2	12.3	12.5	12.0	11.6	11.1
Number live pups						
Day 1	282	321	300	252	279	236
Day 5	NR	NR	NR	NR	NR	NR
Day 8	NR	NR	NR	NR	NR	NR
Day 15	NR	NR	NR	NR	NR	NR
Day 22	258	307	287	240	256	221
Day 29	NR	NR	NR	NR	NR	NR
Number deaths[b]						
Days 0-4	NR	NR	NR	NR	NR	NR
Days 0-22	24	14	13	12	23	15
Viability indices (%)						
Stillborn[b]	2.1	1.0	1.3	0.4	1.2	2.2
Livebirth	97.9	99.0	98.7	99.6	98.8	97.8
Viability (Day 5)	NR	NR	NR	NR	NR	NR
Viability (Day 8)	NR	NR	NR	NR	NR	NR
Viability (Day 15)	NR	NR	NR	NR	NR	NR
Viability (Day 22)	95.5	96.3	96.1	95.7	92.1*(14)	93.7
Viability (Day 29)	NR	NR	NR	NR	NR	NR
Sex ratio (% male)	NR	NR	NR	NR	NR	NR

a Data extracted from the study report, Table 29, 30, and 31, page 123, 124, and 126.
b Calculated by the reviewers from data contained in this table.

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Table 11c. F_2 generation mean litter size and viability.[a]

Observation	Dose Group (ppm)					
	0	10	50	350	1500	7000
Mean litter size						
Day 1	11.9	12.5	11.9	11.1	11.6	12.0
Day 5	11.3	11.8	11.2	10.5	10.9	11.2
Day 8	11.3	11.8	11.2	10.5	10.9	11.2
Day 15	11.3	11.8	11.2	10.4	10.9	11.1
Day 22	11.2	11.7	11.2	10.4	10.9	11.1
Day 29	11.2	11.7	11.2	10.4	10.9	11.1
Number live pups						
Day 1	249	248	249	222	242	228
Day 5	NR	NR	NR	NR	NR	NR
Day 8	NR	NR	NR	NR	NR	NR
Day 15	NR	NR	NR	NR	NR	NR
Day 22	235	223	236	207	218	211
Day 29	NR	NR	NR	NR	NR	NR
Number deaths[b]						
Number deaths[b]						
Days 0-4	NR	NR	NR	NR	NR	NR
Days 0-22	14	25	13	15	24	17
Viability indices (%)						
Stillborn[b]	1.5	1.4	1.0	0.4	2.1	1.9
Livebirth	98.5	98.6	99.0	99.6	97.9	98.1
Viability (Day 5)	NR	NR	NR	NR	NR	NR
Viability (Day 8)	NR	NR	NR	NR	NR	NR
Viability (Day 15)	NR	NR	NR	NR	NR	NR
Viability (Day 22)	94.8	94.2	95.1	94.1	94.6	92.7
Viability (Day 29)	NR	NR	NR	NR	NR	NR
Sex ratio (% male)	NR	NR	NR	NR	NR	NR

a Data extracted from the study report, Table 29, 30, and 31, page 123, 125, and 126.
b Calculated by the reviewers from data contained in this table.

2. Body weights and gains: Pup body weights (Tables 12a and b) were decreased ($p \leq 0.05$ or 0.01) in all F1 dose groups in a dose related manner. Effects were not observed in the F2 pups at doses of 350 ppm and below.

Table 12a. Mean F_1 adjusted (to PND 1) pup weights and body weight gains (g).[a]

Postnatal Day	Dose Group (ppm)					
	0	10	50	350	1500	7000
Males						
Day 1[b]	1.6	1.6	1.6	1.7	1.7	1.7
Day 5	3.3	3.1	3.2	3.3	3.2	3.3
Day 8	5.2	5.0	5.1	5.3	5.0	4.9*(↓6)
Day 15	8.4	8.2	8.2	8.1	8.1	7.1**(↓15)
Day 22	15.6	14.4*(↓8)	14.7	14.5*(↓7)	13.4**(↓14)	11.8**(↓24)
Day 29	26.5	24.5**(↓8)	25.0*(↓6)	24.5**(↓8)	23.6**(↓11)	21.4**(↓19)
Gain (0-29)[c]	24.5	22.2	23.0	23.2	22.5	20.1 (↓18)
Females						
Day 1[b]	1.6	1.6	1.6	1.7	1.6	1.6
Day 5	3.3	3.1*(↓6)	3.1	3.2	3.2	3.3
Day 8	5.2	5.0	4.9	5.1	5.1	4.9
Day 15	8.4	8.1	7.9	8.0	8.1	7.2**(↓14)
Day 22	15.9	14.6*(↓8)	14.6*(↓8)	14.6*(↓8)	13.7**(↓14)	12.4**(↓22)
Day 29	26.0	24.5*(↓6)	24.7*(↓5)	24.4*(↓6)	23.6**(↓9)	21.7**(↓17)
Gain (0-29)[c]	23.8	22.4	23.1	23.2	22.2	20.0 (↓16)

a Data extracted from the study report, Table 34, pages 129 and 130. Percent difference from controls is listed parenthetically.
b Absolute body weight.
c Calculated by the reviewers from data extracted from the study report, Table 34, pages 129 and 130.

Table 12b. Mean F_2 adjusted (to PND 1) pup weights and body weight gains (g).[a]

Postnatal Day	Dose Group (ppm)					
	0	10	50	350	1500	7000
Males						
Day 1[b]	1.9	1.8	1.9	2.0	1.9	1.8
Day 5	3.5	3.5	3.7	3.6	3.6	3.5
Day 8	5.3	5.3	5.5	5.4	5.3	5.2
Day 15	9.1	9.1	9.4	9.2	9.1	8.3*(↓9)
Day 22	15.8	15.7	16.0	15.5	14.2**(↓10)	12.6**(↓20)
Day 29	26.4	26.3	26.3	25.9	24.3**(↓8)	22.0**(↓17)
Gain (0-29)[c]	24.8	23.9	25.0	25.1	22.5	18.8 (↓24)
Females						
Day 1[b]	1.8	1.8	1.9	1.9	1.9	1.7
Day 5	3.5	3.4	3.7	3.6	3.5	3.5
Day 8	5.2	5.2	5.5	5.4	5.2	5.2
Day 15	9.0	9.1	9.4	9.1	9.0	8.5
Day 22	15.7	15.8	16.2	15.8	14.4*(↓8)	13.1**(↓17)
Day 29	25.1	25.3	25.8	25.7	23.7*(↓6)	22.2**(↓12)
Gain (0-29)[c]	23.4	22.9	24.7	24.7	22.1	19.1 (↓18)

a Data extracted from the study report, Table 35, pages 131 and 132. Percent difference from controls is listed parenthetically.
b Absolute body weight.
c Calculated by the reviewers from data extracted from the study report, Table 35, pages 131 and 132.

3. Anogenital distance: Not determined.

4. Offspring developmental landmarks: Data presented in tables 38 and 39 of the test report demonstrated delays in preputial separation in all F1 dose groups. Statistical significance was reached at the 10, 1500, and 7000 ppm dose levels. In the F2 adults, developmental delays were apparent at doses of 350 ppm and above. At the 7,000 ppm dose level, the delay reached a full 3 days and was statistically significant, $p \leq 0.01$.

5. Offspring behavioral tests: Not determined.

6. Offspring postmortem results:

   a) Organ weights: There were differences ($p \leq 0.05$ or 0.01) in liver, kidney, testes, and epididymides weights (↓29-↑33%). In the F0 adult males, statistically significant increases in relative liver weights were noted in males at 350 ppm and above and in females at 1500 ppm and 7000 ppm. Absolute mean weights increased in males at 350 ppm and 7000 ppm and in females at 50 ppm and higher ($p \leq 0.05$ and $p \leq 0.01$).

In F1 males, relative epididymides weights increased at 1500 and 7000 ppm ($p \leq 0.05$). In F1 males statistically significant increases in absolute and relative kidney weights were observed at dose levels of 350 ppm and above. In F1 females, relative kidney weights were statistically significantly increased at dose levels of 50 ppm and above. Relative liver weights also increased in both males and females at 350 and 7000 ppm levels but not at statistically significant levels at the 1500 ppm level.

b) Pathology:

1) Macroscopic examination: There was an increase in the incidence of opaque or cloudy eyes (Table 13) in 7000 ppm (10/33 treated vs 0/30 controls) and 1500 ppm (4/30 treated) F_2 males.

Table 13. Incidence of opaque or cloudy eyes at necropsy in F_1 and F_2 male and female pups.[a]

Observation	Dose Group (ppm)					
	0	10	50	350	1500	7000
F_1 Males						
No. of animals examined	33	35	34	28	32	30
Opaque eyes	0	0	0	0	0	0
Cloudy eyes	1	5	0	1	2	2
Total opaque/cloudy eyes	1	5	0	1	2	2
F_1 Females						
No. of animals examined	32	34	33	29	29	30
Opaque eyes	0	1	1	0	0	1
Cloudy eyes	6	7	3	1	1	1
Total opaque/cloudy eyes	6	8	4	1	1	2
F_2 Males						
No. of animals examined	30	28	31	30	30	33
Opaque eyes	0	0	0	0	2	4
Cloudy eyes	0	1	0	0	2	6
Total opaque/cloudy eyes	0	1	0	0	4	10
F_2 Females						
No. of animals examined	31	29	31	30	30	31
Opaque eyes	0	0	0	0	1	2
Cloudy eyes	1	0	0	0	2	1
Total opaque/cloudy eyes	1	0	0	0	3	3

a Data extracted from the study report, Tables 48 and 49, pages 194 through 209.

2) Microscopic examination: There was an increase in the incidence of minimal to marked unilateral and/or bilateral cataractous changes (Table 14) in all 7000 ppm groups, with the severity ranging from minimal to marked: F_1 males - 4/30 treated vs 0/37 controls; F_1 females - 2/30 treated vs 0/40 controls; F_2 males - 11/33 treated vs 0/30 controls; F_2 females - 2/31 treated vs 0/32 controls. Minimal unilateral cataractous change was also observed in the 1500 ppm F_2 males (2/18 treated vs 0/30 controls). Retinal detachment was observed in one 7000 ppm F_2 male; whether this finding was a direct effect of mesotrione is not clear.. There were no other treatment-related findings.

Table 14. Incidence of unilateral and bilateral ocular cataractous change at necropsy in F_1 and F_2 male and female pups.[a]

Observation		Dose Group (ppm)					
		0	10	50	350	1500	7000
F_1 Males							
No. of animals examined		37	24	11	12	14	30
Unilateral	slight	0	0	0	0	0	2
	total	0	0	0	0	0	2
Bilateral	minimal	0	0	0	0	0	1
	slight	0	0	0	0	0	1
	total	0	0	0	0	0	2
Total uni- and bilateral		0	0	0	0	0	4
F_1 Females							
No. of animals examined		40	21	17	13	13	30
Unilateral	minimal	0	0	0	0	0	1
	slight	0	0	0	0	0	1
	total	0	0	0	0	0	2
Total uni- and bilateral		0	0	0	0	0	2
F_2 Males							
No. of animals examined		30	16	11	11	18	33
Unilateral	minimal	0	0	0	0	2	1
	slight	0	0	0	0	0	1
	moderate	0	0	0	0	0	1
	total	0	0	0	0	2	3
Bilateral	minimal	0	0	0	0	0	3
	slight	0	0	0	0	0	2
	moderate	0	0	0	0	0	1
	marked	0	0	0	0	0	2
	total	0	0	0	0	0	8
Total uni- and bilateral		0	0	0	0	2	11
Retinal detachment, moderate		0	0	0	0	0	1
F_2 Females							
No. of animals examined		32	18	13	12	18	31
Unilateral	minimal	0	0	0	0	0	1
	total	0	0	0	0	0	1
Bilateral	slight	0	0	0	0	0	1
	marked	0	0	0	0	0	0
	total	0	0	0	0	0	1
Total uni- and bilateral		0	0	0	0	0	2

a Data extracted from the study report, Tables 50 and 51, pages 210 through 226.

7. Plasma tyrosine levels: Plasma tyrosine levels (Table 15) were elevated in all treatment groups. The statistical significance of the differences were not determined by the investigators. Increases ranged from 794-5009% of controls in males and from 665-3945% of controls in females.

Table 15. Mean plasma tyrosine levels (nmol/ml) in F_2 pups.[a]

Dosage (ppm)					
0	10	50	350	1500	7000
Males					
26.5	236.8(↑794)	500.3(↑1788)	847.2(↑3097)	818.8(↑2990)	1354(↑5009)
Females					
33.2	253.9(↑665)	415.1(↑1150)	735.2(↑2114)	801.6(↑2314)	1343(↑3945)

a Data extracted from the study report, Table 53, page 228. Percent difference from controls is listed parenthetically.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: There were no adverse effects of mesotrione on reproductive performance, fertility, fecundity, or offspring survival. Pup body weights were reduced in the 1500 and 7000 ppm groups. Mesotrione at 350 ppm had no effect on pup body weight.

B. REVIEWER'S DISCUSSION: In this 2-generation reproduction study, mesotrione was administered in the diet continuously to Alpk:AP$_f$CD-1 mice (26 mice/sex/dose) at dose levels of 0, 10, 50, 350, 1500, or 7000 ppm (equivalent to 0, 2.1/2.4, 10.1/11.7, 71.4/82.5, 306.7/362.7, or 1455.5/1652.3 mg/kg/day [M/F] in the P and F_1 animals). The P animals were exposed to the test substance beginning at approximately 3 weeks of age and exposure lasted for approximately 8 weeks prior to mating. F_1 pups selected (26/sex/dose) to produce the F_2 generation were exposed to the same dosage as their parents beginning on PND 29 and continuously throughout the rest of the study. F_1 animals were administered the test article for approximately 8 weeks prior to mating to produce the F_2 animals. Mating to produce an F_{2b} generation was not performed. Exposure of all animals to the test material was continuous throughout the study.

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

1. Parental toxicity: There were no statistically significant and treatment-related changes in mortality, or reproductive performance observed in the P or F_1 adults. Adjusted (to LD 1) body weights were decreased during lactation in 7000 ppm P dams on LDs 5, 8, and 15 (↓3-13%, $p \leq 0.05$ or 0.01). Body weights were decreased (↓9-12%, $p \leq 0.01$) in 7000 ppm F_1 dams on LDs 1 (absolute) and 15 (adjusted). Food consumption was decreased ($p \leq 0.05$ or 0.01) in the 7000 ppm P dams throughout lactation (↓15-28%) and in 7000 ppm F_1 dams during weeks 2, 3, and 4 of lactation (↓10-33%).

During clinical observation, an increase in the incidence of opaque eyes in 7000 ppm F_1 males (4/26 treated vs 0/26 controls) and females (6/26 treated vs 0/26 controls) was observed. Opaque eyes were also observed in a single animal from each of the following groups: 7000 ppm P males, 10 and 350 ppm F_1 males, and 1500 ppm F_1 females. At necropsy, an associated increase was observed in the incidence of grossly visible opaque/cloudy eyes in 7000 ppm P males (3/26 treated vs 0/26 controls), P females (1/26 treated vs 0/25 controls), F_1 males (5/25 treated vs 0/26 controls) and F_1 females (5/26 treated vs 1/26 controls). In addition, upon histological examination, an increase was observed in the incidence of minimal to marked unilateral and bilateral ocular cataractous change in the 7000 ppm P males (unilateral - 3/26 treated vs 0/26 controls), F_1 males (unilateral - 7/25 treated, bilateral 1/25 treated vs 0/26 controls), and F_1 females (unilateral - 4/26 treated, bilateral 1/26 treated vs 0/26 controls). Retinal detachment with marked cataractous change was also observed in one of the 7000 ppm F_1 males and females; whether this lesion was a primary effect of mesotrione on the eye is unclear.

Absolute kidney weights were statistically significantly increased in F1 males at 350 ppm and above. Percent increases above controls were 11, 8, and 13% for 350, 1500, and 7000 ppm, respectively. Relative kidney weights were also statistically significantly increased at 350 ppm and above in males. Percent increases above controls were 6, 8 and 17% for 350, 1500, and 7000 ppm, respectively. In females, relative kidney weights were statistically significantly increased at 50 ppm and above, but percent increases on ranged from 4 to 9% above controls.

The LOAEL for parental toxicity is 350 ppm (equivalent to 71.4/82.5 mg/kg/day [M/F]) based upon dose related and statistically significant increases in kidney weights in F1 males. The increased kidney weights may be associated with tyrosyluria since tyrosinemia which was observed at all dose levels in F1 animals (exposed in utero) in this study. Ocular lesions in both sexes and decreased body weights in females were observed at higher dose levels. The NOAEL for parental toxicity is 50 ppm (equivalent to 10.1/11.7 mg/kg/day [M/F]).

2. Offspring Toxicity: No treatment-related effects on mortality or viability were observed at any time in the F_1 and F_2 litters.

An increase in the number of pups with ocular discharge was observed in the 7000 ppm dose groups (F_1 and F_2 - 11 pups in 5 litters each vs 0 controls) during clinical examination. A slight increase in the number of pups with ocular discharge was also observed in the other F_1 (1-5 pups/group in 1-3 litters/group in the 10, 50, 350, and 1500 ppm groups) and F_2 litters (2-3 pups/group in 2-3 litters in the 10, 350, and 1500 ppm groups). An increase in the number of pups with opaque eyes (6 pups in 1 litter vs 0 controls) and pups with eye(s) shut (7 pups in 4 litters vs 0 controls) was observed in the 7000 ppm F_2 pups. Body weights were decreased in all 7000 ppm groups: F_1 males from PND 8 to weaning (↓6-24%); F_1 females from PND 15 to weaning (↓14-22%); F_2 males from PND 15 to weaning (↓9-20%); F_2 females from PND 22 to weaning (↓12-17%). Body weights were also decreased in 1500 ppm groups on PND 22 and 29 (↓6-14%). Body weight gains, as calculated by the reviewers, were decreased in both generations in the 7000 ppm groups (↓16-24%).

At necropsy, an increase was observed in the incidence of opaque or cloudy eyes in 7000 ppm (10/33 treated vs 0/30 controls) and 1500 ppm (4/30 treated) F_2 males. Upon histological examination, an associated increase was observed in the incidence of microscopic minimal to marked unilateral and/or bilateral cataractous changes in all 7000 ppm groups, with the severity ranging from minimal to marked: F_1 males - 4/30 treated vs 0/37 controls; F_1 females - 2/30 treated vs 0/40 controls; F_2 males - 11/33 treated vs 0/30 controls; F_2 females - 2/31 treated vs 0/32 controls. Minimal unilateral cataractous change was also observed in the 1500 ppm F_2 males (2/18 treated vs 0/30 controls).

Plasma tyrosine levels were elevated in a dose related manner in all F1 treatment groups (exposed in utero) A statistical assessment of these data was not presented by the study investigators. However, increases ranged from 269-586% of controls in males and from 159-542% of controls in females. In addition, plasma tyrosine levels were even more dramatically elevated in F2 pups. At the 10 ppm level, male tyrosine levels were 794% of controls and females levels were 665% of controls. At the 7000 ppm level, male tyrosine levels were 5009% of controls and females were 3945% of control levels. .

The LOAEL for offspring toxicity for males and females is 10 ppm (LDT) (equivalent to 2.1/2.4 mg/kg/day [M/F]) based on tyrosinemia at all dose levels in F2a pups (exposed in utero). In addition, ocular discharge was observed at all dose levels in F1 pups (and in nearly all F2 pup dose groups) with a 0 incidence in controls and cataractous changes were observed histologically at the high dose level. An offspring toxicity NOAEL was not observed.

Even though a NOAEL for effects observed in offspring was not determined, this reproductive study in the mouse is determined to be **acceptable/guideline (§83-4(b), reproduction)** and satisfies the requirements for a multigenerational reproductive toxicity

study in mice as per the Hazard Identification Assessment Review Committee (March 13, 2001).

C. STUDY DEFICIENCIES: The following deficiencies were noted, but will not change the conclusions of the review: litters were not standardized at PND 4 and no dose rationale was provided. In addition, no offspring behavioral tests were performed and statistical assessments of plasma tyrosine levels were apparently not performed.

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